Clinically Significant Bacteria

Chapter 11
Hemolytic Patterns on Blood Agar
Gram-Positive Cocci

A Staphylococcus

1. Morphology – occur in grape-like clusters
2. *Staphylococcus aureus* – Named for golden color
3. Causes many diseases such as food poisoning and toxic shock syndrome
4. Characteristics
   • a. Grow under high osmotic pressure and low moisture. Grows on Mannitol salt agar and *Staph aureus* ferments mannitol.
   • b. Produce toxins that contribute to pathogenicity, e.g., enterotoxin involved in food poisoning.
   • c. Facultative anaerobe
Gram-Positive Cocci

• A. Staphylococcus
  – 5 Laboratory Identification – Staph aureus
    • Colony – White, creamy, often Beta-hemolytic
    • Bio Chem tests – Catalase +, Coagulase +
    • Use Flow charts for ID
*Staph aureus* on BAP – White, Creamy, Beta - Hemolysis
*Staphylococcus aureus* – Culture Gram Stain
Non-hemolytic Staph – *Staph saprophytics* or *epidermitidis*
Staphylococcus aureus – Direct Smear
Novobiocin susceptibility test to differentiate coagulase-negative isolate from urine sample. *Staphylococcus saprophyticus* is resistant to novobiocin, depicted with no zone of inhibition around the disk.
Catalase Test

positive catalase

negative catalase
Flow Chart for *Staph aureus* Identification

- **Catalase**
  - (+)
  - Staph
    - Coagulase
      - (+)
      - Staph aureus
        - (−)
        - Staphlococcus
          - Novobiocin
            - R
            - Staph saphrophyticus
            - S
            - Staph epi
Gram-Positive Cocci

• B. Streptococcus

  – 1. Morphology – Appear in chains with as few as from 4-6 cocci or as many as 50. One species, *Streptococcus pneumoniae*, occurs in pairs.
  
  – 2. Metabolism – do not use oxygen although most are aerotolerant. See smaller colonies.
  
  – 3. Characteristics
    • a. Produce products that destroy phagocytic cells
    • b. Produce enzymes that digest connective tissue
Gram-Positive Cocci

• B. Streptococcus
  – 4. Classification – Based on action of blood agar.
    • a. Alpha-hemolytic – Produces an alpha-hemolysin that reduces hemoglobin (red) to methemoglobin (green). Causes a greenish zone to surround the colony. *Streptococcus pneumoniae*
    • b. Beta-hemolytic – Produces hemolysins which form a clear zone of hemolysis on blood agar. *Streptococcus pyogenes*
    • c. Gamma-hemolytic – No hemolysis. *Enterococcus faecalis*
Gram-Positive Cocci

• B. Streptococcus
  – 5. Laboratory Identification – Use hemolysis pattern and bio chems. Colony morphology is smaller than Staph.
  – 6. Examples:
    • a. *Streptococcus pyogenes* – scarlet fever
    • b. *Streptococcus pneumoniae* – pneumonia
    • c. *Streptococcus mutans* – dental caries
Streptococcus pyogenes on BAP
Streptococcus pyogenes
Streptococcus pyogenes in Throat Culture

Arrows indicate areas of Beta-hemolysis
Bacitracin Testing for Group A Strep – Streptococcus pyogenes
Bacitracin on Culture of Strep pyogenes
Beta Hemolytic Strep – Strep agalactiae
CAMP Test – Group B from Group A Beta-hemolytic Strep
Streptococcus pneumoniae colonies on blood agar. The colonies demonstrate a characteristic mucoid appearance.
Streptococcus pneumoniae
Alpha Strep or Strep Viridans
Gamma Hemolysis – Enterococcus or Group D Non-enterococcus
Enterococcus in Blood Culture

Red Blood Cells

Gram-positive cocci in pairs and chains suggestive of *Streptococcus*
Enterococcus vs Non-Enterococcus vs Viridans Strep

<table>
<thead>
<tr>
<th></th>
<th>BE</th>
<th>NaCl</th>
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<tbody>
<tr>
<td>Enterococcus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Non-enterococcus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Viridans Strep</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Streptococcus Flow Chart

Strep ID

**Catalase**
(-)
Streptococcus

**α** and **γ**
P Disk Optochin

**S**
Strep pneumo

6.5% Na Cl

(-)
Bile Esculin Agar

(+)
Entero- coccus
PYR test (+)

Group D
Non-enterococcus

(-)
Viridans Strep

**S**
Group A
Beta Strep
Strep pyogenes

Confirm

Strep Latex
PYR test (+)

(+)
Group B
Beta Strep
(Strep agalctiae)

(-)
Beta hemolytic
Strep not group A or B

**R**
CAMP

Beta Hemolytic Colonies
Taxos A Disk
Bacitracin
# Naming of Strep and Type of Hemolysis

