AEROBIC GRAM NEGATIVE COCCI

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General Characteristics

- Habitat – upper respiratory tract, alimentary and urogenital tracts
- Gram negative cocci occurring in pairs (diplococci) having a coffee bean or kidney bean shape (GNDC), may be intracellular
- Fastidious- requiring enriched media for optimal recovery; Capnophilic –requiring CO2 for growth
- Cytochrome oxidase positive, most are catalase positive, non-motile, capnophilic
- Virulence factors: endotoxin, pili, polysaccharide capsule, IgA protease

Organisms

A. Neisseria species

1. Neisseria gonorrhoeae
   a. Morphology and characteristics
      i. Growth requirements
         - Fastidious (requiring enriched media and 2-8% CO₂ to grow)
           o Chocolate agar
           o Modified Thayer Martin: Selective for N. gonorrhoeae, N. meningitidis, and N. lactamica
             - Agar is chocolate agar with antibiotics vancomycin, colistin, nystatin, trimethoprim which inhibits gram positive and gram negative organism as well as yeast and swarming Proteus sp respectively
         - Temperature sensitive (transport at room temp)
         - Susceptible to drying; Requires high humidity
           o Direct inoculation to media gives optimal results
           o Alternatively swabs with transport system can be used
         - Dacron or rayon swabs should be used
           o Calcium alginate and some cotton swabs are inhibitory
         - If long transportation time, send in transport media that provides carbon dioxide atmosphere
           o Several transport systems available that contain selective enriched media and a carbon dioxide atmosphere
             - Jembec, Transgrow, Gono-Pak, Bio-Bag
             - Especially useful for clinics or physician’s office located offsite from lab
         - Genital specimens – routinely screened for N. gonorrhoeae using selective media
         - Other sources such as synovial fluid – caregiver should notify laboratory that Neisseria gonorrhoeae suspected so the correct media can be inoculated
      ii. Gram stain
         - From specimen: often gram negative intracellular diplococci (GNID)
         - Isolated colony: GNDC
      iii. Colony morphology (24 to 48 hours)
         - Chocolate or selective (Thayer Martin/Jembec) agars: small, tan, translucent, and raised
iv. Rapid Identification
- Direct Microscopic Examination
  - Diagnostic for symptomatic male with urethral discharge
  - Intracellular gram negative diplococci
  - Not diagnostic for females – commensal flora resemble GNDC
- Molecular testing
  - Nucleic acid detection (Amplified or nonamplified technologies)
    - Genital or urine specimens only
    - Rapid and sensitive in high risk populations
    - Simultaneous detection for both *N. gonorrhoeae* and *Chlamydia trachomatis*

v. Culture Identification
- **Oxidase = Positive (key reaction)**
- **Carbohydrate utilization**
  - Dextrose = positive
  - Maltose = negative
  - Lactose = negative
  - Sucrose = negative
- DNase = negative
- Nitrate reduction = negative
- Butyrate esterase (Catarrhalis disk) = negative
- Enzyme production detected by chromogenic substrates
  - Isolates must be taken from a Selective media such as Thayer Martin/Jembec
    - Indol butyrate esterase (IB) = negative
    - Hydroxylprolylaminopeptidase (PRO) = positive
    - γ-glutamyl-aminopeptidase (GLUT) = negative
    - β-D-Galactosidase (BGAL) = negative

b. Humans are only natural reservoir; always pathogenic

c. Pathogenesis
i. Port of entry
  - Primary infection usually occurs at columnar and transitional epithelium of urethra and endocervix
  - Conjunctival, pharynx and rectal epithelium may also serve as portal of entry

ii. Progression of infection
  - Two to seven day incubation period
  - Untreated infections can lead to blood-borne dissemination, resulting in infectious arthritis and rarely septicemia

iii. Human to human transfer, primarily venereal spread

iv. Asymptomatic reservoir the most common source of infection (often female)
d. Clinical Significance

