

8- Gram Negative Cocci

Neisseria

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Objectives

- Understand the morphology, epidemiology, pathogenesis, and laboratory diagnosis of *Neisseria gonorrhoeae*
- Understand the morphology, epidemiology, pathogenesis, and laboratory diagnosis of *Neisseria meningitides*

Introduction

Gram -ve cocci

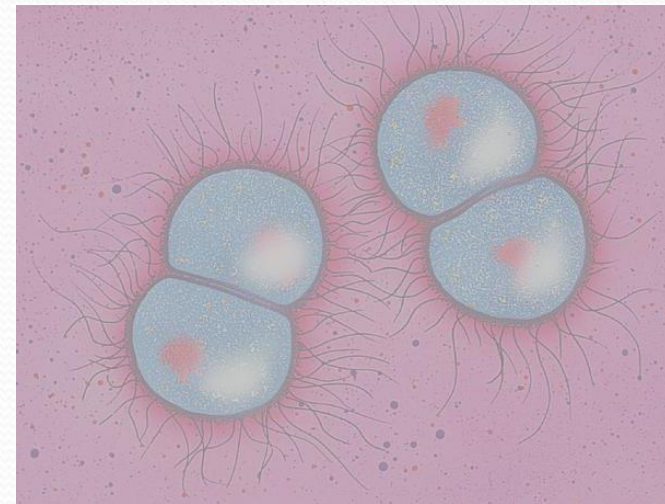
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Moraxella catarrhalis*
- Other *Neisseria species* (non-pathogenic)
N. cineria, *N. lactamica*, *N. polysaccharea*, *N. subflava*,
N. sicca, *N. mucosa*, *N. flavescens*



Neisseria gonorrhoeae
(gonococcus)

