NONFERMENTATIVE GRAM NEGATIVE BACILLI

General Information
- Predominantly opportunistic
- Pathogenicity of the organism is usually related to an altered or already debilitated host (underlying disease, antibiotic therapy, immunosuppressive drugs, intubation, etc.)
- Nomenclature of these organisms changes rapidly due to defining of new genera with the use of molecular techniques (phylogenetic/genotypic classification)

Characteristics of Nonfermentative Gram-negative Bacilli
- Will grow on routine isolation media (BAP, Choc).
- Growth on MacConkey agar is variable and this property used for identification
- Optimal temperatures of incubation range from 22-35°C
- Most require an incubation time of at least 24 hrs., sometimes 48-72 hrs., before they can be identified
- Nonfermenting GNR are distinguished from the Enterobacteriaceae based on:
  - **Oxidase test**
    - Most nonfermenting GNR are oxidase POSITIVE (for oxidase test take organism from BAP or CHOC, not MAC)
  - **Growth on MacConkey agar**
    - Not all nonfermenters grow on MacConkey agar.
    - All nonfermenters that grow on MAC are lactose negative.
  - **Utilization of glucose**
    - Nonfermenting GNR do NOT ferment glucose
      a. Organisms degrade carbohydrates via oxidative rather than fermentative pathways.
      b. Organisms that are unable to utilize carbohydrates as energy sources are termed nonsaccharolytic or asaccharolytic.
- Gram stain morphology should be taken from a non-inhibitory medium (if possible), noting cellular morphology (coccobacillus, rod, coccus) and size (long/short/fat/thin)
- Nonfermenters can rapidly develop resistance to antimicrobials used in treating infection.

Clinically Significant Nonfermentative Gram Negative Bacilli

<table>
<thead>
<tr>
<th>Achromobacter</th>
<th>Chryseobacterium</th>
<th>Ochrobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidovorax</td>
<td>Chryseomonas</td>
<td>Oligella</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>Comamonas</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Agrobacterium</td>
<td>Flavimonas</td>
<td>Psychrobacter</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>Flavobacterium</td>
<td>Roseomonas</td>
</tr>
<tr>
<td>Brevundimonas</td>
<td>Methylbacterium</td>
<td>Shewanella</td>
</tr>
<tr>
<td>Burkholderia</td>
<td>Moraxella</td>
<td>Sphingobacterium</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>Weekella</td>
<td></td>
</tr>
</tbody>
</table>

CLS 418 Clinical Microbiology I
Student Laboratory
Glucose Nonfermenting Gram Negative Rods
Laboratory Identification – one approach

- Organisms characterized as nonfermentative gram-negative rods are first differentiated based upon the combination of the following three reactions:
  
  Glucose oxidizer or Glucose inactive/ asaccharolytic  
  MacConkey agar growth (NLF) or MacConkey agar no growth  
  Oxidase positive or Oxidase negative

Once characterized as a nonfermentative gram-negative rod and placed in one of the above groupings, the organisms are identified using a specific set of differential media (can be conventional or commercial systems).

Speciation of these organisms can be difficult. Reliability of commercial systems with some of these organisms is variable.

**Glucose “O”, MAC growth, Oxidase positive**

**Genus Pseudomonas**

General information:
- *Pseudomonas* is the most commonly isolated nonfermenting GNR
- Distribution is world wide and associated with water and moist environments
- Strict aerobes
- Motile by POLAR monotrichous or multitrichous (tufts) of flagella
- Cytochrome oxidase = positive
- Utilize carbohydrates oxidatively
- Grow on MacConkey agar

**Pseudomonas aeruginosa**

General information
- Most commonly isolated species in the genus
- One of the leading causes of hospital acquired (nosocomial) infections
- Not usually part of normal flora in healthy individuals
- Colonization occurs in the GI tract, throat, nasal mucosa, axillae and perineum
- Produces a pigment: pyocyanin (blue green)  
  pyoverdin (yellow/green or yellow brown, fluorescent)  
  pyocyanin + pyoverdin = characteristic green color  
  pyorubin (red)  
  pyomelanin (brown)
- Cetrimide agar can be used to detect pyocyanin and pyoverdin production and selectively isolate the organism
**Pseudomonas aeruginosa** (cont.)