i. Gonorrhea
   • Male: acute urethritis, prostatitis and epididymitis if untreated
   • Female: may be asymptomatic or exhibit severe discharge
     o Cervicitis --> pelvic inflammatory disease --> scarring of fallopian tubes --> sterility, ectopic pregnancies

ii. Disseminated gonococcal infection (DGI)
   • Spread from the genitourinary tract, rectum or pharynx to the blood stream
     o Cause of infectious arthritis

iii. Pharyngitis; Oral infection

iv. Gonococcal ophthalmia neonatorum; Conjunctiva infection in the newborn

v. Vulvovaginitis in young girls

vi. Rectal

vii. Reportable disease: Report within 7 days venereal infections or ophthalmia neonatorum to the state health dept.

e. Treatment

i. Empiric therapy options.
   • Drug of choice is broad spectrum cephalosporins – ceftriaxone or cefixime
   • Increased resistance to fluoroquinolone seen no longer recommended for treatment

ii. Most patients treated with antibiotics for possible co-infections with Chlamydia.

iii. Eye treatments for infants with 1 hour of delivery

f. Antimicrobial testing

i. CLSI standards for antimicrobial disk susceptibility testing; not routinely performed.

ii. Beta lactamase testing routinely done
2. *Neisseria meningitidis*
   a. Morphology and characteristics
      i. Growth requirements
         - Fastidious (requiring an enriched media such as chocolate agar and 2-8% CO₂ to grow)
         - Temperature sensitive
      ii. Virulence factors: pili, polysaccharide capsule (serotype b the most common) and some virulent strains have IgA1 protease
      iii. Colony morphology
         - Sheep blood agar – bluish-gray colonies
         - Chocolate agar or selective agar (Thayer Martin/Jembec)- small, tan, sometimes mucoid, convex colonies
      ii. Gram stain: GNDC can see intracellular in specimen gram stain
      iii. Culture Identification
         - Oxidase = positive (key reaction)
         - Carbohydrate utilization
           - Dextrose = positive
           - Maltose = positive
           - Lactose = negative
           - Sucrose = negative
         - DNase = negative
         - Nitrate reduction = negative
         - Butyrate esterase (Catarrhalis disk) = negative
         - Enzyme production detected by chromogenic substrates
           - Isolates must be taken from a Selective media such as Thayer Martin/Jembec
             - Indoxyl butyrate esterase (IB) = Negative
             - Hydroxylprolylaminopeptidase (PRO) = variable
             - \( \gamma \)-glutamyl-aminopeptidase (GLUT) = positive
             - \( \beta \)-D-Galactosidase (BGAL) = negative
   b. Clinical Significance
      i. Primary pathogen in humans only; can be commensal; Oral secretions or respiratory droplets of asymptomatic nasopharyngeal carriers are source of infection
      ii. Dissemination into the blood stream leads to septicemia and/or meningitis
      iii. Endemic meningitis
         - Often in college age young adults
         - Especially in closed populations, such as college dormitories and military barracks
         - Classic symptoms include: purpura with petechial rash, fever
      iv. Causative agent of septicemia, septic arthritis, endocarditis, pneumonia, urethritis
      v. Reportable disease: Report immediately cases of meningitis (CSF) or meningococcemia (bloodstream) to the state health dept.
      vi. Treatment and antimicrobial testing/Prevention
         - Penicillin is drug of choice; meningococcemia use 3rd generation cephalosporins
         - CLSI standardized methods available but not routinely performed.
         - Beta lactamase production is rare. Resistance by altered penicillin-binding proteins is emerging.
         - Single dose vaccine is available to 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
3. **Neisseria lactamica** (amica= latin for friend or friendly)
   a. Morphology and characteristics
      i. Gram stain: GNDC
         
      ii. Colony morphology
          - Sheep blood agar – bluish-gray colonies
          - Chocolate agar or selective agar (Thayer Martin/Jembec)- small, tan, convex colonies (very similar to N. meningitis but usually a smaller colony)
         
