Clinical Bacteriology Lecture (Prelims)

Lesson 1: History of Microbiology

Lucretius

- Roman Philosopher
- Believed invisible creatures were responsible for disease

Girolamo Fracastoro

- Physician
- Same beliefs with Lucretius

Francesco Stelluti

- Observed bees and weevils using microscope

Spontaneous Generation Debate (SGD)

- Life could originate from non-living matter
- This is supported by:
    - Aristotle – originator
    - John Needham
    - Lazzaro Spallanzani
    - Felix Pouchet

John Needham

- Boiled nutrient broth briefly
- Left flask uncovered
- Microorganisms grew in broth
- Supported *Spontaneous Generation*

Lazzaro Spallanzani

- Heat killed microbes
- Flasks were sealed tightly
- No microorganisms grew
- Supported *Biogenesis* (life comes from pre-existing life)

(Theory of Spontaneous Generation disproved by):

1. **Francesco Redi**

- Conducted an experiment using meat in jars
- Compared open jars, covered jars, and sealed jars
- Used gauze to cover meat for maggot to unable to grow on meat
- Disproved spontaneous generation for larger organisms

2. **Louis Pasteur**

- French Chemist
- His design of experiment settled the argument on Spontaneous Generation
- Used a Swann-neck flask
- Responsible for Germ Theory of disease
- Disproved theory of abiogenesis

Theory of Biogenesis

- Life could only come from life

(Germ Theory of Disease):

1. **Oliver Wendell Holmes Sr.**

- Believed death following childbirth often caused by material on hands of midwives or attending physicians
- Recommended better hygiene practices

2. **Ignaz Semmelweis**

- "Father of hand hygiene"
- Doctors could spread infection through contaminated hands

3. **John Snow**

- "Father of Epidemiology"
- Setting standards of goof hygiene
- Studied 1854 cholera outbreak in London
- Traced the outbreak to a contaminated water source

4. **John Tyndall**

- Discovered that bacteria exit in two (2) forms:
    a. **Endospore** – heat stable form
    b. **Vegetative** – heat sensitive form
- Need prolonged or intermittent heating (3 days) to destroy heat stable endospore (Tyndallization)
- Developed the process of fractional sterilization using repeating heating called Tyndallization

5. **Robert Koch**

- Confirmed Germ Theory of Disease
- Experimented with medium to grow on bacteria
- Originated use of a two-part dish from growing bacteria (petri-dish)

Koch's Postulate

- Proof that bacteria cause disease
    - Disease = germ
    - Healthy = no germ

I. **Postulate 1:** Microorganism must be found in all organisms suffering from the disease
II. **Postulate 2:** Suspected organism should be grown in pure culture
III. **Postulate 3:** Cells from a pure culture of suspected organism should cause disease in a healthy animal
IV. **Postulate 4:** Organism should be reisolated and shown to be the same as original

Pure culture

- Must be same organism as before

(Exemptions in Pure Culture):

- Polymicrobial
- Virus
- Patient shows no symptoms

Robert Koch

- Discovered Tubercle bacilli
- Discovered cause of anthrax from blood of dead cattle and applied as Koch's postulate

Joseph Lister

- Father of modern antisepsis

Edward Jenner

- Invented the first vaccine
- Made small incisions/punctures with cowpox materials in human arms subjects in order to prevent smallpox

Paul Ehrlich

- Hospital dermatologist
- German doctor wanted to find "magic bullet"
- Developed Salvarsan (arsenic derivative) preparation 606 for syphilis "Salvation for syphilis"
- Chemotherapy treatment using chemical substances

(Discovery of antimicrobials):

1. **Gerhard Domagk**

- German Chemist
- Discovered dye Prontosil (a sulfonamide) was effective against a bacterial infection

2. **Alexander Fleming**

- Discovered Penicillin
- First widely used antibiotic
- Observed that a mold (Penicillium) prevented the growth of bacteria

3. **Ernst Ruska**

- First electron microscope
- Capable of magnifying biological specimens

4. **Jacob and Mond**

- Discovered role of messenger RNA in transferring genetic information from DNA to ribosomes

Lesson 2: Introduction to Microbiology

Microbiology

- Study of microorganisms including viruses
- Broad discipline, includes:
  - Bacteriology
  - Virology
  - Mycology
  - Parasitology
  - Etc.

Anton Van Leeuwenhoek

- Father of protozoology and bacteriology
- Dutch biologist

Microbial world

- Viruses
- Bacteria (Eubacteria)
- Fungi (Yeast and Mold)
- Protozoa
- Microscopic algae

Virus

- Smallest known infectious agent
- Cannot be seen under an ordinary light microscope
- Subcellular microorganism
  - ➢ Have only nucleic acid surrounded by protein coat
  - ➢ Must live and grow in living cells of other organisms
- Nucleoproteins
- Acellular (not composed of cells)
- Neither prokaryotic nor eukaryotic

(Other characteristics of virus):

- Consist of DNA or RNA, but rarely both
- Genome can be double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single stranded RNA (ssRNA)
- Lack cytoplasmic membranes and surrounded by a protein coat
- Obligate intracellular parasites that cannot self-replicate