**Laboratory identification:**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>BAP = Spreading, flat, irregular edge, gray-green, with a metallic sheen, possibly mucoid, usually beta-hemolytic and a grape-like or corn taco-like odor</td>
</tr>
<tr>
<td></td>
<td>MAC = Clear = non-lactose fermenter (NLF)</td>
</tr>
<tr>
<td>Gram stain morphology</td>
<td>Thin gram negative rod</td>
</tr>
<tr>
<td>Pigment production</td>
<td>Pyocyanin, Pyoverdin, Pyomelanin, Pyorubin</td>
</tr>
<tr>
<td>Glucose utilization</td>
<td>Glucose oxidizer (KIA = K/K)</td>
</tr>
<tr>
<td>Oxidase</td>
<td>Positive</td>
</tr>
<tr>
<td>42°C Growth</td>
<td>Growth</td>
</tr>
<tr>
<td>Arginine dihydrolase (ADH)*</td>
<td>Positive</td>
</tr>
<tr>
<td>Lysine decarboxylase (LDC)*</td>
<td>Negative</td>
</tr>
<tr>
<td>Ornithine decarboxylase (ODC)*</td>
<td>Negative</td>
</tr>
<tr>
<td>Gelatin</td>
<td>Variable</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Positive</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

*Moeller based

**Quick Identification:**

- Oxidase positive, growth on MAC
- Colony morphology BAP: spreading, flat with serrated edges, beta-hemolytic, may exhibit metallic sheen
- Exhibit of bright bluish-green, red or brown diffusible pigment
- "Grape-like" smell

**Virulence Factors**

- Pili (attach to cell surface)
- Lipopolysaccharide (endotoxin)
- Exotoxin A (inhibits protein synthesis)
- Proteolytic enzymes (destroy tissue)
- Extracellular slime (inhibit phagocytosis)
- Intrinsic antimicrobial resistance

**Clinical Significance**

- **Type of patient infected by *Pseudomonas aeruginosa***:
  - Leukocytopenic
  - Immunosuppressed
  - Extensive burns
  - Antibiotic therapy
  - Cystic fibrosis patients
  - Immature immunologic system
  - Presence of indwelling foreign devices
  - IV drug abuser

- **Types of infections associated with *Pseudomonas aeruginosa***:
  - Infected burn wounds
  - Nosocomial, such as pneumonia
  - Chronic pulmonary disease in Cystic Fibrosis patients
  - Septicemia
  - "Swimmer's ear" = otitis externa
  - Folliculitis (whirlpools, spas, swimming pools)
  - Corneal ulcers / Keratitis
  - Urinary tract infection
  - Osteomyelitis
**Pseudomonas aeruginosa (cont.)**

**Antibiotic Therapy**
- Resistant to many commonly used antibiotics (penicillin, ampicillin, first & second generation cephalosporins)
- Sensitive: aminoglycosides, some third generation cephalosporins, anti-pseudomonal penicillins (ticarcillin, piperacillin) and quinolones
- Aminoglycoside results are greatly affected by the concentration of Ca\(^+\) and Mg\(^++\) in the test medium (especially tobramycin)

**Chryseobacterium meningosepticum**

**General information**
- The organism is found in soil, water and hospital environments (water fountains, reservoirs in equipment), and on plants and foodstuffs. Not part of normal human flora. Can cause nosocomial infections.

**Laboratory identification**
- Colony morphology (24 hours, 35\(^\circ\)C in ambient air or increased CO\(_2\)):
  - **BAP** = Smooth fairly large colonies, may have a pale yellow pigment
  - **MAC** = Growth (ambient air only)
- Gram morphology: thin GNR, sometimes with swollen ends, may include filamentous forms
- **Oxidase** = positive
- **Glucose** = oxidizer (usually delayed, may initially appear to be a nonfermenter)
- **Indole** = positive (may be weak, use Ehrlich's method)

**Clinical Significance**
- Neonatal meningitis and sepsis (highly pathogenic for premature infants) high mortality rate and chance of nursery epidemics
- Bacteremia associated with implanted catheters
- Other opportunistic infections

**Antibiotic Therapy**
- MIC determinations are recommended for clinically significant isolates
- Disk diffusion testing is unreliable
- Susceptible (usually) to penicillin, vancomycin (unusual for a gram-negative organism), SXT, fluoroquinolones, piperacillin/tazobactam
Glucose “O”, MAC growth, Oxidase variable

**Burkholderia cepacia**

General Information
- Natural distribution is being intensively studied due to pathogenicity in cystic fibrosis (CF) patients, recovered from water sources, detergents, disinfectants
- 2nd to *Pseudomonas aeruginosa* in isolation from CF patients with respiratory infections