      iii. Culture Identification
          - Oxidase = positive (key reaction)
          - Carbohydrate utilization
            - Dextrose = positive
            - Maltose = positive
            - Lactose = positive (only Neisseria that will do this)
            - Sucrose = negative
          - DNase = negative
          - Nitrate reduction = negative
          - Butyrate esterase (Catarrhalis disk) = negative
          - Enzyme production detected by chromogenic substrates
            - Isolates must be taken from a Selective media such as Thayer Martin/Jembec
              - Indolyl butyrate esterase (IB) = negative
              - Hydroxylprolylaminopeptidase (PRO) = n/a
              - γ-glutamyl-aminopeptidase (GLUT) = n/a
              - β-D-Galactosidase (BGAL) = positive (only Neisseria that will do this)

   b. Clinical Significance
      i. Normal flora of nasopharynx (more frequently in young children than adults)
      ii. May be protective against N. meningitidis infections
      iii. Growth on selective GNDC media requires identification to differentiate from pathogens

4. **Other Neisseria species**
   a. Morphology and characteristics
      i. Gram stain: GNDC
         
      ii. Colony morphology
          - Several species with grow on sheep and chocolate agars with various colony morphologies
         
      iii. Culture identification
          - Colonies isolated from respiratory sources showing a yellow color can be reported as normal respiratory flora without additional testing performed
          - Colonies isolated from selective media or sterile body sites should be differentiated from pathogenic Neisseria
          - Sterile sites may required full species identification with multi-test systems as they may be a causative agent of disease
         
      iv. Clinical Significance
          - Usually normal flora
          - Commensal Neisseria sp. are rarely implicated in disease but have been implicated in a variety of infections
            - Meningitis, endocarditis, prosthetic heart valves, bacteremia, pneumonia, empyema, bacteriuria, osteomyelitis and ocular infections
B. Other Gram Negative Cocci
   1. *Moraxella (Branhamella) catarrhalis*
      a. Morphology and characteristics
         i. Gram stain
            • From specimen: GNDC, Intracellular gram negative cocci (GNID) in respiratory specimens is suggestive of *M. catarrhalis*
            • From colony: GNDC
         ii. Colony morphology
            • Sheep blood /Chocolate agar - smooth, opaque, gray to white colonies
            • “Hockey puck” – colony remains intact when pushed across plate with loop
            • Thayer Martin agar – usually no growth, but colistin resistant strains will grow
         iii. Culture Identification
            • Oxidase = positive (key reaction)
            • Carbohydrate utilization
              o Dextrose = negative
              o Maltose = negative
              o Lactose = negative
              o Sucrose = negative
            • DNase = positive
            • Nitrate reduction = positive
            • Butyrate esterase (Catarrhalis disk) = positive
              o Tributyrin is the substrate used to detect butyrate esterase activity
            • Enzyme production detected by chromogenic substrates
              o Isolates must be taken from a Selective media such as Thayer Martin/Jembec
                • Indoxyl butyrate esterase (IB) = positive
                • Hydroxylprolylaminopeptidase (PRO) = n/a
                • γ-glutamyl-aminopeptidase (GLUT) = n/a
                • β-D-Galactosidase (BGAL) = n/a
      b. Clinical Significance
         i. Normal flora of the upper respiratory tract
         ii. Opportunistic upper respiratory pathogen especially in children and the elderly
            • In children, third most common cause of otitis media and maxillary sinusitis
         iii. Can cause lower respiratory infections; acute bronchitis, pneumonia,
         iv. Severe infection seen in elderly and immunocompromised patients
            • Endocarditis, meningitis and bacterial tracheitis
      c. Antimicrobial Testing
         i. Beta lactamase testing, most do not produce resistance through other mechanisms so beta lactamase testing is sufficient. Most are beta lactamase positive.
         ii. Susceptibility testing is not needed to guide therapy; many beta-lactam antibiotics maintain activity.