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Lancefield Group Antigen</th>
<th>Hemolysis α, β, none</th>
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</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Group A Strep</td>
<td>A</td>
<td>Beta</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>Group B Strep</td>
<td>B</td>
<td>Beta</td>
</tr>
<tr>
<td><em>Streptococcus bovis</em></td>
<td>Group D Nonenterococcus</td>
<td>D</td>
<td>Alpha, None</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Group D Enterococcus</td>
<td>D</td>
<td>Alpha, Beta, none</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Pneumococcus</td>
<td>___</td>
<td>Alpha</td>
</tr>
<tr>
<td><em>Streptococcus anginosus</em></td>
<td>α- Strep. Viridans</td>
<td>___</td>
<td>Alpha, none</td>
</tr>
</tbody>
</table>
Gram-Negative Cocci

• A. Usually inhabit mucous membranes
• B. Diplococci – Some look like short fat rods
• C. ID – *Neisseria* and *Moraxella* – Oxidase + and use CTA Sugars. *Moraxella* is Dnase +.
• C. Examples
  – 1. *Neisseria gonorrhoea* – Gonorrhea
    • a. Aerobic but requires CO$_2$ and chocolate agar for growth
    • b. Attaches via fimbriae
  – 2. *Neiseria meningitidis* – Meningitis
    • a. Will grow on blood agar
  – 3. *Moraxella catarrhalis* – Pneumonia
    • a. Moraxella are strictly aerobic and shaped like coccobacilli
  – 4. *Moraxella lacunata* – Pink eye (conjunctivitis)
  – 5. Veillonella
    • a. Anaerobe
    • b. Component of dental plaque
    • c. Part of normal flora of mouth
Neisseria gonorrhoea
Neisseria meningitidis - Culture
Neisseria meningitidis – CSF
Moraxella catarrhalis - Culture
DNase Test – Moraxella is Positive
Veillonella
Endospore-Forming Gram-Positive Rods

• A. Bacillus – Common environmental inhabitants
  – 1. aerobes or facultative anaerobes
  – 2. Large straight sided rods
  – 3. Example – *Bacillus anthracis* (Anthrax)

• B. Clostridium
  – 1. Obligate anaerobe
  – 2. Examples
    • a. *Clostridium tetani* (tetanus)
    • b. *Clostridium botulinum* (botulism)
    • c. *Clostridium perfringens* (gas gangrene)
Clostridium tetani
Clostridium botulinum

Arrows indicate representative spores (pink)
Clostridium perfringes
(a) *Bacillus thuringiensis*
Figure 11.17b

(b) *Bacillus cereus* germinating

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Nonsporing Gram-Positive Rods

A. Lactobacillus

1. Metabolism – Lack a cytochrome system and are unable to use oxygen as an electron acceptor. Aerotolerant and produce lactic acid from simple carbohydrates. Acidity creates an ecological niche by inhibiting competing organisms.

2. Site – In humans are found in vagina, oral cavity and intestinal tract.

3. Industrial uses – Sauerkraut, pickles, and yogurt

Lactobacillus sp.
Nonsporing Gram-Positive Rods

B. *Listeria monocytogenes*

- 1. Psychotroph – Can survive refrigerator temperature. Also can survive inside phagocytic cells.
- 2. Involved in food contamination, mainly dairy products.
- 3. Threat of still birth or serious damage to fetus.
- 4. Medium, translucent Beta-hemolytic colonies. Small rod. Catalase +
Listeria monocytogenes
Nonsporing Gram-Positive Rods

- C. Corynebacterium (club shaped cells)
  - 1. Morphology – Tend to be pleiomorphic in cell shape
  - 2. White medium colony
    - Catalase +
  - 3. Metabolism – may be aerobic, anaerobic, or microaerophiles
  - 4. Example – *Corynebacterium diphtheriae* (diptheria)
Corynebacterium diphtheriae - Sputum
Nonsporing Gram-Positive Rods

D. Anaerobic Diphtheroid – *Propionibacterium acnes*
   – 1. Found in skin, causes acne

E. Actinomyces sp. – Filamentous anaerobic bacteria
   – 1. Fragment into Coryneform cells
   – 2. *Actinomyces israeli* – Actinomycosis affecting the head, neck, or lungs.
Proprionibacterium acnes – Anaerobic Diphtheroid
Actinomyces israeli - Filamentous anaerobic rod
Fermenting Gram-Negative Rods

• A. Enterobacteriaceae (Enterics)
  – 1. Can’t distinguish one from the other on the basis of gram stains. Are straight and short sided – some bipolar staining.
  – 2. Facultatively Anaerobic
  – 3. Most inhabit the intestinal tract of humans and animals.
  – 4. Some present only as agents of disease
  – 5. Active fermenters of glucose and other carbohydrates, oxidase negative, nitrate positive
  – 6. Many techniques for isolation and identification because of clinical importance
Fermenting Gram-Negative Rods

