GENERAL MICROBIOLOGY
Microbiology

The science that deals with organisms causing infectious diseases
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prokaryotes</th>
<th>Eukaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear Membrane</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Chromosome Number</td>
<td>Diploid</td>
<td>Haploid</td>
</tr>
<tr>
<td>Histone</td>
<td>70 S</td>
<td>80 S</td>
</tr>
<tr>
<td>Ribosome</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Mitosis</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Membrane bound organelles</td>
<td>Premature nucleus</td>
<td>True nucleus</td>
</tr>
</tbody>
</table>

Prokaryotes: Premature nucleus
Eukaryotes: True nucleus
General Bacteriology

- This includes:
  - Morphology of bacteria
  - Ultra structure of bacterial cell
  - Growth characters of bacteria
  - Nutrition of bacteria
  - Bacterial products
  - Metabolism of bacteria
  - Antimicrobial agents
Size, Shape & Arrangement

**Size**
- Measured by micron.
- Smallest bacteria is “Serratia” = 0.2 micron

**Shape**
- Cocci (Spherical) e.g. Staphylococci
- Bacilli (Cylindrical) e.g. Diphtheria
- Spiral
  - One curve e.g. Vibrio
  - More than one curve e.g. Spirilla and Spirochetes

**Arrangement**
- Single, Pairs, Tetrads, Bunches, Chains, Angular
Figure 2–1. Bacterial morphology. A: Cocci: in clusters, eg, Staphylococcus (A-1); chains, eg, Streptococcus (A-2); in pairs with pointed ends, eg, Streptococcus pneumoniae (A-3); in pairs with kidney bean shape, eg, Neisseria (A-4). B: Rods (bacilli): with square ends, eg, Bacillus (B-1); with rounded ends, eg, Salmonella (B-2); club-shaped, eg, Corynebacterium (B-3); fusiform, eg, Fusobacterium (B-4); comma-shaped, eg, Vibrio (B-5). C: Spirochetes: relaxed coil, eg, Borrelia (C-1); tightly coiled, eg, Treponema (C-2). (Modified and reproduced, with permission, from Joklik WK et al: Zinsser Microbiology, 20th ed. Originally published by Appleton & Lange. Copyright © 1992 by The McGraw-Hill Companies.)
Bacterial Structure (E/M)

- **Surface structure:**
  - Capsule
  - Cell wall
  - Cell membrane

- **Internal structure:**
  - Nuclear body
  - Ribosomes
  - Inclusion bodies
  - Flagella
  - Fimbriae
  - Mesosomes
Bacterial cell structure

- Capsule
- Cell Wall
- Plasma Membrane
- "Mesosome"
- Flagellum
- Pili
- Cytoplasm
- Cytoplasmic Inclusion
- Nucleoid & DNA
- Ribosome
- Endospore
Cell Wall

- It is a rigid layer covering the bacterial cell, and resting over the cell membrane

Chemical Structure

**Peptidoglycan:**
Backbone of alternating N-acetyl glucosamine (G) and N-acetyl muramic acid (M)

**Tetrapeptide side chain:**
A chain of 4 amino acids (??)

**Peptide cross bridge:**
5 amino acids
Gram positive cell wall

- Teichoic Acid
- Thick Layer of Highly Crosslinked Peptidoglycan
- Plasma Membrane
Gram negative cell wall

- Outer Membrane
  - Lipopolysaccharide Layer
  - Phospholipid Layer
  - Thin Peptidoglycan Layer
  - Periplasmic Space
- Plasma Membrane (Inner Membrane)
- Porin
Chemical Structure
### G+ve & G-ve cell wall differences:

<table>
<thead>
<tr>
<th></th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptidoglycan</strong></td>
<td>Several layers up to 20 layers</td>
<td>One or two layers</td>
</tr>
<tr>
<td><strong>Thick</strong></td>
<td></td>
<td>Very thin</td>
</tr>
<tr>
<td><strong>90% of cell wall material</strong></td>
<td></td>
<td>5-20% only</td>
</tr>
</tbody>
</table>
| **Special structure** | Techoic acid Polysaccharides | - Lipoprotein  
- Outermembrane  
- Periplasmic membrane  
- Lipopolysaccharide (endotoxin) |
Functions:

- Preservation of shape of cell
- Protection against high osmotic pressure
- Antigenic
  - Techoic acid in G+ve bacteria
  - Lipopolysaccharides in G-ve bacteria
- Toxicity of bacterial cell
  - Lipid A in LPS of G-ve bacteria is endotoxin
- Permeability of cell
  - Outer membrane of G-ve bacteria is barrier for large molecules
- Staining reaction
- Target action for antibiotics as penicillins and cephalosporins
Cytoplasmic membrane

- It is a thin elastic membrane inner to cell wall.
- It is very thin and porous
- It consists of lipoprotein (70% protein, 30% phospholipids) and small amount of CHO.
- Functions:
  - Chemo tactic function
  - Excretion of hydrolytic enzymes and toxins
  - Cell wall synthesis
  - Transport and permeability
Mesosomes

- They are inward invagination of cytoplasmic membrane inside cytoplasm.

- Functions:
  - Increase surface area
  - Site of attachment of chromosome in cell division
  - Excretion of extra cellular enzymes as *penicillinase*
Capsule

- It is a well defined layer surrounding cell.

- It is made of firm gelatinous material, consisting of large amount of water and small amount of solids.

- Chemical structure:
  - Most species: Polysaccharide
  - Anthrax: Polypeptide
Capsule by india ink stain
Capsule continue

- **Demonstration:**
  - Light microscope (L/M): unstained halo
  - Negative stain by India ink
  - Electron microscope (E/M)
  - Serological demonstration by Ag/Ab reaction

- **Function:**
  - Protection against attack by antibacterial agents.
  - Protection against phagocytosis
  - Determination of virulence
  - Antigenic: K antigen
Flagella

- They are long hollow helical filaments, attached to cytoplasm
- Organ of motility
- Very tall
- Diameter about 12-20 nm
- Demonstration:
  - Hanging drop method
  - L/M using mordant
  - E/M
Flagella

- **Structure:**
  - Flagellin protein

- **Types:**
  - Monotrichous
  - Amphitrichous
  - Lophotrichous
  - Peritrichous
Flagella

**Functions:**

- **Organ of Motility**
  - Increase rate of uptake of nutrients
  - Colonization site
  - Aerobic bacteria migrate towards higher conc. of oxygen
  - Penetration of pathogenic bacteria through viscid mucous
- **Antigenic (H antigen)**
Fimbrae (Pilli) \((\text{Pilli} = \text{hairs})\)

- They are filamentous appendages that differ from flagellae.

**Difference than flagellae:**
- Occur in motile & non-motile strains.
- More numerous (50-100 / cell).
- Much shorter & thinner.
- Straight \((\text{flagellae are spiral})\).
- Bacteria with fimbrae undergo reversible variations.
Fimbriae (Pilli)

- **Demonstration:**
  - Only by E/M

- **Functions:**
  - Organ of Adhesion
  - Hemagglutination of some G-ve bacilli
  - Sex fimbriae in conjugation and bacteriophage
  - Virulence (colonization Ag) (Surface virulent factor)
Nuclear bodies

- No nuclear membrane
- Made of DNA
- There is single chromosome
- Seen by E/M
Inclusion granules

- They are round granules observed in cytoplasm in many bacteria.
- Not permanent nor essential.
- Represent some metabolic products or store CHO, lipid or protein.

Example:
- Volutin granules, commonly seen in diphtheria, (also called metachromatic granules)
Ribosomes

- House of protein synthesis
- Made of RNA and protein
- 2 subunits:
  - 50 S
  - 30 S
- Whole ribosome = 70 S
- Site of translation of mRNA into polypeptide chain.
Bacterial Spores

- Resistant form of bacteria under certain unfavorable conditions (starvation, heat, chemicals)
- Occurs outside the body
- Process:
  - Nuclear material moves to one spot
  - Then surrounded by thick spore membrane
- Shape:
  - Oval or rounded
  - Bulging or non-bulging
Bacterial Spores

- **Structure:**
  - Bacterial DNA
  - Small amount of cytoplasm
  - Peptidoglycan
  - Very little amount of water
  - Thick keratin coat, responsible for resistance of spore

- Resistance may be due to dipicolinic acid (*Ca^{++} ion chelator*)
Figure 2–8. Bacterial spores. The spore contains the entire DNA genome of the bacterium surrounded by a thick, resistant coat. (Modified and reproduced, with permission, from Tortora G, Funk B, Case C: Microbiology: An Introduction, 5th ed. Benjamin/Cummings, 1995.)
Germination

- On exposure of spore to water and appropriate nutrients, specific enzymes degrade coat
- Water and nutrients enter
- Germination occurs into metabolizing and reproducing cell
Germination is **NOT** a means of reproduction; since one cell produces one spore which germinates into one cell.