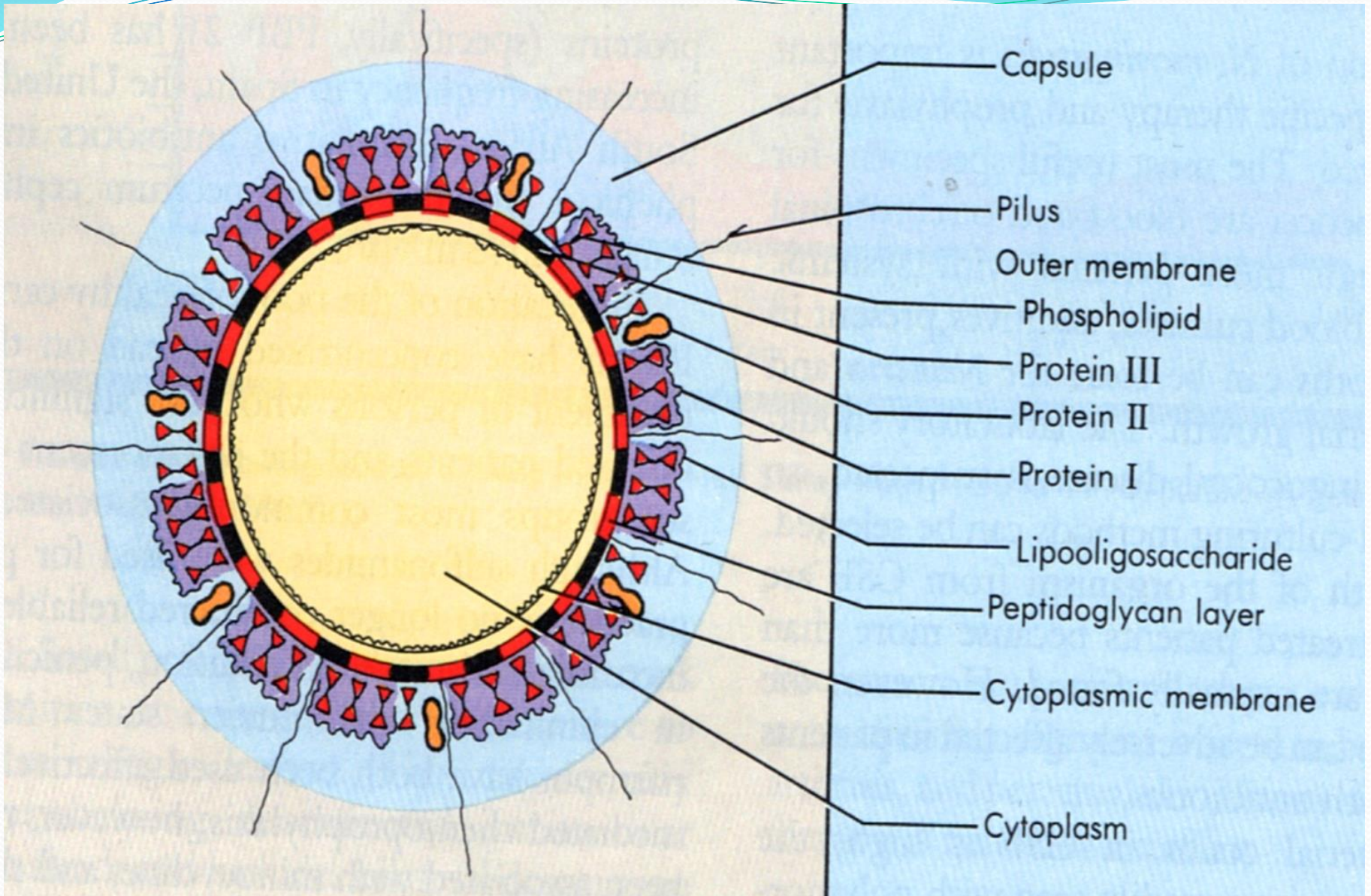
General Characteristics

- Gram-negative cocci often arranged in pairs (diplococci) with adjacent sides flattened
- Oxidase positive
- Most catalase positive
- Non-motile, non spore forming
- Sensitive, aerobic but grow better with low CO₂ and susceptible to cool temperatures, drying and fatty acids



Structure

- Pili (N-methylphenylalanine)
- Outer membrane:
 1. Phospholipids
 2. Proteins: Outer membrane proteins (OMP I, OMP II, Opa)
 3. Lipopolysaccharide (LPS) mainly as lipooligosaccharide (LOS)
- Antigenic variation: Pili, OMPs, LOS



Epidemiology

- *Neisseria gonorrhoeae* is a common source of infection in humans
- Not part of normal human flora, only found in mucous membranes of genitalia, anorectal area, oropharynx or conjunctiva during infection
- Transmission primarily by sexual contact or from infected mother during birth
- Asymptomatic carriage is a major reservoir
- Lack of protective immunity and therefore reinfection, partly due to antigenic diversity of strains

Pathogenesis

1. Attachment and invasion: pili and outer membrane protein help the bacteria to attach to intact mucous membrane epithelium. Invasion of submucosal epithelial cells through parasite-directed endocytosis
 2. Survival and multiplication in the submucosa, establish infection in the sub-epithelial layer
 3. Spread and dissemination: The bacteria cause local cell injury and inflammatory response. Local spread to adjacent structures or systemic dissemination through blood (bacteremia)
- Most common sites of inoculation:
 - Cervix (cervicitis) or vagina in the female
 - Urethra (urethritis) or penis in the male

Virulence Factors

- *N. gonorrhoea* secretes IgA protease that inhibit IgA antibodies function
- Outer membrane proteins:
 1. Prevents phagolysosome and promotes intracellular survival
 2. Mediates firm attachment to epithelial cells and subsequent invasion into cells
 3. Protects other surface antigens from bactericidal antibodies
- Lipooligosaccharide (LOS) (Lipid A) has endotoxin activity
- Acquisition of antibiotic resistance:
 1. Plasmid-encoded beta-lactamase production
 2. Chromosomally-mediated changes in cellular permeability inhibit entry of antibiotics

Clinical Presentation

- **In Men:**

Urethritis: Most infections among men are acute and symptomatic with purulent discharge & dysuria (painful urination) after 2-5 day incubation period