• A. Enterobacteriaceae
  – 7. Include motile (peritrichous) and non-motile species; some have fimbriae (adherence), and pili (genetic exchange).
  – 8. Among important genera are Escherichia, Salmonella, Klebsiella, Serratia, Proteus, Yersinia, and Enterobacter •a. *Escherichia coli* Examples: used as an indicator of fecal contamination, UTI’s, Food contamination (O157:H7), common gut inhabitant
  – 9. Each of these can cause a wide variety of infections or some such as *Salmonella typhi*, cause a specific disease called typhoid fever.
MacConkey Agar
TSI – Triple Sugar Iron Agar
Gram Negative Rod – Enteric Group
Gram negative rod – enteric Sputum
Klebsiella pneumoniae – Mucoid Colony
Proteus mirabilis – Swarming on Blood Agar
Proteus mirabilis – Swarming on Blood Agar
(a) *Proteus mirabilis* with peritrichous flagella

(b) A swarming colony of *Proteus mirabilis*; note concentric rings of growth

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Non-Fermenting Gram-Negative Rods

• A. Pseudomonas aeruginosa
  – 1. Aerobe
  – 2. Slender rod on gram stain. Has polar flagella
  – 3. Causes a wide variety of infections – UTI’s, wounds, burns. Cause 1 in 10 nosocomial infections.
  – 4. Excrete extra cellular, water soluble pigments
  – 5. Common in soil and can grow at refrigerator temperature (food spoilage and medication contamination).
  – 6. Large genetic capacity allows for many unusual traits. Able to decompose chemicals such as pesticides in soil, can grow in antiseptic solutions, soap, cap-liner adhesives, etc.
  – 7. Very resistant to antibiotics
  – 8. Oxidase +
  – 9. Use TSI/KIA media to put into non-fermenter group
Other Gram-Negative Rods (unusual, fastidious, anearobic)

• A. Pasteurellaceae
  – 1. Facultative anaerobes
  – 2. Example – *Pasteurella multocida* – Isolated from dog and cat bites. Really a pathogen of domestic animals.
    • Identification- same as enteric, but ox +
  – 3. Example – *Hemophilus influenza* – Inhabitant of upper respiratory tract and causes a variety of diseases such as meningitis in children and pneumonia in adults.
    • Requires chocolate agar. – Requires Hemin & NAD to grow. Have special ID procedure.
    • Small rod on gram stain. Brownish small colonies on chocolate.
Hemophilus influenza on Chocolate Agar
Direct smear of CSF from a child, showing abundant gram-negative, pleomorphic coccobacilli characteristic of H. influenzae. The background shows degenerating inflammatory cells. Gram stain, High-power view.
Hemophilus influenza – Satellitism. Note Colony Growth around Staph streak

- Staphylococcus aureus (X) on blood agar
- Satellite colonies of Haemophilus influenzae growing adjacent to the Staphylococcus aureus
Other Gram-Negative Rods
(unusual, fastidious, anearobic)

• B. *Bacteroides sp.*
  – 1. Anaerobe
  – 2. Live in human intestinal tract (1 billion per gram of feces), oral cavity, and genital tract.
  – 3. Cause deep wound infections, especially from punctures and bowel perforations

• C. *Fusobacterium sp.*
  – 1. Anaerobe
  – 2. Long, slender, pointed rods (spindle shaped)
  – 3. Causes some dental abscesses
Bacteroides – Gram Negative Anaerobe
Fusobacterium nucleatum – Anaerobe
Spirochetes

• A. Morphology – Long, thin, helical cells that move by means of axial filament (endoflagella). Wound around the body of the cell between an outer sheath and the body. Cell moves by rotating filament like a corkscrew.

• B. Examples
  – 1. *Treponema pallidum* (syphilis, an STD)
  – 2. *Borrelia burgdorferi* (Lyme Disease transmitted by a tick)

• Do not use culture methods for these organisms – Use serologies.
Treponema pallidum from tissue section, Levadit’s stain
Borrelia burgdorderi – Spirochete – Lyme Disease
Figure 11.24 - Overview

(a) Cross section

(b) Treponema pallidum

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Aerobic/Microaerophilic, Motile, Helical/Vibroid Gram-Negative

• A. Morphology – Helical, but lack an axial filament. Do not have a complete turn. Has a single flagella at one or both ends. Are rigid helices or curved rods.