Protozoans

- Unicellular eukaryotic organism within kingdom Protista that obtain nutrition through ingestion
- Larger than bacteria

Eukaryotes	Prokaryotes
- Have membrane-bound organelles - True nucleus - Humans, animals, plants	- Does not have cytoplasmic organelles - Do not have true nucleus - Bacteria

Metazoans

- Multicellular

Fungi

- Eukaryotic organism with rigid cell wall containing chitin
- Yeast
- Have true nucleus
- Avirulent microorganism; deadly for immunocompromised patients
- Can cause systemic infection
- Heterotrophic eukaryotes that obtain nutrients through absorption (cannot produce all of its nutrient)

Hyphae

- Long, thread-like filaments that make up the body of a fungus
- Individual fungal threads

Mycelia

- Mass or network of interconnected hyphae that forms the main vegetative body of a fungus
- Spreads through substrate to absorb nutrients and support fungal growth

(Two types of Fungi):

1. **Single-celled**

- Yeast
- Reproduce by budding
- Does not form hyphae or mycelia

2. **Multicellular**
- Molds
- Large, fuzzy, multicellular organisms
- Produce spores
- Can reproduce asexually and sexually

Chitin
- Makes cell wall of fungi rigid

Bacteria
- Single-celled prokaryotic organisms
- Reproduce very fast (8 hours for *E. coli*) through binary fission
- Classified as prokaryotes
- Lack nuclear membrane and a true nucleus, mitochondria, ER, and Golgi bodies

Characteristics	Prokaryote	Eukaryote
Size	0.4 – 2 um in diameter 0.5 – 5 um in length	10-100 um >10 um in length
Nucleus	No nuclear membrane; nucleoid region of cytosol	Membrane-bound
Genome		
Location	Nucleoid, at mesosome	Nucleus
Chromosomal DNA	Circular; complexed with RNA	Linear; complexed with basic histones and proteins
Extrachromosomal DNA	Plasmids	In mitochondria, chloroplasts, and cytoplasm
Reproduction	Asexual (binary fission)	Sexual and asexual
Membrane-bound organelles	Absent	All
Golgi bodies	Absent	Present in some
Lysosomes	Absent	Present in some; contain hydrolytic enzymes
Endoplasmic Reticulum	Absent	Present; lipid synthesis, transport
Mitochondria	Absent	Present in most
Chloroplasts for photosynthesis	Absent	Present in algae and plants
Ribosomes	Present	Present
Size	70S consisting of 50S and 30S esubunits	80S consisting of 60S and 40S subunits

Electron transport for energy	Cell membrane; no mitochondria present	Inner membrane of mitochondria and chloroplasts
Sterols in cytoplasmic membrane	Absent except in Mycoplasmataceae	Present
Plasma membrane	Phospholipid bilayer; lacks carbohydrates	Phospholipid bilayer; contains glycolipids and glycoproteins
Cell wall, if present	Peptidoglycan in most bacteria	Cellulose, phenolic polymers, lignin (plants), chitin
Glycocalyx	Present	present
Cilia	Absent	Present
Flagella, if present	Simple flagella	Complex flagella
Pili and Fimbriae	Present	Absent

(Classifications):

I. **Shape**

- **Cocci** (round)
- **Bacilli** (elongated)
- **Spirillum** (rigid, exoflagella)
- **Spirochetes** (flexible, endoflagella)
    - Leptospira – Leptospirosis
    - Treponema – Syphilis
    - Borrelia – Lyme disease

II. **Ability to retain dyes**

III. **Ability to grow with or without air (aerobic/anaerobic)**

IV. **Biochemical reactions**

(Classification of microorganism):

Taxonomy

- Organizing, classifying and naming things
- Formal system originated by Carl Von Linne
- Identifying and classifying organisms according to specific criteria
- Each organism placed into a classification system
    - **Domain**
    - **Kingdom** – Prokaryotae

- **Phylum** – Gracilicutes
- **Class** – Scotobacteria
- **Order** – Eurobacteria
- **Family** – Enterobacteriaceae
- **Genus** – Escherichia
- **Species** – Coli

(3 Domains):