- **In women:**

Cervicitis: mild pain, discharge, dysurea but mostly asymptomatic

Pelvic Inflammatory Disease: ectopic pregnancy and infertility

Disseminated Gonococcal Infection: bacteremia

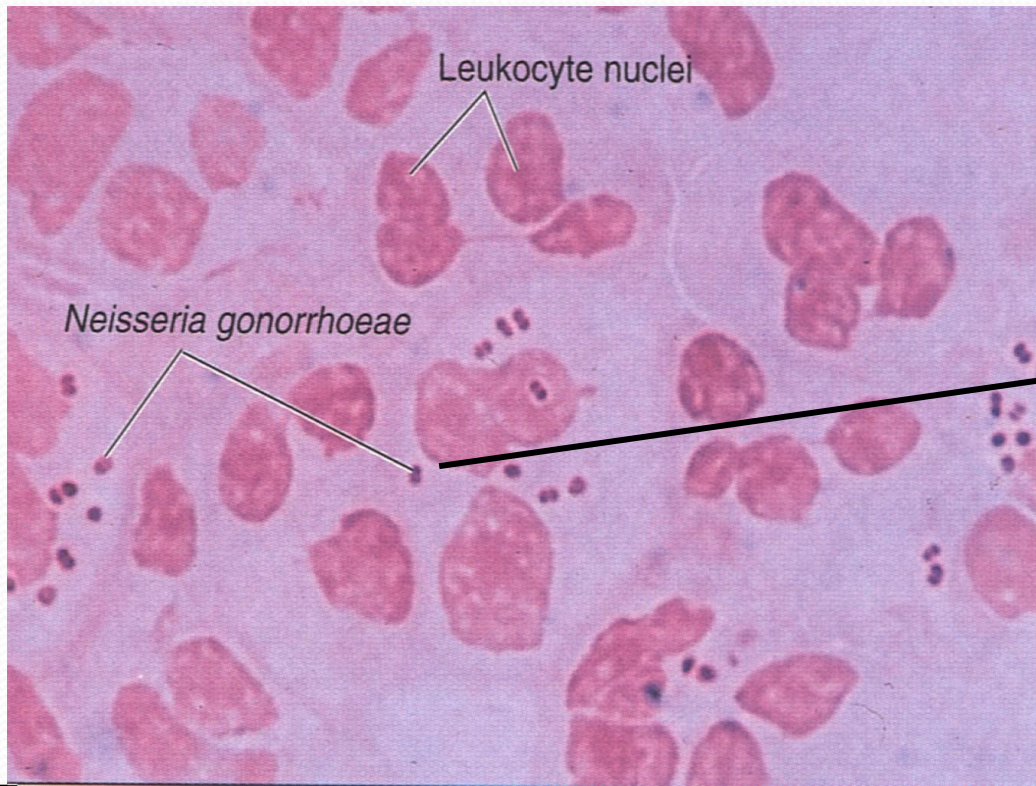
Laboratory Diagnosis

Specimen collection and transportation

- Specimens: urethral discharge, cervical swab, blood
- Should be processed immediately (no more than 6 hours)
- Do not expose to high temperature or dry conditions
- Cotton swab should include charcoal to inhibit fatty acid formation
- Transport in media with increased CO₂ using special packaged system that contain CO₂ generation system

1. Gram Stain

- Gram -ve diplococci inside polymorphonuclear leukocytes
- Gram stain: 95% specific and sensitive in men, 50% - 70% in women



2. Culture

Media:

