MICROBIOLOGY

Lab. 8

Gram-negative cocci
(Neisseriae)
Learning objectives:

After this lab. You must be able to:

- Differentiate between *Neissereiae spp.*
- Describe the two species microscopically and culturally.
- List types of clinical infections these organisms produce.
- Predict g-ve diplococci agents causing clinical cases.
- Diagnose the *Neisseria* spp. In clinical sample.
- Know the prevention ways of each organism.
Neisseriae spp.:

The genus *Neisseriae* contain two important pathogens:

- **N. meningitidis** - cause meningitis and meningococcemia (the most severe form of meningococcemia is life-threatening Waterhouse-Friderichsen syndrome)
- **N. gonorrhoea** - causes gonorrhoea, neonatal conjunctivitis (*ophthalmia neonatrum*) and pelvic inflammatory disease (PID).
Ophthalmia neonatrum

Waterhouse-Friderichsen syndrome
Important properties:

- Aerobic
- Gram –ve diplococci (each coccus is shaped like a kidney or coffee bean with concave side faced each other).
- Oxidase +
- Most catalase +ve
- Non motile
- Grow well on chocolate agar (blood heated to 80 °C), but not on blood agar because the growth is inhibited by toxic trace metal and fatty acids found in certain media including blood.
Neisseriae meningitidis:

Important properties:

- capsulated.
- Have 3 virulence factors: polysaccharide capsule, endotoxin (LPS), and IgA protease.
- Ferment maltose and glucose
- Transmitted by airborne droplets.
Laboratory diagnosis:

- **Specimen:** blood and CSF.
- **Microscopic:** presumptive diagnosis can be made if G–ve diplococci are seen in a smear of CSF inside the PMN (intracellular)
- **Culture:** chocolate agar at 37°C in a 5% CO₂.
- **Biochemical tests:**
  - Oxidase +ve.
  - Maltose fermentation +ve.
- **Immunological assay:** latex agglutination test detects capsule polysaccharide in CSF.
Oxidase test:

\[
\text{Cytochrome } c_{\text{(reduced)}} + O_2 \xrightarrow{\text{cytochrome oxidase}} \text{Cytochrome } c_{\text{(oxidized)}} + H_2O
\]

\[
\text{Cytochrome } c_{\text{(oxidized)}} + \text{oxidase reagent} \xrightarrow{\text{reduced}} \text{Cytochrome } c_{\text{(reduced)}} + \text{oxidase reagent} \xrightarrow{\text{oxidized}} \text{(colorless)} \xrightarrow{\text{dark purple}}
\]

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Maltose and glucose fermentation
Treatment:

• Penicillin is the treatment of choice

Prevention:

- **Chemoprophylaxis:** rifampin or ciprofloxacin
- **Immunization:**
  - **Conjugated** (the four capsular polysaccharide types conjugated to a carrier; diphtheria toxoid)
  - **Non-conjugated:** only the four capsular polysaccharide without the carrier.
Neisseriae gonorrhoea:

Important properties:

- Have no capsule.
- Have 3 virulence factors: pili, endotoxin (LOS; a modified form of endotoxin), and IgA protease.
- Ferment glucose but not maltose.
- Transmitted by sexual contact (killed by drying).
Laboratory diagnosis:

- **Specimen:** urethral discharge and cervical swab.
- **Microscopic:** in men the finding of G –ve diplococci within PMNs in a urethral discharge specimen is sufficient for diagnosis but in women culture should be done.
- **Culture:** Thayer-Martin medium (chocolate agar containing antibiotics; vancomycin, colistin, trimethoprim, and nystatin to suppress the normal flora) at 37 °C in a 5% CO₂.
- **Biochemical tests:**
  - Oxidase +ve
  - Maltose fermentation -ve
- **Nucleic acid based tests.**
211 Carbonydrate utilization test for Neisseria. Neisseria gonorrhoeae ferments only glucose. (Neisseria sugar medium with phenol red indicator, 18 h at 37°C)
Laboratory diagnosis:
Neisseria gonorrhoea in a urethral discharge
N. gonorrhoea on Thayer-Martin agar
Treatment:

- Penicillin is no longer drug of choice due to:
  - Chromosomally-mediated resistance because of (1- reduced uptake of the drug, 2- altered binding site)
  - Plasmid-encoded beta-lactamase production (penicillinase-producing *N. gonorrhoea- PPNG*)

- Ceftrixone is the treatment of choice in uncomplicated cases
- In combination of tetracycline, doxycycline or azithromycin in dual infection with *C. trachomatis*
- Follow-up.
Prevention:

- **Chemoprophylaxis** of newborns against ophthalmia neonatrum with 1% silver nitrate, 0.5% erythromycin eye ointment.

- Measures to limit epidemic include education, aggressive detection, uses of condoms, and the prompt treatment of symptomatic patients and their partners.
Thank you