GRAM POSITIVE & GRAM NEGATIVE BACTERIA

(Foundation Block, Microbiology)

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Department of Pathology, Microbiology Unit
Objectives:
By the end of this lecture, the student should able to:

• Recall the general basic characteristics of bacteria
• Differentiate between gram positive and gram negative bacteria.
• Recall the different groups, genera and species of gram positive bacteria (cocci and bacilli (rods))
• Recall the different groups, genera and species of gram negative bacteria (cocci and bacilli (rods))
• Recall the common infections and diseases caused by these organisms
• Recall the common identification characteristics of these groups and organisms
• Recall the different non gram sustainable bacteria
Bacterial cells
GRAM STAIN

• Developed in 1884 by the Danish physician Hans Christian Gram

• An important tool in bacterial taxonomy, distinguishing so-called Gram-positive bacteria, which remain coloured after the staining procedure, from Gram-negative bacteria, which do not retain dye and need to be counter-stained.

• Can be applied to pure cultures of bacteria or to clinical specimens

Top: Pure culture of *E. coli* (Gram-negative rods)
Bottom: *Neisseria gonorrhoeae* in a smear of urethral pus (Gram-negative cocci, with pus cells)
CELL WALL

Gram positive cell wall
- Consists of
  - a thick, homogenous sheath of peptidoglycan 20-80 nm thick
  - tightly bound acidic polysaccharides, including teichoic acid and lipoteichoic acid
  - cell membrane
- Retain crystal violet and stain purple

Gram negative cell wall
- Consists of
  - an outer membrane containing lipopolysaccharide (LPS)
  - thin shell of peptidoglycan
  - periplasmic space
  - inner membrane
- Lose crystal violet and stain pink from safranin counterstain
<table>
<thead>
<tr>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellum</td>
<td>Flagellum</td>
</tr>
<tr>
<td>Capsule</td>
<td>Capsule</td>
</tr>
<tr>
<td>Pilus</td>
<td>Peptidoglycan</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Outer membrane</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>Cytoplasmic membrane</td>
</tr>
<tr>
<td>Cytoplasmic membrane</td>
<td>Periplasmic space</td>
</tr>
</tbody>
</table>
The Gram Stain

Crystal violet

Gram's iodine

Decolorise with acetone

Counterstain with e.g. methyl red

Gram-positives appear purple

Gram-negatives appear pink
<table>
<thead>
<tr>
<th>Step</th>
<th>Microscopic Appearance of Cell</th>
<th>Chemical Reaction in Cell Wall (very magnified view)</th>
</tr>
</thead>
</table>
| 1. Crystal violet | Gram (+) Gram (-) | Gram (+)  
Both cell walls affix the dye  
Dye crystals trapped in wall  
Crystals remain in cell wall  
Red dye has no effect |
| 2. Gram's iodine | Gram (+) Gram (-) | Gram (-)  
No effect of iodine  
Cell wall partially dissolved, loses dye  
Red dye stains the colorless cell |
| 3. Alcohol | Gram (+) Gram (-) | Gram (-)  
No effect of iodine  
Cell wall partially dissolved, loses dye  
Red dye stains the colorless cell |
| 4. Safranin (red dye) | Gram (+) Gram (-) | Gram (-)  
No effect of iodine  
Cell wall partially dissolved, loses dye  
Red dye stains the colorless cell |
Gram-positive cocci

Gram-positive rods

Gram-negative cocci

Gram-negative rods
Gram positive bacteria

Cocci

- Aerobic / facultative
- Anaerobe
  - Staphylococci
  - Streptococci
  - Enterococci

Bacilli

- Anaerobe
  - Peptostreptococci

Aerobic / facultative anaerobe

- Cornybacterium
- Listeria
- Nocardia
- Latobacillus, Bacillus

Anaerobic

- Clostridium
Gram-Positive

**cocci**

- **bacilli**
  - Corynebacterium
  - Clostridium
  - Listeria
  - Bacillus

- **Staphylococcus**
  - catalase+
    - **S. aureus**
      - coagulase+
        - **S. epidermidis**
          - Novobiocin-sensitive
          - Novobiocin-resistant
    - **S. saprophyticus**
      - coagulase--