• B. Examples
  – 1. *Campylobacter jejuni* (diarrheal diseases)
  – 2. *Helicobacter pylori* (ulcers)
Helicobacter pylori – Ulcers
Helicobacter pylori

Circles indicate *H. pylori* bacterium or groups of bacteria
Helicobacter pylori
Aerobic/Microaerophilic, Motile, Helical/Vibroid Gram-Negative

• C. Vibrio
  – 1. Morphology – Slightly curved rod
  – 2. Found mostly in aquatic environment
  – 3. Example
    • a. *Vibrio cholerae* (cholera)
    • b. *Vibrio parahaemolyticus* (gastroenteritis from raw and undercooked shellfish)
Gram stained morphology of *Vibrio cholera* with many comma shaped bacilli. The smear was counterstained with carbol fuchsin.
Rickettsias and Chlamydias and Related Organisms

• A. Metabolism – Both are obligate intracellular (as are viruses) parasites. Resemble and are classified as bacteria. Main difference between the two is mode of transmission.

• B. Rickettsias
  – 1. Morphology – Rod-shaped bacteria or coccobacilli (0.8-2.0 μm long). Pleiomorphic. Gram-negative and divide by binary fission.
  – 2. Transmission – Ticks and fleas
  – 3. Examples – Several cause fevers. Have to use serology to differentiate
    • a. *Rickettsia rickettsii* (Rocky mountain spotted fever)
    • b. *Rickettsia typhi* (Endemic murine typhus)
    • c. *Rickettsia prowazekii* (Epidemic typhus)
    • d. *Coxiella burnetii* (Q-fever)
Richettsia rickettsii – Geimsa stain – Rocky Mountain Spotted Fever
Figure 11.1 - Overview

(a) Rickettsial cell

(b) Rickettsials in chicken embryo cell

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Rickettsias and Chlamydyias

C. Chlamydyias

1. Have a developmental cycle – the infectious form, elementary body attaches to a host cell and is phagocytized and housed in a cell vacuole. Within the host cell the body becomes a larger less infective reticulate body that divides successively. Eventually these condense into infective elementary bodies that are released to infect surrounding cells.
Chlamydia Life Cycle

1. The bacterium's infectious form, the elementary body, attaches to a host cell.
2. The host cell phagocytizes the elementary body, housing it in a vacuole.
3. The elementary body reorganizes to form a reticulate body.
4. The reticulate body divides successively, producing multiple reticulate bodies.
5. The reticulate bodies begin to convert back to elementary bodies.
6. The elementary bodies are released from the host cell.

(a) Life cycle

(b) Chlamyphila psittaci
Rickettsias and Chlamydiases

• **C. Chlamydiases**
  
  – 2. Morphology – Gram-negative coccoid bacteria (0.2-1.5μm)
  
  – 3. Transmission – Interpersonal contact or airborne respiratory routes (Do not require ticks or fleas)
  
  – 4. Examples

  • a. *Chlamydia trachomatis* – trachoma - blindness, nongonococcal urethritis (NGU), lymphogranuloma venereum (STD)
  
  • b. *Chlamydia psittaci* – psittacosis (Parrot fever) – From parrots and other birds (chicken and turkey farms)
Chlamydia trachomatis – Trachoma & Sexually Transmitted Diseases

To be seen each cell are two inclusions with elementary bodies. (Giemsa stain)

(Foto: Prof. Eiko Petersen, Universität Freiburg/Brsg.)
Mycoplasmas

• A. Morphology – Do not form cell walls and so are very pleiomorphic. Can form filaments that resemble fungi. Small, ranging from 0.1-0.25μm.

• B. Will not readily grow on artificial media. To grow, sterols must be provided.

• C. Example
  – 1. *Mycoplasma pneumoniae* – Cause of “walking pneumonia”. Also called PAP (primary atypical pneumoniae)
Mycobacteria

- A. Aerobic, non-spore forming rods
- B. “Myco” suggests fungus because occasionally has filamentous growth.
- C. Have a distinctive gram-negative-like cell wall – outermost lipopolysaccharide layer is replaced with mycolic acid that forms a waxy, water-resistant layer. Resistant to stress of drying, acts as barrier to antimicrobials (pathogenic mechanism).
- D. Stains acid-fast
- E. Examples
  - 1. *Mycobacteria tuberculosis* (TB)
  - 2. *Mycobacteria leprae* (leprosy)
*Mycobacterium tuberculosis* growing on Lowenstein-Jensen (LJ) medium. The medium contains egg, mineral salts and malachite (green). Growth appears after about 4 weeks incubation as granular buff-colored colonies.
Mycobacterium tuberculosis – Acid Fast Stain
Mycobacterium leprae – Acid Fast Stain
Nocardia

• A. Resemble Actinomyces, but are aerobic
• B. Reproduction – Produce rudimentary filaments which can fragment into short rods.
• C. Cell Wall – Resembles that of *Mycobacteria*, therefore are acid-fast
• D. Example
  – 1. *Nocardia asteroides* – Mycetoma – a localized destructive infection of feet or hands.
Norcardia asteroides – Rudimentary filaments
An Acid Fast Organism