Examples of spore-forming bacteria:
- Closteridium *(anaerobic)*
- Bacillus *(aerobic)*
Growth Requirements of bacteria

- Nutrition
- Gases
- Moisture
- Temperature
- pH
- Others
Bacterial Nutrition

- Bacteria can be classified into:
  - Autotrophic
    - They can assimilate inorganic sources of carbon (CO$_2$) as only source of carbon skeleton
    - As saprophytic bacteria
  - Heterotrophic
    - They require organic sources of carbon and are unable to use CO$_2$ only as source of carbon
    - As pathogenic bacteria
Bacterial Nutrition

• **Types of nutrients:**
  • **Basic elements**
    • **Major elements:**
      – Carbon
      – Nitrogen
      – Water
    • **Minor elements:**
      – Phosphorus
      – Sulphur
      – Magnesium
      – Potassium
      – Calcium
  • **Essential metabolites and growth factors:**
    • Nucleotides and vitamins
### Gases: Oxygen

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Oxygen need</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obligatory aerobes</td>
<td>Grow only in presence of $O_2$</td>
<td>Mycobacterium T.B.</td>
</tr>
<tr>
<td>Facultatative anaerobes</td>
<td>Grow in presence or absence of $O_2$</td>
<td>Pathogenic bacteria (most of them)</td>
</tr>
<tr>
<td>Obligatory anaerobes</td>
<td>Can’t grow in presence of $O_2$</td>
<td>Clostridium group</td>
</tr>
<tr>
<td>Microaerophilic</td>
<td>Grows best in presence of little amount of $O_2$</td>
<td>Corynebacterium acne</td>
</tr>
</tbody>
</table>
Gases: Carbon dioxide

- Normal atmospheric CO$_2$ (0.03%) is sufficient for most bacteria.
- Some bacteria need higher conc. (5-10%) for:
  - Stimulation of growth
    - Streptococcus pneumoniae
    - Neisseria
    - Brucella abortus
  - Capsule formation
    - Yersinia pestis (Pasteurella pestis)
    - Anthrax
  - Enterotoxin formation
    - Staphylococcus aureus
Moisture

• Large amount of bacteria is made of water, so high amount of water is needed in any media used for bacterial culture

• Example:
  • Mycobacterium T.B. needs high conc. of moisture
Temperature

- 37°C is optimum temperature for most pathogenic bacteria
- Growth between 10°C – 42°C is called temperature range
- Growth below minimum temperature is called psychrophilic
- Growth above minimum temperature is called thermophilic
pH (Hydrogen ion concentration)

- Most pathogenic bacteria grow in optimum pH 7.4
- Some bacteria tolerate alkaline media, called alkalophilic e.g. vibrio cholera
- Some bacteria tolerate acidic media, called acidophilic e.g. lactobacillus
Other factors

• As:
  • Light
  • Mechanical factors (Supersonic)
  • Osmotic pressure
Bacterial products

- Bacterial enzymes
- Bacterial pigments
- Bacterial toxins
- Others
Bacterial enzymes

- Protein in nature, produced only by living cells
- Act under special pH and temperature
- Actions:
  - May act as proteolytic, saccharolytic or lipolytic enzymes
  - Respiratory enzymes as dehydrogenase and oxidase
<table>
<thead>
<tr>
<th>Endopigment</th>
<th>Exopigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remains bound to organism and does not diffuse into surrounding medium</td>
<td>Diffuses into surrounding medium</td>
</tr>
<tr>
<td><strong>Red pigment == Serratia</strong></td>
<td><strong>Pseudomonas produces:</strong></td>
</tr>
<tr>
<td><strong>Violet pigment == Chromobacterium</strong></td>
<td><strong>Blue pigment (Pyocyanin)</strong></td>
</tr>
<tr>
<td><strong>Golden yellow ==</strong></td>
<td><strong>Yellow pigment (Flourescens)</strong></td>
</tr>
</tbody>
</table>
## Bacterial Toxins