- **Steptococcus**
  - catalase-
    - β-hemolytic (clear)
    - γ-hemolytic
    - α-hemolytic (green)
      - pyogenes
      - agalactiae
      - Enterococcus
      - pneumoniae
      - Viridans
      - Group A
      - Group B
      - E. faecalis
      - E. faecium
      - optochin-sensitive
      - optochin-resistant
      - bile-soluble
      - not bile-soluble
      - capsule
      - no capsule
      - mutans, sanguis
      - quellung+
Gram positive cocci in chain
Streptococci

Gram positive cocci in clusters
Staphylococci

Catalase
Beta-hemolytic
Alpha-hemolytic
Non-hemolytic
<table>
<thead>
<tr>
<th>Gram Positive Cocci</th>
<th>O2</th>
<th>Arrangement</th>
<th>Example</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td></td>
<td>Gram + cocci in chains</td>
<td>Strep.pneumoniae Alpha hemolysis</td>
<td>Pneumonia meningitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase negative</td>
<td>Group A strep Beta hemolysis</td>
<td>Pharyngitis (Sore throat) Rheumatic fever</td>
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<td></td>
<td>Group B strep Beta hemolysis</td>
<td>Neonatal infection</td>
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<td></td>
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<td></td>
<td>Enterococcus</td>
<td>Urine, blood infection</td>
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<td></td>
<td></td>
<td></td>
<td>Non hemolysis</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td></td>
<td>Gram + cocci in clusters</td>
<td>Staph aureus</td>
<td>Soft tissue bone joint blood and heart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catalase +</td>
<td>Coagulase – staph Staph epidermidis</td>
<td>Catheter related infections</td>
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<tr>
<td>Aerobic</td>
<td>Gram + cocci in chains</td>
<td>Peptostreptococcus</td>
<td>Brain abscess</td>
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<tr>
<td>Anaerobic</td>
<td></td>
<td>Gram + cocci in chains</td>
<td>Neisseria and Moraxella</td>
<td>Genital, Meninges and respiratory</td>
</tr>
<tr>
<td>Aerobic</td>
<td>Gram – cocci in pairs</td>
<td>Neisseria and Moraxella</td>
<td>Genital, Meninges and respiratory</td>
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</tr>
<tr>
<td>Anaerobic</td>
<td>Gram – cocci</td>
<td>Veillonella</td>
<td>Rare cause infection</td>
<td></td>
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<tr>
<td>Gram Positive Bacilli</td>
<td>Aerobic</td>
<td>Spore forming</td>
<td>Bacillus antherasis</td>
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<td>Non-Spore forming</td>
<td>Listeria</td>
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<td></td>
<td>Corynebacterium</td>
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<tr>
<td>Anaerobic</td>
<td>Spore forming</td>
<td></td>
<td>Clostridia</td>
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<tr>
<td></td>
<td>Non-Spore forming</td>
<td></td>
<td>Eubacterium</td>
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<tr>
<td>Gram negative Bacilli</td>
<td>Aerobic</td>
<td>Sugar fermenter</td>
<td>E. coli</td>
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<tr>
<td></td>
<td>Enteric Bacteria</td>
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<td></td>
<td>Oxidase -</td>
<td></td>
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<tr>
<td></td>
<td>Sugar fermenter</td>
<td></td>
<td>Vibrio cholerae</td>
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<tr>
<td></td>
<td>Oxidase +</td>
<td></td>
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<tr>
<td></td>
<td>Non fermenter</td>
<td></td>
<td>Pseudomonas</td>
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<tr>
<td></td>
<td>Oxidase +</td>
<td></td>
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<tr>
<td></td>
<td>Non fermenter</td>
<td></td>
<td>Acinetobacter</td>
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<tr>
<td></td>
<td>Oxidase -</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>fastidious</td>
<td></td>
<td>Haemophilus influenzae</td>
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<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
<td>Bacteroides</td>
<td></td>
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<tr>
<td>Non Gram Stainable</td>
<td></td>
<td></td>
<td>Spirochetes</td>
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<td>Mycoplasma</td>
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</table>
Gram-positive Cocci

- **Staphylococci**
  - Catalase-positive
  - Gram-positive cocci in clusters

- **Staphylococcus aureus**
  - coagulase-positive, most important pathogen

- **Staph. epidermidis**
  - and other coagulase negative staphylococci e.g. S saprophiticus
Streptococci

- Catalase-negative
- Gram-positive cocci in **chains or pairs**
- Divided by type of hemolysis.