Laboratory Identification
- Colony morphology:
  - BAP: Smooth, slightly raised, may be mucoid, usually non-pigmented, strong earthy odor (may produce bright yellow pigment on iron containing media)
  - MAC: Punctate and tenacious, may become dark pink-red after 4-7 days
- Specific selective/differential media (polymyxin B is selective ingredient) used for CF patient respiratory specimens
  - OFPBL agar: Yellow colonies (due to lactose utilization)
  - PC agar: Pink colonies (due to lactose utilization)
- Gram negative rod
- Oxidase = positive (weak)
- Growth at 42°C = variable
- Glucose = oxidizer
- ADH (Moeller based) = negative
- **LDC (Moeller based) = POSITIVE**
- ODC (Moeller based) = variable
- **Polymyxin B = RESISTANT**

Clinical Significance
- Organism can be isolated from numerous water sources, detergent solutions, IV fluids, and disinfectants
- Organism can grow in povidine-iodine, quaternary ammonium compounds, and chlorhexidine
- Opportunistic pathogen primarily related to nosocomial infections and cystic fibrosis patients

Antibiotic Therapy
- Resistant to aminoglycosides
- **Sensitive to trimethoprim-sulfamethoxazole (drug of choice)**
- CLSI: if susceptibility done with disk diffusion only report ceftazidime, meropenem, minocycline and SXT
Glucose “O” or inert, MAC growth, Oxidase negative

**Stenotrophomonas maltophilia**

**General Information**
- Third most commonly encountered nonfermenter in clinical specimens
- Ubiquitous in nature
- Important nosocomial pathogen
- Not considered part of normal human flora, can quickly colonize the respiratory tract of hospitalized patients

**Laboratory identification**
- Colonies are pale yellow to lavender green on blood agar (good growth at 24 hours)
- Gram negative bacilli
- **Oxidase: NEGATIVE**
- Glucose = oxidizer (weak)
- Growth at 42 C = negative
- **Maltose = strong oxidizer**
- ADH (Moeller based)= negative
- **LDC (Moeller based)= POSITIVE**
- ODC (Moeller based) = negative
- **DNase = positive**
- Polymyxin B = sensitive
  Organism may strongly oxidize maltose even if glucose oxidation is slow or negative

**Clinical Significance**
- Infections are usually nosocomial in origin and occur in compromised hosts
- Type of infections caused:
  - Pneumonia
  - Urinary tract infections
  - Wound infections
  - Bacteremia (often catheter related)

**Antibiotic Therapy**
- Organism is inherently resistant to most of the commonly used anti-pseudomonal drugs
- Inherently susceptible to trimethoprim-sulfamethoxazole
- CLSI recommends broth dilution, common antimicrobials include ticarcillin-clavulanate, levofloxacin and tetracyclines
- If disk diffusion performed, only report minocycline, levofloxacin and SXT
Glucose “O” or asaccharolytic, MAC growth, Oxidase negative

Genus *Acinetobacter*

General information
- After the genus *Pseudomonas*, it is the most frequently isolated nonfermenter
- Ubiquitous in soil, H₂O and sewage
  - Can be part of normal skin flora
  - Normal flora of the vaginal tract
- Organism can survive on moist and dry surfaces
- Hospital environment – isolated from ventilators, humidifiers, catheters
- Strict aerobe

Laboratory identification
- Colony morphology:
  - BAP = Translucent to opaque, never pigmented, convex
  - MAC = Colorless to slightly pink
- Gram stain morphology: plump gram negative coccobacilli, often appear to be diplococci
  - **Can be confused with *Neisseria* sp on Gram stain
  - May resist decolorization
- **Oxidase: NEGATIVE**
- **Nitrate: negative (use for neg. control for nitrates)**
- Glucose: variable (oxidizer or asaccharolytic)
- The species most frequently isolated are:
  - *Acinetobacter baumannii* complex or “saccharolytic *Acinetobacter***
    - Glucose oxidizer
    - Nonhemolytic
  - *Acinetobacter lwofii* or “asaccharolytic *Acinetobacter***
    - Glucose non-oxidizer
    - Nonhemolytic

Clinical significance
- Generally considered nonpathogenic to healthy individuals
- Associated with opportunistic and nosocomial infections of the respiratory tract, urinary tract, wounds and blood
- *Acinetobacter baumannii* noted for wound infections in soldiers from Mideast region (Iraq)
  - isolates are very resistant to antibiotics

Antibiotic Susceptibility
- Resistant to a variety of antibiotics (especially *A. baumannii*)
- Susceptibility testing must be performed to determine treatment
Glucose asaccharolytic, MAC growth, Oxidase positive

