Aerobic Gram Negative Cocci

General Characteristics
- Normal flora of upper respiratory, alimentary and genitourinary tracts
- Gram stain: GNDC
- Oxidase positive
- Catalase positive
- Non-motile
- Fastidious
- Capnophilic

Aerobic GNDC

General Characteristics
- Virulence factors
  - Pathogenic species
    - Endotoxin, pili, polysaccharide capsule, IgA protease

Aerobic GNDC

Neisseria gonorrhoeae
- Fastidious
  - Enriched media
    - Chocolate agar
    - Modified Thayer Martin Agar
      - Chocolate agar that is selective
        - Contains antibiotics to inhibit non-pathogenic organisms
          - Vancomycin, colistin, nystatin, trimethoprim
        - Selective for N. gonorrhoeae, N. meningitidis, and N. lactamica
      - Capnophilic
        - 2-8 % CO₂

Aerobic GNDC

Neisseria gonorrhoeae
- Culture immediately
  - Temperature sensitive
  - Transport at room temp.
  - Susceptible to drying
  - Direct inoculation optimal
- Dacron or Rayon swabs
  - Calcium alginate or some cotton swabs are inhibitory

Aerobic GNDC

Neisseria gonorrhoeae
- Transport media if delay
  - Selective enriched media with transport system (carbon dioxide atmosphere)
  - Examples:
    - Jembec
    - Transgrow
    - Gono-Pak
    - Bio-Bag
  - Offsite clinics and physician’s offices
**Neisseria gonorrhoeae**

- Genital specimens
  - Routinely screened for using selective media
- Other specimens
  - Synovial fluid, eye, rectal, etc.
  - Physician must notify laboratory that *Neisseria gonorrhoeae* is suspected
  - Chocolate agar used in culture set up

**Specimen Gram Stain**

- Intracellular GNDC

**Colonial Morphology**

- Chocolate agar/Selective agar such as Thayer-Martin
  - Small, tan, translucent, and raised

**Rapid Identification**

- Molecular testing
  - Nucleic acid detection
    - Allows for simultaneous detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*
    - Co-infections not uncommon
    - Two most common STDs after HPV

- Molecular testing
  - Nucleic acid detection
    - Genital or urine specimens
    - Urine: Non invasive
    - Rapid and sensitive in high risk populations
    - Additional info in textbook
Culture Identification

- Oxidase = pos
- Carbohydrates
  - Glucose = pos
  - Maltose = neg
  - Lactose = neg
  - Sucrose = neg
- DNase = neg
- Nitrate = neg
- Butyrate esterase = neg

Neisseria gonorrhoeae

Pathogenesis

- Port of entry
  - Columnar or transitional epithelium
    - Infects any site with these cells and then can spread
      - Primarily acquired by sexual contact
        » Urethra, endocervix, anal canal, pharynx and conjunctiva
      - Co-infections can be seen with other sexually transmitted diseases
        » Syphilis and/or Chlamydia
- Human to human transfer
  - Primarily a venereal disease
  - Asymptomatic reservoir the most common source of infection
    - Female reservoir
- Progression of infection
  - 2 to 7 days incubation
  - Untreated infections
    - Blood-borne dissemination, infectious arthritis or rarely septicemia
- Male
  - Acute urethritis, prostatitis and epididymitis if untreated
- Female
  - May be asymptomatic or exhibit severe discharge
    - Cervicitis pelvic inflammatory disease scarring of fallopian tubes sterility, ectopic pregnancies
Other Infections

- Disseminated gonococcal infection
  - Spread from the genitourinary tract, rectum or pharynx to the blood stream
  - Infectious arthritis
- Pharyngitis
- Gonococcal ophthalmia neonatorum
- Vulvovaginitis
- Rectal

Reportable disease

- Report within 7 days venereal infections or ophthalmia neonatorum to the state health dept.