<table>
<thead>
<tr>
<th>Character</th>
<th>Exotoxin</th>
<th>Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusibility</td>
<td>Diffusible</td>
<td>Non-diffusible</td>
</tr>
<tr>
<td>Heating at 60-80</td>
<td>Destroyed</td>
<td>Stable</td>
</tr>
<tr>
<td>Antigenicity</td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>Toxicity</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Nature</td>
<td>Protein</td>
<td>LPS</td>
</tr>
<tr>
<td>Source</td>
<td>Some G+ve and G-ve</td>
<td>Most G-ve</td>
</tr>
<tr>
<td>Effect of formaline</td>
<td>Change to toxoid</td>
<td>Not affected</td>
</tr>
<tr>
<td>Location of genes</td>
<td>Plasmid or bacteriophage</td>
<td>Chromosome</td>
</tr>
</tbody>
</table>
Other products

- Coagulase
- Leucocidin
- Haemolysin
- Hyaluronidase
Bacterial reproduction & Growth Curve

• Common method of reproduction is simple binary fission

• Growth curve shows 4 phases:
  • Lag phase
  • Log phase
  • Stationary phase
  • Decline phase
Rate of Death > Rate of division

Growth curve

- **Lag phase**
- **Log phase**
- **Stationary phase**
- **Decline phase**

When organism is introduced into suitable medium, it does not multiply immediately, but increases in size and changes metabolism to prepare for reproduction.
Bacterial Metabolism

• Metabolism means all chemical processes within a cell:
  • Anabolism
  • Catabolism

• ATP is formed by:
  • Oxidation, where energy is stored as high energy phosphate bond
  • Oxidative phosphorylation (respiratory chain)
    • NAD == 3 ATP
    • FAD == 2 ATP
**Bacterial Metabolism**

- **Carbon & Energy sources of Bacteria:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Energy source</th>
<th>Carbon source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photoautotrophs</td>
<td>Light</td>
<td>CO$_2$</td>
</tr>
<tr>
<td>Photoheterotrophs</td>
<td>Light</td>
<td>Organic</td>
</tr>
<tr>
<td>Chemoautotrophs</td>
<td>Oxidation of inorganic substance</td>
<td>CO$_2$</td>
</tr>
<tr>
<td>Chemoheterotrophs</td>
<td>Oxidation of organic substances</td>
<td>Organic</td>
</tr>
</tbody>
</table>
Chemoheterotrophic bacteria

- Bacteria which are unable to use CO$_2$ as only source of carbon, and must obtain energy from organic substrates by fermentation.
- Other type of metabolism is respiration.
Fermentation

- During fermentation, immediate products formed by catabolism of organic substrate serve as final electron acceptor.
- Result is acid and gas, so detected by:
  - Acid base detector (acid detection)
  - Inverted Durham tube (gas detection)
- Gas-Liquid chromatography is used for rapid identification of some obligate anaerobes.
Respiration (Aerobic oxidation)

- Glucose $\rightarrow$ 2 Pyruvic acid (Glycolysis)
- Pyruvic acid $\rightarrow$ 3 CO$_2$, NADH, FADH (Kreb’s cycle)
- Respiratory chain:
  - All NAD and FAD $\rightarrow$ ATP
- Results:
  - Glycolysis = 2 ATP + 6 ATP (from 3 NADH)
Anti-microbial agents
Antimicrobial agents

- Include:
  - Antibiotics
  - Antiviral drugs
  - Antifungal drugs
  - Antiprotozoal agents
Antibiotics

- Mechanism of action may be:
  - Inhibition of cell wall synthesis
  - Alteration of cell membrane permeability
  - Inhibition of protein synthesis
  - Inhibition of nucleic acid synthesis
  - Others
Cell wall inhibitors

- **B- Lactams**
- **Glycopeptides**
  - Vancomycin
- **Polypeptides**
  - Cycloserine
  - Bacitracin
B-Lactams

- **Penicillins**
  - Classic e.g. penicillin G
  - Penicillinase resistant penicillin e.g. cloxacillin
  - Broad spectrum e.g. ampicillin
  - Ureidopenicillin e.g. piperacillin

- **Cephalosporins**
  - 1\textsuperscript{st} generation e.g. cephaloridine (velosef)
  - 2\textsuperscript{nd} generation e.g. cefaclor
  - 3\textsuperscript{rd} generation e.g. cefotaxime
Mechanism of action:

- **Penicillins:**
  - Inhibit terminal cross link of peptidoglycan
  - Bind to cell receptor penicillin binding protein, which are transpeptidase
  - Removal of inhibitors of autolytic enzymes