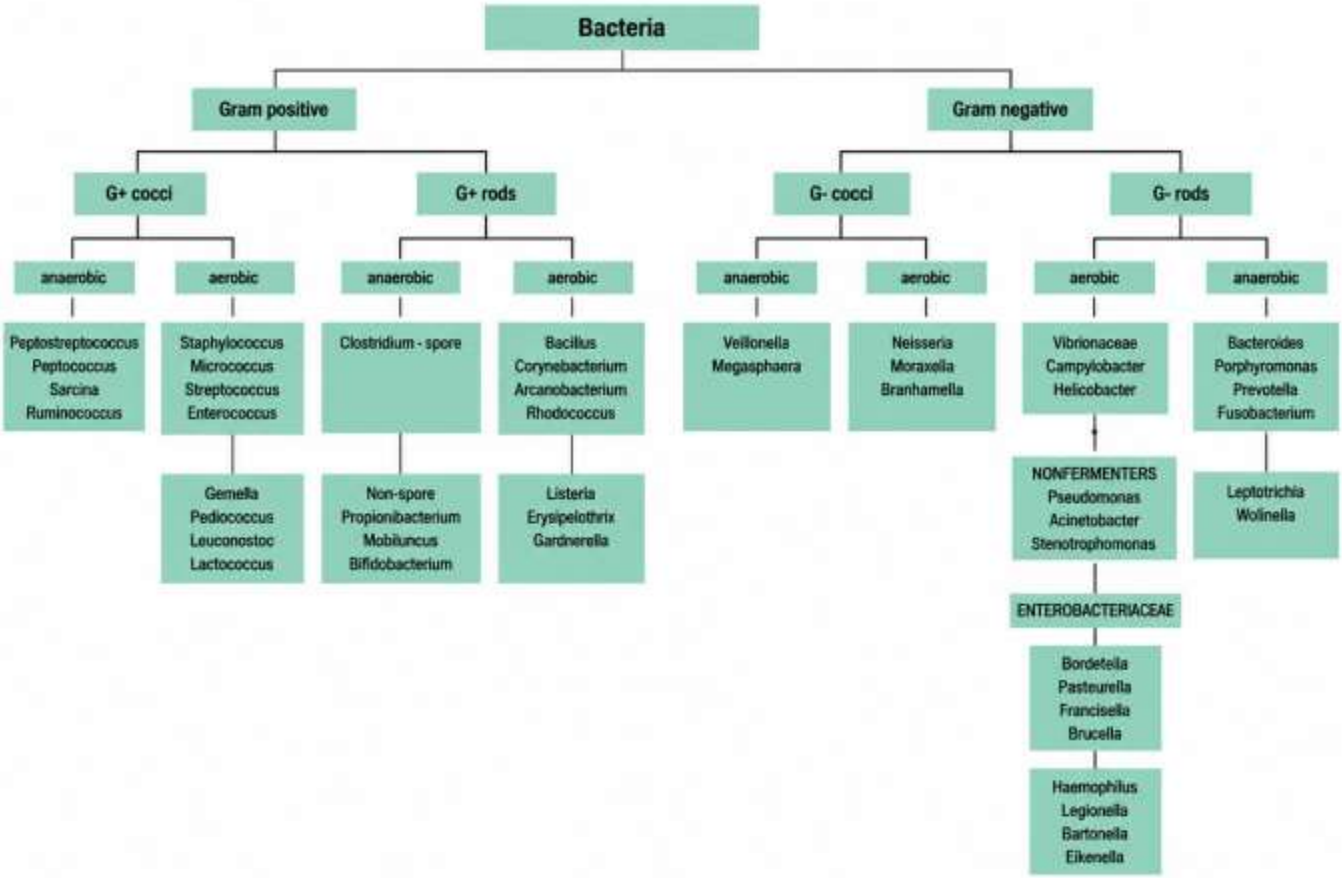
1. **Eubacteria**
- True bacteria
- Peptidoglycan
- Occupied by prokaryote

2. **Archaea**
- Odd bacteria that live in extreme environments, high salt, heat
- Do not have peptidoglycan in cell wall
- Unicellular
- Occupied by prokaryote

3. **Eukarya**
- Have nucleus and organelles
- Human, animal, plants
- Can be unicellular or multicellular

Naming Microorganism

- Binomial (scientific/nomenclature)
    a. ***Genus*** – noun, always capitalized
    b. ***Species*** – adjective, lower case
- Both italicized, underlined



Crystal Violet

- Violet-colored stain
- Gram positive (+)
- If you use crystal violet but it did not stain, therefore it's gram (-)

Safranin

- Red-colored stain
- Gram negative (-)
- If you use safranin but it did not stain, therefore it's gram (+)

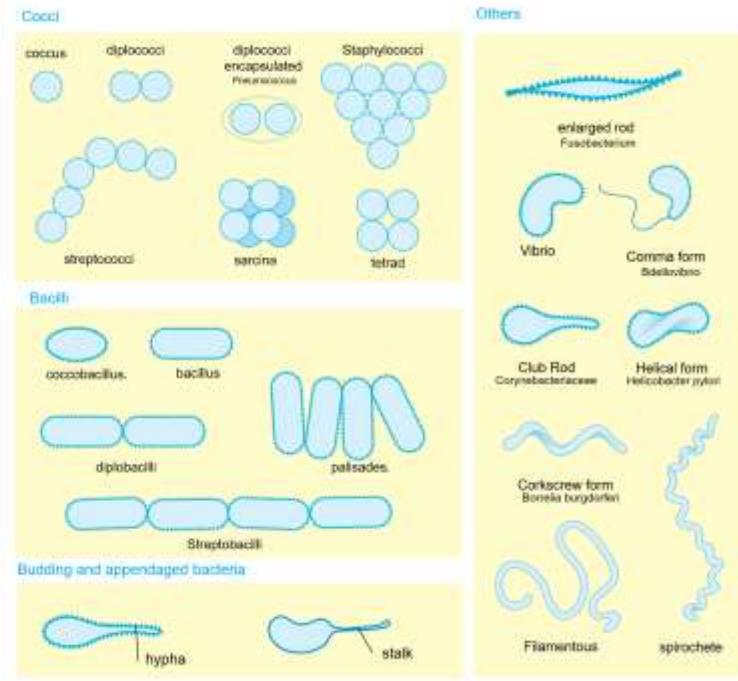
(Bacterial morphology, structure, and classification):