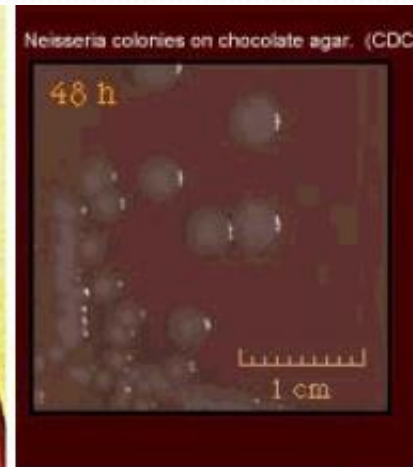
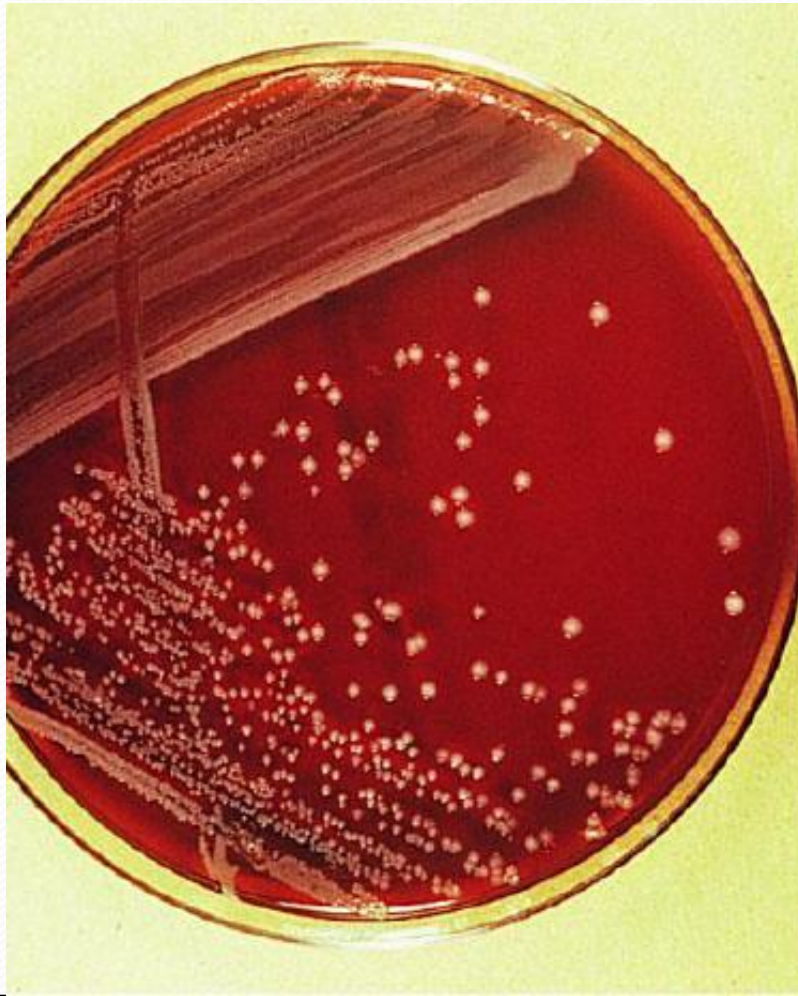
- Thayer Marten Media (TM): Enriched chocolate agar with antimicrobial colistin (to inhibit G- bacilli) nystatin (to inhibit yeast) and vancomycin (to inhibit G+ bacteria)
- Modified Thayer Marten Media (MTM): as above plus trimethoprim (to inhibit *proteus*)
- Martin Lewis medium (ML): same as above except that anisomycin is substituted for nystatin and vancomycin concentration is increased

Incubation conditions:

- Prewarm the media
- Incubate at 35-37 °C for 73 hours
- CO₂ enriched ex candle jar (5-7%)
- Humid atmosphere ex sterile gauze pad soaked with sterile water in the bottom of candle jar

Colonial appearance:

Small, grayish white, convex, translucent, shiny, with smooth or irregular margins



3. Biochemical Tests

- Oxidase positive
- Glucose fermentation positive (while maltose and lactose fermentation is negative)
- Nitrite reduction negative



4. Immunological Tests

- Commercially available particle agglutination tests using specific monoclonal antibodies are available and used mainly for confirmation of colony growth
- Immunological assays is less useful due to antigenic variations