Treatment

- Treatment
  - Empiric
    - Drug of choice is broad spectrum cephalosporins – ceftriaxone or cefixime
    - Most patients treated for co-infections with Chlamydia
  - Eye treatments for infants within 1 hour of delivery

Antimicrobial testing

- Antimicrobial testing
  - CLSI standards available but not routinely done
  - Beta-lactamase is done

Neisseria meningitidis

- Fastidious
  - Chocolate agar and 2-8 % CO₂
  - Will grow on Thayer Martin agar
- Temperature sensitive
  - Keep specimen at room temp until set up
  - Culture immediately
  - Delay in culture set up should be avoided.

Virulence factors

- Pili
- Polysaccharide capsule
  - Serotype b the most common
- IgA1 protease
  - Increased invasiveness
Colony Morphology

- Sheep blood agar – bluish-gray colonies
- Chocolate agar or selective agar such as Thayer Martin – small, tan, sometimes mucoid, convex colonies

Specimen Gram Stain

- GNDC

Culture Identification

- Oxidase = pos
- Carbohydrates
  - Glucose = pos
  - Maltose = pos
  - Lactose = neg
  - Sucrose = neg
- DNase = neg
- Nitrate = neg
- Butyrate esterase = neg

Culture Identification

- Enzyme production detected by chromogenic substrates
  - Isolates from selective media only
  - Bact/Neisseria Card (Remel) is one kit available
    - Indolyl butyrate esterase (IB) = negative
    - Hydroxylprolylaminopeptidase (PRO) = variable
    - γ-glutamyl-aminopeptidase (GLUT) = positive
    - β-D-Galactosidase (BGAL) = negative
  - Growth on selective media aids in identification

Pathogenesis

- Primary pathogen in humans only
  - Asymptomatic carriers
  - Source of infections – oral secretions or respiratory droplets of asymptomatic nasopharyngeal carriers
  - Dissemination into the blood stream leads to septicemia and/or meningitis

Pathogenesis

- Endemic meningitis
  - Often in college age young adults
  - Esp. in closed populations, such as college dormitories and military barracks
  - Symptoms include:
    - Purpura with petechial rash (classic)
    - Other symptoms of meningitis
      - Fever, lethargy, irritability, headache, photophobia, stiff neck, and seizures
**Neisseria meningitidis**

**Pathogenesis**
- Septicemia
- Septic arthritis
- Endocarditis
- Pneumonia
- Urethritis

**Reportable Disease**
- Report immediately cases of meningitis or meningococcemia to the state health dept.

**Treatment and Antimicrobial testing**
- Treatment
  - Penicillin
  - 3rd generation cephalosporins for meningococcemia
- Antimicrobial testing
  - CLSI standards; Not routinely done
  - Beta lactamase production is rare

**Prevention**
- Single dose vaccine
  - Polysaccharide capsular antigens A, C, Y and W-135
  - Does not protect against the most common serotype b
- Prophylaxis treatment given to close contacts of patient with meningococcal meningitis
  - Rifampin or a sulfonamide

**Neisseria lactamica**

**Aerobic GNDC**

**Neisseria lactamica**

**Colony Morphology**
- Blood agar – bluish-gray colonies
- Chocolate or selective agar such as Thayer-Martin - small, tan, convex colonies
  - Non pathogen that grows on selective agar
**Culture Identification**

*Neisseria lactamica*

- Oxidase = pos
- Carbohydrates
  - Glucose = pos
  - Maltose = pos
  - Lactose = pos
  - Sucrose = neg
- DNase = neg
- Nitrate = neg
- Butyrate esterase = neg

*Neisseria lactimica*

- Enzyme production detected by chromogenic substrates
  - Isolates from selective media only
    - BactNeisseria Card (Remel) is one kit available
      - Indoxyl butyrate esterase (IB) = negative
      - Hydroxylprolylaminopeptidase (PRO) = n/a
      - γ-glutamyl-aminopeptidase (GLUT) = n/a
      - β-D-Galactosidase (BGAL) = positive
    - NOTE: BGAL is the enzyme that breaks down lactose