- **Alpha hemolytic:**
  - *S. viridans*- oral flora - infective endocarditis
  - *S. pneumoniae*- important cause of community acquired pneumonia

- **Beta hemolytic:**
  - *S. pyogenes*, group A streptococcus
    - Important cause of pharyngitis and cellulitis
S. pneumoniae
GRAM POSITIVE BACILLI

• A-Spore forming
• B-Non spore forming

Spore forming are divided into:

Aerobic spore forming most important is *Bacillus spp. (e.g. anthracis, that causes anthracis)*

Anaerobic spore forming

*Clostridium spp.*
GRAM POSITIVE BACILLI

Anaerobic gram positive bacilli

- **C. tetani** - Tetanus
  - Gas gangrene

- **C. perfringens** - Gas gangrene

- **C. botulinum** - botulism
  - Descending weakness-->paralysis
  - diplopia, dysphagia-->respiratory failure
GRAM POSITIVE BACILLI

Aerobic gram positive bacilli

- Corynebacterium diphtheriae
  - Fever, pharyngitis, cervical LAD
  - thick, gray, adherent membrane
  - sequelae --> airway obstruction, myocarditis
Gram-Negative Cocci

- **Neisseria gonorrhoeae**
  - *The Gonococcus*
- **Neisseria meningitidis**
  - *The Meningococcus*
- Both Gram-negative intracellular diplococci
- **Moraxella catarrhalis**
Gram-Negative Rods

• Enteric Bacteria they ferment sugars most important are;
  – *E. coli*
  – *Salmonella*
  – *Shigella*
  – *Yersinia and Klebsiella pneumoniae*
  – *Proteus*
Gram-Negative Rods

- **Fastidious GNRs**
  - *Bordetella pertussis*
  - *Haemophilus influenzae*
  - *Campylobacter jejuni*
  - *Helicobacter pylori*
  - *Legionella pneumophila*

- **Anaerobic GNRs**
  - *Bacteroides fragilis*
  - *Fusobacterium*
Non fermentative gram negative rods i.e. they do not ferment sugars e.g.

- Oxidase positive: Pseudomonas, causes infection in immunocompromised patients
- Oxidise negative non fermentative e.g. Acinobacter spp.
Oxidise positive comma shaped and also fermentative most important is *Vibrio cholerae* that causes cholera which is a disease characterized by severe diarrhea and dehydration.
Non-Gram-stainable bacteria

- Unusual gram-positives
- Some spirochaetes (e.g. Treponema pallidum (cause of syphilis))
- Bacteria with no cell wall
- Obligate intra-cellular bacteria
Unusual Gram-positives

- **Mycobacteria**
  - Contain mycolic acid in cell wall
Non-Gram-stainable bacteria

No cell wall
• Mycoplasmas
  – Smallest free-living organisms
  – No cell wall
  – *M. pneumonia, M. genitalium*

Obligate intra-cellular
• Chlamydia
  – *C. pneumoniae, C. trachomatis*
• Rickettsia
Gram Positive

Cocci
- Aerobic
  - Staphylococcus
  - Enterococcus
  - Streptococcus
- Anaerobic
  - Peptostreptococcus
  - Bacillus
  - Corynebacterium
  - Listeria

Bacilli
- Aerobic
  - Clostridium
- Anaerobic
  - Neisseria
  - Moraxella
  - Veillonella

Gram Negative

Cocci
- Aerobic
  - E.coli
  - Klebsiella
  - Citrobacter
  - Salmonella
  - Shigella
  - etc
- Anaerobic
  - Veillonella

Bacilli
- Aerobic
  - Neisseria
  - Moraxella
  - Veillonella
- Anaerobic
  - E.coli
  - Klebsiella
  - Citrobacter
  - Salmonella
  - Shigella
  - etc

Mycoplasma, Chlamydia, Rickettsia
- Spirochaetes
- Mycobacterium

Bacteroides