- Largest bacterium known, *Thiomargarita namibiensis (0.1 – 0.3 mm)*, is found in ocean sediment
- Most bacteria range in size from 0.4 – 2 um, they occur in three basic shapes
    - **Cocci** (spherical)
    - **Bacilli** (rod-shaped)
    - **Spirochetes** (spiral)

1. **Cocci**
- 1 um in diameter
- Plural of *coccus*
- spherical

(arrangements of cocci):

a. **Cocci** – single
b. **Diplococci** – in pairs
c. **Streptococci** – in chains
d. **Tetrad** – divide into two planes and occur into four
e. **Sarcina** – divide in three planes
f. **Staphylococci** – in cluster; multiple planes



2. **Bacilli**
- Plural form of bacillus
- 0.5 – 1 um in width, 3 um in length
- Vary greatly in size and length, long filamentous rods

(arrangements of bacilli):

a. Single bacillus
b. **Diplobacilli** – in pairs
c. **Streptobacilli** – in chain
d. **Coccobacillus** – short and fat
e. **Palisades** – seen side-by-side
f. **Fusiform** – tapered, pointed ends

Pleomorphic
- When a species differs in size and shape within a pure culture, bacterium is termed pleomorphic

3. **Spiral**

a. **Vibrio** – comma shaped
b. **Spirillium** – exoflagella
c. **Spirochete** – endoflagella
   - ➢ *Treponema*
   - ➢ *Borrelia*
   - ➢ *Leptospira*

(Essential Structures of Bacteria):

1. **Cell wall**

- maintains shape of the cell and prevents bursting of the cell from high osmotic pressure inside.
- Two major types of cell wall:
  a. **Gram-positive**
  b. **Gram negative**

Feature	Gram Positive Cell Wall	Gram Negative Cell Wall
Peptidoglycan Layer	Thick	Thin
Number of Layers	One main cell wall layer	Two layers (thin peptidoglycan + outer membrane)
Outer Membrane	Absent	Present
Teichoic Acid	Present	Absent
Lipoteichoic Acid	Present	Absent
Lipopolysaccharide	Absent	Present
Endotoxin (Lipid A)	Absent	Present
Porins	Absent	Present
Periplasmic Space	Absent	Present
Barrier to harmful substances	Less effective	Outer membrane acts as a protective barrier
Sensitive to Penicillin	More sensitive	Less sensitive
Main surface components	Peptidoglycan, Teichoic Acid, Lipoteichoic Acid	Outer membrane with proteins, phospholipids, and LPS
Gram Stain Color	Purple/Violet	Pink/Red

(Other informations stated):

- *If there's an infection in Gram negative and cell wall is broken, endotoxin (a form of poison) will release causing shock or fever.*
- *Teichoic acid is not present in Gram negative cell wall*

Peptidoglycan

- Consists of glycan (a polysaccharide) and chains of alternating N-acetyl-D-glucosamine (NAG) and N-acetyl-D-muramic acid (NAM)
- Short peptides, each consisting of four (4) amino acid residues, are attached to a carboxyl group on each NAM residue

- Since peptidoglycan layer is the principal component of Gram (+) cell wall, antimicrobial agents that target Gram (+) organisms such as penicillin are effective by preventing synthesis of peptidoglycan

Lipoteichoic Acid

- Anchored to the plasma membrane
- Strengthens wall of Gram (+)
- Antigenicity
- Prevents cell wall breakdown

Mycoplasma

- Cell wall-less bacteria
- Has a flexible cell membrane

(Wall-less forms of bacteria):

- Other bacteria are treated with:
    I. Enzymes that are lytic for cell wall
    II. Antibiotic that interferes with biosynthesis of peptidoglycan, wall-less bacteria

Spheroblast

- Has outer membrane

Protoblast

- No outer membrane

2. **Cell membrane**

- Regulates movement of materials into and out of cells
- Site of biosynthesis of DNA, cell wall polymers and membrane lipids
- Electron transport chain is found here
- Electron transport and oxidative phosphorylation
- Excretion of hydrolytic exoenzymes

3. **Cytoplasm**

- Contains genetic elements
- Composed largely of water, protein, nucleic acid, lipids and small amounts of sugar and salt
    - **a. Ribosomes**
        - Numerous
        - 15-20 nm with 70S
        - Distributed throughout the cytoplasm

        - ➢ Sensitive to streptomycin and erythromycin site of protein synthesis

   b. **Plasmids**
      - ➢ Extrachromosomal genetic elements
   c. **Inclusions**
      - ➢ Sources of stored energy
   d. **Nucleus**
      - ➢ Lacks nuclear membrane
      - ➢ No nucleoli, hence known as nucleic material/nucleoid