Genus *Alcaligenes/Achromobacter*

**General information**
- Occur in water, soil and moist areas of the hospital environment such as respirators, hemodialysis systems and IV solutions
- May be found on skin and in the GI tract

**Laboratory identification**

Genus:
- Gram negative coccobacilli
- Oxidase = positive
- Grow on MacConkey agar
- Glucose = oxidizer (weak) or asaccharolytic
- Lysine decarboxylase = negative
- Basically biochemically inert. Only a few characteristics differentiate the species

*Alcaligenes faecalis* (asaccharolytic)
- Colony morphology = flat, thin, spreading, rough with an irregular edge
- May have a fruity odor = green apple odor

*Achromobacter (Alcaligenes) xylosoxidans ssp. xylosoxidans* (saccharolytic)
- Glucose = oxidizer (weak)
- Xylose = oxidizer (strong)

**Clinical Significance**
- *Alcaligenes faecalis* = opportunistic pathogen, isolated from blood, sputum and urine
- *Achromobacter xylosoxidans ssp. xylosoxidans* = nosocomial septicemia and severe pulmonary symptoms in cystic fibrosis patients and intubated children

**Antibiotic Therapy**
- Susceptibility patterns vary
Glucose asaccharolytic, MAC variable, Oxidase positive

Genus Moraxella

General information:
- Normal flora of skin and mucous membranes

Laboratory identification:
- Colony morphology: BAP = tiny pinpoint colonies at 24 hrs (some species pit the agar)
- Most strains will grow slowly or not at all on MacConkey
- Gram stain morphology = plump, gram negative coccobacilli; may appear as diplobacilli
  May resist decolorization
- **Moraxella (Branhamella) catarrhalis** is a gram negative diplococci and is discussed with the Neisseria
- Oxidase: positive
- Glucose: non-oxidizer (asaccharolytic)
- Indole: negative
- Highly susceptible to penicillin

Clinical Significance
- Nosocomial and opportunistic infections
- Most significant isolates are recovered from the eye or respiratory tract

Antibiotic Therapy
- Most are sensitive to low concentrations of penicillin
- Check for beta lactamase production
**SUMMARY**

<table>
<thead>
<tr>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colony morphology</strong></td>
</tr>
<tr>
<td><strong>Gram morphology</strong></td>
</tr>
<tr>
<td><strong>Glucose utilization</strong></td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
</tr>
<tr>
<td><strong>Pigment</strong></td>
</tr>
<tr>
<td><strong>Growth at 42°C</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Oxidase</th>
<th>42°C C</th>
<th>ADH*</th>
<th>ODC*</th>
<th>LDC*</th>
<th>Gelatin</th>
<th>Polymyxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>+</td>
<td>Growth</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>Sensitive</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>-</td>
<td>No growth</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

*Moeller based

<table>
<thead>
<tr>
<th>Glucose Utilization</th>
<th>Growth on Mac</th>
<th>Oxidase</th>
<th>Motility</th>
<th>Gram Morph.</th>
<th>Polymyxin B Susceptibility</th>
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</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>“F” Good</td>
<td>-</td>
<td>+/- peritrichous</td>
<td>Large GNR</td>
<td>NA</td>
</tr>
<tr>
<td>Burkholderia. cepacia</td>
<td>“O” Good (3 days)</td>
<td>+/- Motile, polar multi</td>
<td>Thin GNR</td>
<td>Resistant</td>
<td></td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>“O” (weak) Good</td>
<td>-</td>
<td>Motile, polar multi</td>
<td>Thin GNR</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Chryseobacterium</td>
<td>“O” Variable</td>
<td>+</td>
<td>Nonmotile</td>
<td>Thin GNR, swollen ends</td>
<td>Resistant</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>“O” or “N” Good</td>
<td>-</td>
<td>Nonmotile</td>
<td>GNCB</td>
<td>NA</td>
</tr>
<tr>
<td>Alcaligenes/ Achromobacter</td>
<td>“N” or “O” Good</td>
<td>+</td>
<td>Motile, peritrichous</td>
<td>GNCB/GNR</td>
<td>NA</td>
</tr>
<tr>
<td>Moraxella</td>
<td>“N” Variable</td>
<td>+</td>
<td>Nonmotile</td>
<td>GNCB</td>
<td>NA</td>
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<td>Eikenella</td>
<td>“N” Neg.</td>
<td>+</td>
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<td>Thin GNR</td>
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</tbody>
</table>