5. Antimicrobial Susceptibility Tests

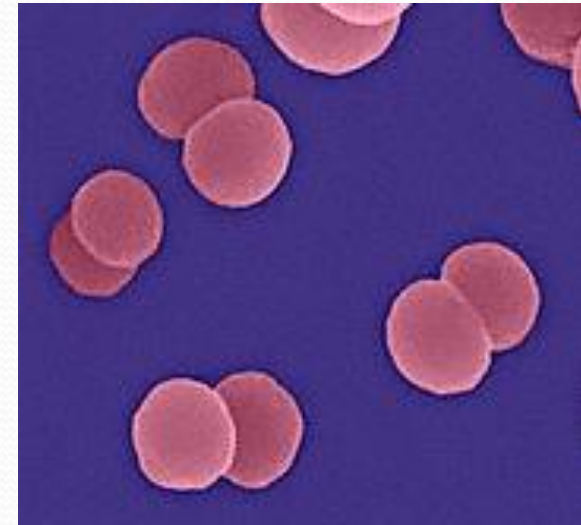
- Resistance to penicillin is quite common due to production of beta-lactamase
- Resistance to ceftriaxone is not described
- Treatment with ceftriaxone is effective and does not require routine susceptibility testing



Neisseria meningitidis
(meningococcus)

General Characteristics

- Encapsulated small, gram-negative diplococci
- Oxidase positive
- Catalase positive
- Can be a member of the normal flora of the upper respiratory tract
- Causes life-threatening disease when the bacteria invade the blood or cerebrospinal fluid
- CO₂ enhances growth but is not absolutely required
- Less sensitive than *Neisseria gonorrhoeae*
- Have a well developed highly antigenic capsule



Structure

- Pili: attachment and enhance virulence
- Outer membrane:
 1. Porins
 2. Outer membrane proteins (OMP)
 3. Lipooligosaccharide (LOS)
- Capsule contains polysaccharide with more than 13 known antigenic types
- Types A, B, C, Y & W₁₃₅ are more commonly associated with human disease

Epidemiology

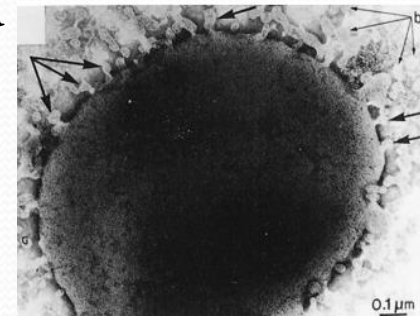
- *Neisseria meningitidis* found as nasopharyngeal flora in 10% of healthy individuals
- Transmission occurs by inhalation of respiratory droplets through close contacts with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)
- The most common cause of meningitis in under 20 and the second most common cause after *pneumococci* in all ages
- Usually cause sporadic cases but can be associated with outbreaks

Virulence Factors

- Pili-mediated, receptor-specific colonization of nonciliated cells of nasopharynx
- Antiphagocytic polysaccharide capsule allows systemic spread in absence of specific immunity
- Toxic effects mediated by hyperproduction of lipooligosaccharide (Endotoxin)