4. **Nuclear materials**

(Particular Structures of Bacteria/Non-essential):

1. **Capsule**

- Surrounds entire bacteria
- Made up of polysaccharides
- Avoids phagocytosis (bacteria avoids)

2. **Flagella**

- Motility structure
- Thin diameter
- 20 nm
- Long with some having a length 10 times the diameter of cell
- Cannot be seen in light microscope due to its small diameter unless special stain is applied
- Structure can be identified based on appearance
  a. ***Monotrichous***: 1 flagellum
  b. ***Peritrichous***: flagella around bacteria
  c. ***Lophotrichous***: many flagella on one side
  d. ***Atrichous***: no flagella
  e. ***Amphitrichous***: flagella on both sides
- Elongated bacteria (bacilli) only move because they have flagella

Mordant Tannic Acid

- Special stain used so that flagella will be visible
- Thickens flagella

3. **Pili**

a. **Common pili (fimbria)**
   - Attachment to surfaces and host cell
   - Shorter, straighter and thinner than flagella
   - ***Pilus***: singular
   - ***Pili***: many

b. **Sex pili**
   - Hollow structure used for bacterial conjugation
   - Not all bacteria undergo conjugation
   - This is how bacteria can transfer from one bacterium to another (ex: *Neisseria gonorrhoeae* has fimbria that makes it pathogenic)

Plasmid

- Mostly carry antibiotic resistance genes

4. **Spores**

- Located inside cytoplasm
- Elongated bacteria (bacilli) only have this
- Aerobic (bacillus) and anaerobic (clostridium) both have spores
- All spore forming bacteria are elongated
- When spore becomes moist or encounter favorable conditions it can vegetate again

Sporulation

- Creating spore
- When dried out, it becomes Spore Germination

Endospore

- Resting structures formed by bacteria for survival during adverse environmental condition

Germination

- Process by which a dormant bacterial spire returns to an active vegetative cell when environmental conditions become favorable

Dipicolinic Acid (DPA)

- Responsible for resistance against degrees
- Resist adverse environmental conditions

Endoflagella (axial filaments)

- Rotation of these filaments causes corkscrewing motion by spirochete

Phototaxis

- Movement of an organism response to light
- Moves towards light (positive response)
- Moves away from light (negative response)

Chemotaxis

- Movement of an organism in response to chemical substances
- Moves toward attractant chemicals (run)
- Moves away from harmful chemicals (tumble)

(Movement of flagellated bacteria):

a. **Clockwise**: bacteria run
b. **Counterclockwise**: bacteria tumble

Lesson 3: Physiology, Metabolism, and Genetics of Cells

Metabolism

- Sum of all chemical reactions within a living organism

Microbial Metabolism

- Transformation of energy
- Biochemical reactions bacteria use to break down organic compound
- Can be regulated in cell either by regulation of the production of an enzyme itself or by regulation of the activity of enzyme

ATP (Adenosine Triphosphate)

- Energy storing nucleotide
- Consists of Adenine, Ribose, and 3-phosphate groups
- ATP = ADP + P; energy is released to drive anabolic reactions

Oxidation

- Loss of electron

Reduction

- Gain of electron

NADH

- Special molecule cells used to carry electrons (often in H atoms)
- Potential energy, another way to transport energy
- Two important carriers:
  a. Nicotinamide Adenine Dinucleotide (NAD+) – adds electron and hydrogen during cellular respiration
  b. FAD (Flavin Adenube Dinucleotide – oxidized form; accepts electrons and hydrogen atoms; becomes FADH2 after being reduced

(Basic Metabolic reactions):

Anabolism

- Synthesis
- Energy is generated during catabolism of a substrate

Catabolism

- Breakdown
- Bacteria use biochemical pathways to catabolize carbohydrates and produce energy by two mechanisms:
  a. Fermentation
  b. Aerobic respiration (oxidation)

Exogenic

- Produce more energy than they can consume

Endogenic

- Consume more energy than they produce

Fermentation

- Anaerobic process carried out by:
  - Obligate
  - Facultative
  - Aerotolerant anaerobes
- Electron acceptor is an organic compound
- Less efficient in energy generation than respiration because beginning substrate is not completely reduced
- All of energy in substrate is not released
- Generates NAD
- Useful for identification of anaerobic bacteria

- End-product determination is also used in:
    - Voges-Proskauer (VP)
    - Methyl Red tests
        - ✓ Two important tests used in identifying members of Enterobacterales

Aerobic Respiration (oxidation)

- Efficient energy generating process
- Molecular oxygen is the final electron acceptor
- Obligate aerobes and Facultative anaerobes undergo aerobic respiration
- Anaerobic respiration energ yielding than aerobic respiration

Glucose

- Starting carbohydrate for bacterial fermentation or oxidation
- When bacterua use other sugars as a carbon source, they first convert sugar to glucose, which is processed by one of three pathways
- These pathways are designed to generate pyruvic acid
- Three major biochemial pathways bacteria use to break down glucose to pyruvic acid:
    1. ***Embden-Meyerhof-Parnas (EMP) glycolytic pathway***
    2. ***Pentose Phosphate Pathway***
    3. ***Entner-Dourdoroff Pathway***
- Pyruvate can be further catabolized either fermentatively or oxidatively

