Non-fermenting Gram Negative Bacilli

Objectives:
1. Give the general characteristics of the pseudomonads and other nonfermenting Gram-negative rods.
2. Associate infections/diseases & symptoms with the causative agents within the nonfermenting GNR.
3. Describe characteristics unique to the pseudomonads and describe colonial morphology on RAF & MAC.
4. Differentiate between fluorescent pseudomonads.
5. Review the most common non-fermenters in the clinical laboratory: 
P.aeruginosa, Acinetobacter spp., & Stenotrophomonas maltophilia
- indicate associated infections & biochemical characteristics of each.
6. Differentiate between the miscellaneous non-fermenting Gram-negative bacilli by summarizing the unique biochemical characteristics of each. Review the clinical significance of each bacterium studied.
7. Analyze key identifying biochemical test results to correctly identify the associated genus and species described.

General Characteristics:
- 15% of GNR isolated organisms from clinical infections
- Organisms are ubiquitous
- Soil, water, plants, decaying vegetation, food
- Considered opportunists
- Nosocomial/hospital acquired
- Saline, catheters, dialysate fluid, nebulizers, etc.
- Typical but variable resistance
- Chlorhexidine
- Quaternary ammonium compounds
General Characteristics:

- Nosocomial infections
- Septicemia
- Meningitis
- Wound infections
- Surgery and trauma infections
- Osteomyelitis
- Increased risk factors include
  - Immunocompromised individuals
  - Implantation of foreign objects
  - Trauma
  - Infusion of fluids

Nonfermentative GNR
- Do not ferment carbohydrates

Oxidizers
- Saccharolytic
  - Break down the carbohydrates oxidatively

Nonoxidizers
- Asaccharolytic or nonsaccharolytic
  - Considered to be biochemically inert

TSI K/K
- For detection of small amounts of acids due to oxidation or fermentation
- No growth in anaerobic environment

NLF GNR Characteristics:
- Environmental opportunists
- Cause nosocomial infections
- Not fastidious organisms
- Can grow between 22 and 25 degrees C
- GNR or GNCB
- Oxidase: Positive
- TSI: K/K
- Resistant to antibiotics
Hugh Leifson:
- Hugh-Leifson OF media
- Detection of an organism's ability to use carbohydrates oxidatively or fermentatively
- Detects weak acidity
- Lower amount of peptone
- Fermentation
  - Pyruvic acid transfers its own electrons to organic compounds
  - Large amounts of mixed acids are produced
  - Occurs in an anaerobic environment
- Oxidation
  - Pyruvic acid enters the Kreb cycle
  - Transfers its own electrons to oxygen to form water
  - Citric acid is produced
  - Weak acid
  - Occurs ONLY in the presence of air

Hugh Leifson:
- Set two tubes
  - One tube is aerobic
    - Atmospheric oxygen for oxidation
  - One tube is anaerobic (overlayed with mineral oil)
    - For fermentation to take place
- Interpretation
  - Yellow color
  - Bromthymol blue pH indicator
  - Only tests a single carbohydrate (such as glucose)
- Results
  - Acid production in both tubes
  - Fermenter
  - Acid in aerobic tube
  - Oxidizer
  - No acid production in either tube
  - Non-oxidizer

Hugh Leifson:
- Reactions
  - Glucose fermenter
    - Acidity in closed and open tube
    - Klebsiella oxytoca
    - E. coli
  - Glucose oxidizer
    - Acidity in open tube only
    - Pseudomonas aeruginosa
    - Inactive
      - No acidity in either tube
      - Alcaligenes faecalis
Pseudomonads:
- Thin Gram Negative Rods (GNR)
- Motile (exception is B. mallei)
- Oxidase
  - Positive (weak and variable)
- Catalase
  - Positive
- Grow on MAC
- Oxidizers
- TSI: K/K
- Reduce Nitrates

Pseudomonads:
- Fluorescent group
  - *P. aeruginosa*
  - *P. putida*
  - *P. fluorescens*
  - *P. veronii*
  - *P. mosselii*
  - *P. monteilii*
  - Pyoverdin (Fluorescent pigment yellow-green)
  - Pyocyanin (bluish pigment)
  - Only *P. aeruginosa*
  - Combination of the two
  - Characteristic green
- Stutzeri group
  - *P. stutzeri*
  - *P. mendocina*

Pseudomonas aeruginosa:
- Most common species
- Normal enteric flora in only about 4-12% of individuals
  - Not commonly isolated from stools
- Causes 5-15% of nosocomial infections
  - Bacteremia, respiratory (pneumonia), UTIs
  - Bacteremia assoc. with ecthyma gangrenosum of the skin
- Commonly seen in burn wounds
- Assoc. with cystic fibrosis
- Pulmonary infections
- Swimmer’s ear
  - Otitis externa
- Jacuzzi or hot tub syndrome
- Necrotizing skin rash
Pseudomonas aeruginosa:

- Virulence Factors
  - Lipopolysaccharide endotoxin
  - Motility
  - Pili
  - Capsule
  - Exotoxin
    - Proteases, lecinthinase, elastase, Dnase, hemolysins
  - Exotoxin A
    - Blocks protein synthesis
  - Slime factor
    - Polysaccharide polymer alginate
  - Resistance to antimicrobials