- **Cephalosporins:**
  - Inhibit terminal cross link of peptidoglycan
Antimicrobials acting through inhibition of cell membrane

- **Polymyxins:**
  - Peptides which incorporate itself inside protein and phospholipids causing free passage of substances outside and inside cell

- **Nystatinin and Amphotericin B:**
  - Combine with sterols in cell membrane causing rupture and leakage of cytoplasm contents
Antimicrobials acting against protein synthesis:

- Aminoglycosides (Neomycin, Kanamycin, Streptomycin ... etc)
- Tetracyclins
- Macrolides
- Chloramphenicol

Mechanism of action:

- Inhibition of 30 S (Aminoglycosides and tetracyclins) and 50 S (Macrolides and chloramphenicol)
- Aminoglycosides act on specific receptors on P12 on 30S
Resistance to drugs:

- **Penicillins:**
  - Organism produce B-lactamase
  - Absence of penicillin receptors
  - Failure of drug to activate autolytic enzymes

- **Aminoglycosides:**
  - Organism produce adenylating, phosphorylating, acetylating enzymes
  - Absence of specific receptors for drug
Inhibitors of nucleic acid synthesis

Inhibitors of precursor synthesis
- Sulfonamides
- Trimethoprim

Inhibitors of DNA synthesis
- Quinolones
- Flucytosine

Inhibitors of RNA synthesis
- Rifampicin
Inhibitors of nucleic acid synthesis

Mechanism of action:
- Sulfonamide:
  - Competitive inhibition of PABA
- Trimethoprim:
  - Inhibition of oxidation of nicotinic acid to tetrahydrofolic acid
- Rifampicin:
  - Inhibit RNA synthesis
Other mechanisms of action:

- **Isoniazide**
  - Mycolic acid synthesis inhibitor
- **Metronidazole**
  - DNA strand break
- **Ethambutol**
  - Inhibition of arabino-glactan
- **Griseofulvin**
  - Mitotic spindle formation inhibitor
Causes of failure of antimicrobial chemotherapy

- Viral or mixed infection may be not susceptible to antimicrobial agents
- Failure to use laboratory
- Errors of laboratory
- Wrong choice of antibiotics
- Wrong route of administration
- Inadequate dose of drug
- Inadequate duration of treatment by drug
- Antimicrobial resistance
- Antagonistic antibiotic
Drug resistance

- Means unresponsiveness of organism to drug
- Mechanism:
  - Organism produces enzyme that destroy drug (B-lactamase)
  - Organism changes permeability to drug
  - Organism alters structural target for drug (P12)
  - Organism alters metabolic pathway (PABA)
Drug resistance

• **Origin of resistance:**
  - **Non-genetic**
    - As inactive TB and L-form
  - **Genetic**
    - **Chromosomal**
      - Spontaneous mutation in gene responsible for resistance
    - **Extrachromosomal (Plasmid)**
      - Genes control often formation of enzymes capable of destroying drugs
Combination of antimicrobial drugs

- **Advantages:**
  - Treat serious infection
  - Delay or prevent resistance
  - Treat mixed infection
  - Synergism

- **Disadvantage:**
  - Drug interactions
  - High cost
  - Superinfection
  - Antagonism

- **Mechanism:**
  - Sequential block of metabolic pathway by 2 drugs
  - One drug enhances uptake of another drug
  - One drug facilitates entry of another drug
  - One drug inhibits enzyme which destroys the other drug
Antimicrobial chemoprophylaxis

- **Prophylaxis in person of normal susceptibility:**
  - Rheumatic fever, by long acting penicillin
  - Meningitis, by rifampicin
  - Plague, by tetracycllin
- **Prophylaxis in person of increased susceptibility:**
  - Heart disease
  - Leukemia
  - Recurrent UTI
  - Chronic respiratory diseases
- **Prophylaxis in surgery:**
  - Lower limb amputation
  - Wound sepsis
  - Orthopedic surgery
Factors to select drug of choice

- In vitro sensitivity test
- Narrow / Broad spectrum
- Determination of drug resistance
- Determination of side effects and toxicity
- Pharmacodynamics of drug
- Drug must be effective in vivo and in vitro
- Drug combination
- Drug interactions
- Patient factors:
  - Pregnancy
  - Age
Good Luck!

Dr Sahar Taher