EMP Glycolytic Pathway (Glycolysis)

- Major pathway in conversion of glucose to pyruvate
- Generates reducing power in form of NADH2
- Generates energy in the form of ATP
- Anaerobic; does not require oxygen
- Used by many bacteria, including all members of the order Enterobacterales

Pentose Phosphate Pathway

- Alternative to EMP pathway for carbohydrate metabolism
- Consion of glucose to ribulose-5-phosphate
- Provides pentoses for nucleotide synthesis
- Produces glyceraldehyde-3-phosphate, which can be converted to pyruvate
- Generates NDH, which provides reducing power for biosynthetic reactions
- May be used to generate ATP (yield is less than EMP pathway)
- Used by heterolactuc fermenting bacteria:
    a. *Lactobacilli* and *Brucella abortus*

Entner-doudoroff Pathway

- Converts glucose-6-phosphate to pyruvate and glyceraldehyde phosphate
- Generates one (1) NADPH per molecule of of glucose, but uses one (1) ATP
- Aerobic processed used by:
    A. ***Pseudomonas***
    B. ***Alcaligenes***
    C. ***Enterococcus fecalis***
    D. ***Other bacteria lacking ceetain glycolytic enzymes***

Carbohydrate Catabolism

- Organisms catabolize carbohydrates as primary energy source for anabolic reactions

I. ***Aerobic Cellular Respiration***

- Complete breakdown of glucose to carbon dioxide, water, and a lot of ATP (most common pathway)

II. ***Anaerobic Respiration and Fermentation***

- Only partially break down glucose, into pyruvic acid and organic waste products and a little ATP; incomplete degradation

Krebs Cycle

- Most important pathway for the complete oxidation of a substrate
- Also known as Tricarboxylic Acid Cycle
- Pyruvate is oxidized, carbon skeletons for biosynthetic reactions are created
- Guanosine-triphosphate (GTP) is generated and electrons aee donated to form NADH and FADH2, which enter electron transport chain to generate energy in form of ATP

Aerobic Cellular Respiration

(4 Sub-pathways of Cellular Respiration):

1. **Glycolysis** – occurs in cytoplasm of bacterial cell wall
2. **Synthesis of acetyl CoA**
3. **Krebs Cycle** – occurs in cytoplasm of prokaryotes
4. **Electron Transport Chain**

Pentose Phosphate Pathway (PPP)

- Hexose monophosphate shunt
- Provides a means of breakdown of 5-carbon sugars aa well as glucose
- Provides important intermediate pentoses used in synthesis of nucleic acids and glucose from carbon dioxide in photosynthesis and certain amino acids

Entner-Doudoroff Pathway

- Produces 2 molecules of NADPH and 1 molecule of ATP for each molecule of glucose
- Not in Gram-positive bacteria
- Bacteria that have enzymes for this pathogen can metabolize glucose withot glycolysis or PPP
- Pyruvic acid needs to transform to acetyl CoA to enter Krebs Cycle

Electron Transport Chain

- Most ATP made in cellular respiration come from the stepwise release of energy through series of redox reaction between molecules known as electron transport chain
- Must occur in the membrane

Proton Gradient

- Type of ion gradient (difference in ion concentration on either side of a membrane)

(Creation of a proton gradient):

- Energy from each electron being passed down the chain is used to pump protons (H+) from one side of the membrane to the other

For every NADH = <u>**3 ATP**</u>
1 molecule of glucose = <u>**38 ATP**</u>

Anaerobic Cellular Respiration

- Cells that do not have access to oxygen, or obligate anaerobes can make ATP by using something other than oxygen as an electron receptor
    - ***Nitrate***
    - ***Sulfate***
    - ***CO2***
- Net gain (anaerobic): <u>**2 ATP**</u>
- Net gain (aerobic): <u>**38 ATP**</u>

Replication – duplication of chromosomal DNA for insertion into a daughter cell
Transcription – synthesis of mRNA by enzyme RNA polymerase using one strand of DNA
Translation – synthesis of a specific protein from mRNA code
Protein expression – also refers to synthesis of protein
Codon – group of three nucleotides in an mRNA molecule that signifies specific amino acid

Source	ATP Yield
(Glycolysis)	
1. Oxidation of Glucose to Pyruvic Acid	2 ATP
2. Production of 2 NADH	6 ATP
(Preparatory Step	
1. Formation of Acetyl CoA produces 2 NADH	6 ATP
(Krebs Cycle)	
1. Oxidation of succinyl CoA to succinic acid	2 GTP
2. Production of 6 NADH	18 ATP
3. Production of 2 FADH	4 ATP

Total = **38 ATP**

Metabolic Pathway of Energy used

- Microbes of ATP used to provide energy for transport of substances across plasma membranes

Lipid Biosynthesis

- Cell synthesizes fats by joining glycerol and fatty acid
- Glycerol portion is derived from dihydroxyacetone phosphate (glycolysis intermediate)

(Bacterial Genetics)

Bacterial Genome

- Single, circular chromosome of dsDNA
- Haploid chromosome
- Chromosome is not enclosed by a membrane but located in the nucleoid that is about 1/3 of interior volume
- Proteins aid in holding chromosome in this nucleoid space, filled with genetic material and devoid of ribosomes
- Chromosome fit inside bacteria because it is supercoiled
- Contains all information needed for cell growth and replication

Genes

- Specific DNA sequences that code for amino acidnsequence in one protein
- May be sliced or combined with other polypeptides to form more than one protein.