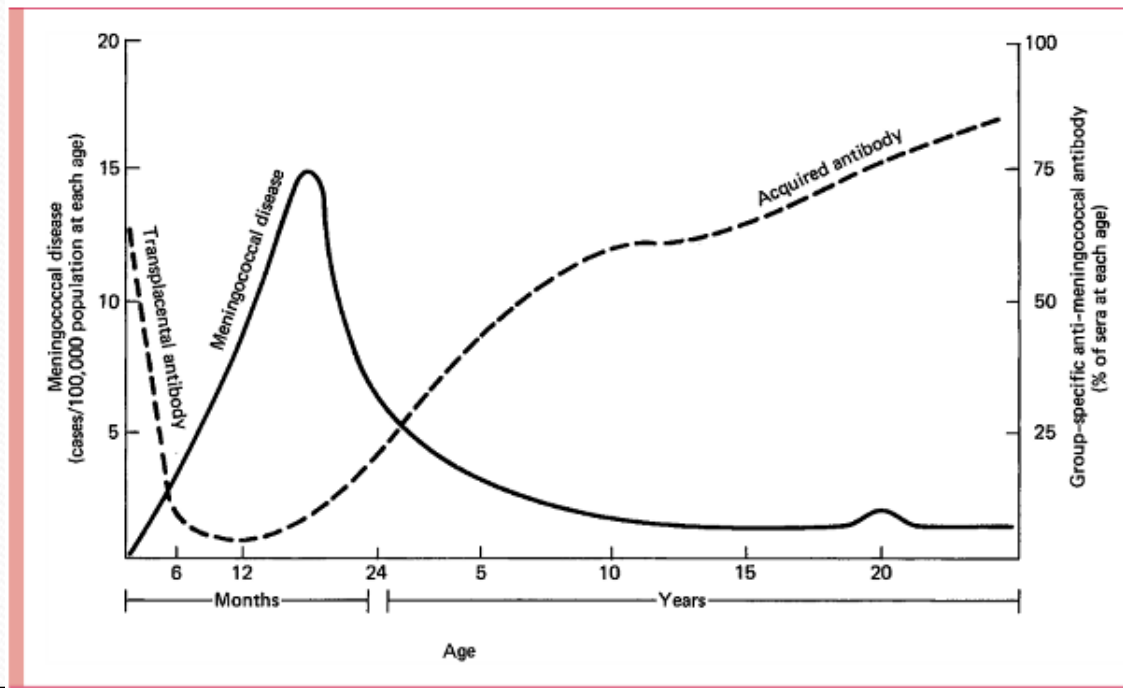
Pathogenesis

- Pili/fimbriae facilitate attachment to mucosal epithelium and invasion of submucosa
- Specific receptors for bacterial fimbriae on nonciliated columnar epithelial cells in nasopharynx of host
- Organisms are internalized into phagocytic vacuoles, avoid intracellular killing
- Replicate intracellularly and migrate to subepithelial space
- Once bacterial reach blood survival is mediated by production of polysaccharide capsule
- Endotoxin release and blebbing mediates systemic manifestation like shock
- Primarily infect CNS to cause acute purulent meningitis with meningococcal bacteremia and systemic manifestation



Immunity

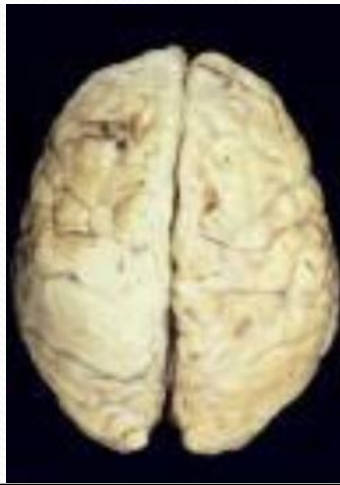
- Immunity to meningococcal infection is related to group specific antipolysaccharide antibody which is bactericidal and facilitate phagocytosis
- Infection, carrier state or other polysaccharide stimulate antibodies production
- Absence of antibody correlates with susceptibility



Clinical Presentation

Meningitis:

- Fever, fatigue, weakness
- CNS: convulsion, motor disability, loss of consciousness
- Thrombocytopenia results in bleeding and skin petechiae
- Disseminated intravascular coagulation (DIC)
- Fatal if not treated early (death within 6 hours of initial presentation)