**Clinical Significance**

*Neisseria lactimica*

- Normal flora of the nasopharynx
- Growth on selective GNDC media
  - Differentiate from pathogens

*Other Neisseria species*

- Respiratory cultures
  - Yellow colonies
  - No further work-up, normal flora
- Selective media or sterile sites
  - Should be differentiated from pathogenic *Neisseria*
- Sterile sites may require full identification

**Morphology and characteristics**

*Other Neisseria species*

- Gram stain = GNDC
- Colony morphology – growth on sheep or chocolate agar with varies colonies morphologies

**Clinical Significance**

*Other Neisseria species*

- Usually normal flora
- Commensal *Neisseria* species
  - Rarely implicated in disease
  - Meningitis, endocarditis, prosthetic heart valves, bacteremia, pneumonia, empyema, bacteriuria, osteomyelitis and ocular infections
**Moraxella catarrhalis**

- From specimen = intracellular GNDC
  - Respiratory specimen
- From colony = GNDC

**Aerobic GNDC**

**Colony Morphology**

- Sheep blood or Chocolate agar
  - Smooth, opaque, gray to white colonies
- "Hockey Puck" – colony remains intact when pushed across plate with loop or stick
- Thayer-Martin agar - variable

**Culture Identification**

- Oxidase = pos
- Carbohydrates
  - Glucose = neg
  - Maltose = neg
  - Lactose = neg
  - Sucrose = neg
- DNase = pos
- Nitrate = pos
- Butyrate esterase = pos

**Clinical Significance**

- Normal flora of the upper respiratory tract
- Opportunistic upper respiratory pathogen esp. in children and the elderly
  - Third most common cause of otitis media and maxillary sinusitis

**Enzyme production detected by chromogenic substrates**

- Isolates from selective media only
  - BaclNeisseria Card (Remel) is one kit available
    - Indoxyl butyrate esterase (IB) = positive
    - Hydroxyprolylaminopeptidase (PRO) = N/A
    - γ-glutamyl-aminopeptidase (GLUT) = N/A
    - β-D-Galactosidase (BGAL) = N/A
**Clinical Significance**

- Acute bronchitis
- Pneumonia
- Severe infections seen in elderly and immunocompromised
  - Endocarditis, meningitis, and bacterial tracheitis

**Antimicrobial testing**

- Most are beta lactamase positive
  - Currently no other mechanisms of resistance
- Many beta lactams maintain activity

---

**Definitive Identifications**

- *Neisseria* and *Moraxella* species
  - Clinically relevant species can be identified by using rapid multi-test systems
  - Use combination of tests described today:
    - IDS-RapID-NH (Remel)
    - HNID (Dade Behring)
    - Crystal Neisseria/Haemophilus (Siemens)
    - NHI Card (bioMerieux)

---

**Summary**

**Aerobic GNDC**

- **Specimen handling and processing**
  - GNDC
    - Most are fastidious
    - Require enriched media and 2-8 % CO₂
    - Culture immediately
    - *N. gonorrhoeae* used transport media with carbon dioxide atmosphere

**Growth characteristics**

- Selective media
  - Thayer Martin
    - Growth of *N. gonorrhoeae, N. meningitidis*, and *N. lactamica*
- Identification
  - Key reactions
    - GNDC, oxidase positive
    - Carbohydrate utilization
    - Butyrate esterase and other enzyme detections

---

**Antimicrobial testing**

- Most are beta lactamase positive
  - Currently no other mechanisms of resistance
- Many beta lactams maintain activity

**Summary**

- **Clinical significance**
  - *N. gonorrhoeae*
  - *N. meningitidis*
  - *Moraxella catarrhalis*
- Antimicrobial testing
  - Beta lactamase
  - CLSI standards but not routinely performed
Summary

• Flowcharts

Who am I?

Neisseria gonorrhoeae

Who am I?

Neisseria meningitidis

Day 7

• Remember Key Points quiz.....