Genome Size

- Average bacterial chromosome in between 0.16 Mb and 10 Mb
- Free-living bacteria have longer genomes and more genes than bacteria that are obligate parasites
- Free-living bacteria bacteria have more genes than parasites

Plasmids
- Small, circular, extrachromosomal, double-stranded DNA strands found in some bacteria
- Independently replicating
- Do not carry any genes essential for normal bacterial growth or function
- Some plasmids carry genes that provide a selective advantage in the environment
- Some plasmids carey genes that allow for
    - Bacterial conjugation
    - Antibiotic resistance
    - Antibiotic production
- Genes that code for antimicrobial resistance often located on plasmids
- Located in the cytoplasm of cell and are self-replicating and passed to daughter cells

Binary Fission
- Division into two (2) cells
- Split into two
- Asexual type or reproduction
- Mother and daughter cells are clones

(3 different ways of bacteria):

1. **Vertical Gene Transfer**
- Transfer of DNA from a parent to daughter cells during binary fission
- Passes genetic information from one generation to another

2. **Horizontal Gene Transfer**
- Transfer of DNA between bacteria of same generation, not from parent to offspring
- Allows bacteria to rapidly acquire new traits, such as antibiotic resistance

3. **Mutation**
- Change in DNA sequence that occurs spontaneously or is induced by environmental factors
- Create new genetic variation that can be inherited during vertical gene transfer
- Often result in a change in the coded protein or in the prevention of its synthesis

(3 ways for bacterial diversity):

1. **Conjugation**
   - Transferring of genes from plasmids
   - Ducting should take long to transfer genes
   - Direct transfer of DNA between bacteria through a sex pilus

2. **Transformation**
   - Ability of some cells to take up freely floating DNA found in environment
   - DNA either has to be incorporated into the host cell
   - Uptake of free DNA from environment

3. **Transduction**
   - Transfer of bacterial genes by a bacteriophage from one cell to another

Competent cells/bacteria

- Have the ability to pick-up free-floating DNA in the surrounding
- Most are Gram-positive

(usually starve bacteria to become competent)

Bacteriophage

- DNA virus
- Consist of genetic material (DNA/RNA) surrounded by a protein coat

(DNA transfer through Bacteriophage):

- When phage particles infect another bacterial cell, it injects its genome into the bacterial cell, leaving protein coar ourside.

Specialized Transduction

- Restricted set of genes are transferred only

Lambda phage

- Infects *E. coli*

Generalized Transduction

- Chunks of genes are transferred
- Phage (third party) transfer DNA

Discovery of Transduction

- First, they ruled out transformation by verifying that culture was feee of contaminating DNA
- Since there was no contamination, there was no way for bacteria to take up freely floating (naked) DNA from environment
- Then they ruled out conjugation by helping cells isolated from one anotherby a thin membrane.
- If the cells couldn’t touch, there was no way for bacteria to conjugate
- When they still detected genetic recombination, it was time to do something
- Eventually, they discovered the tiny bacteriophage particles in the culture and uncovered the details of transduction

(2 Types of Bacteriophage):

Virulent	**Temperate**
- Use bacterium as a viral synthesis factory - Break down bacterial cell machinery to produce more phages - Progeny phages are released from the bacterium as the cell lyses	- Can be virulent - Phage genome may integrate into bacterial chromosome as a prophage - Bacteria that contain a prophage are said to be lysogenic for that phage

Viral DNA

- Gene (specific) is included to resemble as a new phage

Tamed DNA

- Infect bacterial cell but does not destroy immediately but insert genome

Lysogeny

- Phage DNA incorporates into bacterial genome, where it is replicated along with bacterial chromosomal DNA when the cell divides.
- Phage refers to as being temperate

(Microbial Growth Requirements):

1. **Physical**

- Correct temperature
- pH
- Osmotic pressure

Correct temperature

- Most bacteria grow only within a limited range of temperature
- Optimum: best/ideal temperature
- ***37°:*** ideal temperature for bacterial growth because it mimics the body temperature (most bacteria that are pathogenic is mesophilic)

Psychrophiles

- Cold-loving organisms
  a. ***Psychrotrophs*** – prefer to live at much lower temperature (0-15°)
  b. ***Moderate psychrophiles*** – 20-30°
  c. ***Mesophiles –*** love body temperature; 25-40°
  d. ***Thermophiles*** – 50-60°
  e. ***Hyperthermophiles*** – above 80°; archaeans

pH

- Hydrogen potential
  a. Acidophiles
    - Live on acidic environment
    - *Campylobacter pylori*
  b. Alkalidophiles
    - Grows best in basic environment
    - *Vibrio colera*