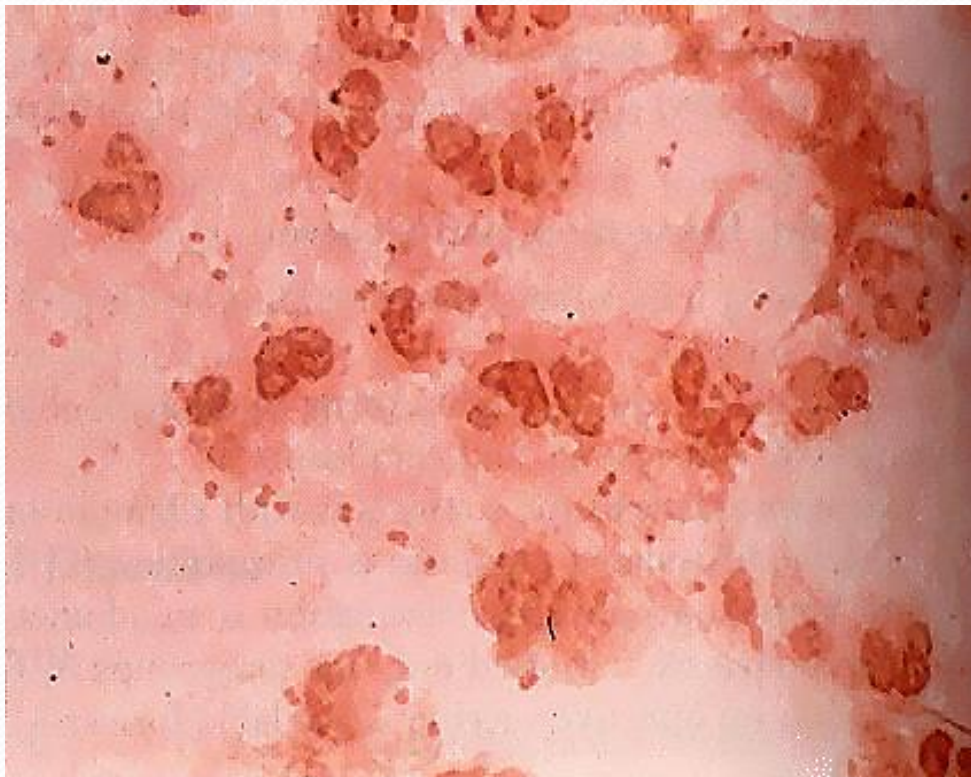
Laboratory Diagnosis

Specimen collection and transportation

- Specimens: pharyngeal swab, cerebrospinal fluids, skin lesions and blood
- Less sensitive compared to *Neisseria gonorrhoeae*, however quick handling is required to establish the diagnosis early
- Transport in media with increased CO₂ using special packaged system that contain CO₂ generation system

1. Gram Stain

- Large numbers of encapsulated, small, gram-negative diplococci (flattened along adjoining side) and polymorphonuclear leukocytes (PMN's) can be seen microscopically in cerebrospinal fluid (CSF)



2. Culture

Media:

- Thayer Marten Media (TM)
- Modified Thayer Marten Media (MTM)
- Blood or Chocolate agar

Incubation conditions:

- Incubate at 35-37 °C for 18 hours
- CO₂ enriched ex candle jar (5-7%)
- Humid atmosphere ex sterile gauze pad soaked with sterile water in the bottom of candle jar

Colonial appearance:

- Medium, smooth, round, moist, gray to white, encapsulated strains are mucoid

3. Biochemical Tests

- Oxidase positive
- Glucose and maltose fermentation positive (while lactose fermentation is negative)
- Nitrite reduction negative

4. Immunological Tests

- The routine detection of *Neisseriae meningitidis* capsular polysaccharide antigen in body fluids (CSF) is not recommended

5. Antimicrobial Susceptibility Tests

- Resistance to penicillin is very rare and accordingly penicillin is still the drug of choice
- Chloramphenicol or cephalosporins can be used as alternatives
- Routine susceptibility testing is of limited value

Diagnosis

Neisseria gonorrhoeae and *Neisseria meningitidis* Flow Chart



Microscopic Appearance
Kidney bean shaped Gram negative diplococci



Growth in Culture
Use chocolate agar or Thayer Martin agar
Fastidious
Capnophilic
Will not grow at 22°C



Positive Oxidase Test

Sugar Utilization



Glucose Maltose

Glucose positive
Neisseria gonorrhoeae



Glucose Maltose

Glucose Positive, Maltose Positive
Neisseria meningitidis

Prophylaxis and vaccination

- Chemoprophylaxis of close contacts (if susceptible)
- Polyvalent vaccine containing serogroups A, C, Y, and W₁₃₅ is effective for immunoprophylaxis as an adjunct to chemoprophylaxis
- Serogroup B is only weakly immunogenic and protection must be acquired naturally from exposure to cross-reacting antigens



Thank you....