Cystic Fibrosis

- Cystic fibrosis is a hereditary disorder characterized by lung congestion and infection and malabsorption of nutrients by the pancreas.
Pseudomonas aeruginosa:

- Treatment
  - Resistant to numerous antibiotics
    - Such as Pencillin, ampicillin, cephalosporins, SXT, chloramphenicol
  - Typically susceptible to
    - Aminoglycosides
    - Carbencillin
    - Piperacillin
    - Ticarcillin
    - 3rd and 4th generation cephalosporins
      - Cefotaxime and cefepime
      - Carbapenems and fluoroquinolones
  - Severe infections require double therapy
    - Cefotaxime, piperacillin or carbapenem with
      - An aminoglycoside

Identification - Pseudomonas aeruginosa:

- GNR
- Beta-hemolytic
- Green metallic sheen
  - Pyocyanin
- Fluorescent
- Pyoverdin
- Tortilla odor
  - Fruity grape like
  - "Slime"
- Growth at 42 degrees C
- Oxidizer (glucose)
- Oxidase positive
- ADH
  - Positive
- Citrate
  - Positive
- Acetamide utilization
- Gelatin
  - Positive
- Nitrate
  - positive
Identification

- *P. aeruginosa*
  - Produces pyocyanin
  - Green pigment metallic sheen
  - Grows at 42 degrees
- *P. fluorescens*
  - No pyocyanin produced
  - No growth at 42
  - Gelatin hydrolysis positive
- *P. putida*
  - No pyocyanin produced
  - No growth at 42
  - Gelatin hydrolysis negative

Identification - *Pseudomonas stutzeri:*

- Saprophyte
- Rarely causing infection
- Oxidase positive
- Growth at 42 degrees C
- Motile
- Colonies
  - Wrinkly, leathery, adherent
- Yellow-brown pigment
- Nitrate to nitrite to N2
- Pyocyanin and Pyoverdin
  - Negative

Burkholderia

- Previously
  - *Pseudomonas cepacia*
- Plant pathogen
  - Typically onions and bulbs
- Considered a low grade nosocomial pathogen
- Causes pneumonia
- Cystic fibrosis patients
- Chronic granulomatous disease
Identification - Burkholderia cepacia

- Oxidase +
- Oxidizer
- Glucose, maltose, lactose and mannitol
- LDC +
- OD =
- ONPG +
- Nitrate reduction =
- Growth at 30 degrees C
- Motility (polar tufts)
- Non-fluorescing yellow or green color
- Non-wrinkled colonies
- Polymyxin B resistance
- Resistant to aminoglycosides

Identification - Stenotrophomonas maltophilia

- 3rd most common nonfermenting GNR
- Ubiquitous
- Not normal flora
- Seen in nosocomial infections
  - Venous catheter
  - Pneumonia
  - Endocarditis
  - Wounds
  - Bacteremia
  - Rare meningitis
  - UTI
- Antibiotic resistance
  - Susceptible to SXT
  - Susceptible to ticarcillin-clavulanate, levofloxacin, tetracycline

Identification - Stenotrophomonas maltophilia

- Oxidase =
- Catalase +
- DNAse +
- Esculin +
- Gelatin +
- LDC +
- Glucose & maltose +
- Susceptible to SXT
### Acinetobacter

- **Family Moraxellaceae**
- **Ubiquitous**
  - Soil, water, and foodstuffs
  - Seen on ventilators, humidifiers and catheters
- **Typically considered insignificant or a contaminant**
  - Specimens: urine, feces, vaginal, respiratory
- **Causes opportunistic infections**
  - UTI, pneumonia, endocarditis, septicemia, meningitis, cellulitis

### Acinetobacter

- **Acinetobacter baumannii**
  - Saccharolytic
  - Seen in clinical infections more than other species
  - Resistance to antimicrobials
- **Acinetobacter lwoffii**
  - Asaccharolytic
  - Less virulent
  - Colonization or contamination

### Acinetobacter

- **GNCB**
- **MAC - Purple color**
- **Oxidase**
- **Catalase**
- **Nonmotile**
- **Better growth at 30–35 degrees C**
- **Better growth at a pH of 5.5–6.0**
- **Carb utilization**
  - A. baumannii – saccharolytic
  - A. lwoffii - asaccharolytic
Moraxella
- Opportunist
- Causes otitis media and sinusitis in children
- Normal oral flora
  - M. catarrhalis
    - GNCB
    - Nonmotile
    - Strict aerobe
    - Oxidase +
    - Catalase +
    - Asaccharolytic
    - Dnase +
    - Butyrate esterase +
    - Penicillin susceptibility

Flavobacterium
- Ubiquitous
  - Soil and water
  - Not normal flora in humans
  - Assoc. with nosocomial infections
    - Hospital equipment and patients
  - Neonatal meningitis or septicemia
    - Newborns
  - Pneumonia, endocarditis and bacteremia
    - Adults

Alcaligenes and Achromobacter
- Family Alcaligenaceae
  - Oxidase +, grow on MAC, motile
- Water
  - Swimming pools
  - Tap water
  - Dialysis fluids
- Causes
  - Otitis media, meningitis, pneumonia, UTI, wounds, bacteremia
- Resistant to disinfectants
  - Chlorohexidine and quaternary ammonium compounds
- Alcaligenes faecalis
  - Asaccharolytic
  - Achromobacter xylosoxidans ssp. Xylosoxidans
  - Oxidizer