Osmotic Pressure

- Check pH after you check medium
- Distilled water is used to prevent contamination

2. **Chemical**

- Sources of C, N, S, P, trace elements, O_2 and organic growth factors
  a. **Carbon** – structural backbone of living matter
  b. **Nitrogen, Sulfur, and Phosphorus** – protein synthesis (N and S), DNA and RNA synthesis, (K, Mg, Ca) are needed for co-enzymes

c. **Trace elements** – Fe, Cu, Mg, Zn; naturally present in water

Oxygen

- Metabolic systems require oxygen for aerobic respiration
- Microbes that use molecular oxygen produce more energy from nutrients than microbes
    a. ***Obligate aerobe*** – requires oxygen to survive
    b. ***Facultative anaerobe*** – could be aerobe or moderate anaerobe; oxygen is nutrient and can grow with or without O_2
    c. ***Obligate anaerobe*** – do not like oxygen
    d. ***Aerotolerant anaerobe*** – tolerates oxygen; no benefit nor harm
    e. ***Microaerophiles*** – love small amount of oxygen (can tolerate 5-10% O_2); should be found in the middle; contains little enzyme
    f. ***Capnophiles*** – love carbon dioxide
        - Campylobacter
        - Haemophilus

Chemoautotrophs

- CO_2
- Inorganic

Chemoheterotrophs

- Organic compounds

Phototrophs

- Source of energy is light
- Photosynthetic bacteria (green-sulfur bacteria)

Photoheterotrophs

- Organic compound
- CO_2 carbon source

Turbidity

- Sign of bacterial growth

Some bacteria are aerobe or anaerobe

- Because of the **enzyme** that aerophiles contain:
    - **Catalase**
    - **Superoxide dismutase (SOD)**

(Toxic Form of Oxygen):

- Single oxygen ($^1O_2$) is normal O_2 boosted into a higher energy state and extremely reactive
- Superoxide free radicals (O_2) formed in small amounts during normal respiration of organism (SOD neutralizes this

$$\mathbf{O_2 + O_2 + 2H^+ \rightarrow H_2O_2 + O_2}$$

- Peroxide anion (O_2^{2-}) toxic form which is neutralize by catalase converts it to water and oxygen

$$\mathbf{2H_2O_2 \rightarrow 2H_2O + O_2}$$

- Peroxidase can also convert it to water

(Organic Growth Factors):

- Vitamins which function as co-enzymes
- Other organic growth factors:
    - **Amino acids**
    - **Purine**
    - **Pyrimidine**
- May be synthesized by bacteria themselves or should be obtained from environment

(Anaerobic Growth System):

Sodium Thioglycolate

- Chemically combine with dissolve oxygen and deplete oxygen

Palladium Catalyst

- Sodium Thioglycolate should have a pinkish layer (resazurin layer)
- Reheat medium to derive oxygen

Candle Jar Plate

- Old way of making oxygen-free environment by candle extinction method

Anaerobic Chamber

- Provided with air lock and glove ports
- Used if the laboratory is culturing all anaerobes

Generation Time

- Time required for a cell to divide
- Most bacteria's doubling time: 1-3 hours; others: 24 hours or more
- ***Escherichia coli GT***: <u>6-8 hours</u>

- ***Mycobacterium tuberculosis***: long period; weeks

(4 Phases of Bacterial Growth):

1. **Lag Phase**
- When bacteria are adjusting to a new environment
- Period of intense metabolic synthesis of enzymes or molecules

2. **Logarithmic Phase/Exponential Growth**
- Cells begin to divide
- Bacteria are most active and sensitive
- Peak
- Shortest phase
- This is where you test bacteria

3. **Stationary Phase/Equilibrium**
- Microbes that are dying are equal with new cells

4. **Death Phase/Decline**
- Number of deaths exist in cells

(Reason for knowing bacteria's growth):

- Antibiotic susceptibility testing
- Supply nutrients to keep them alive (preservation) in the process of subculture

(Direct Measurement of Growth):

1. **Plate Count**
- Measures numbers of viable cell (Pour or Plate)

2. **Filtration**
- At least 100 ml of water are passed through a thin membrane filter whose pores are too small to allow bacteria to pass

3. **Direct Microscopic Count**
- No incubation time
- Reserved for application in which time is the primary consideration:
    - **Breed count**
    - **Petroff-Hauser**
    - **Electronic Cell Count**

(Estimating Bacterial Numbers by Indirect Method):

I. **Turbidity**

- By using spectrophotometer

II. **Metabolic Act**

- Assumes amount of certain metabolic product is in direct proportion to the number of bacteria present

III. **Dry Weight**

- For filamentous bacteria and mold
- Fungus is removed from growth medium, filtered to remove extraneous material and dried in a dessicator

(Controlling of Growth Media):

Normal microbiota

- Staphylococcus epidermis: normal residence of skin
- Normal bacteria
- Do not harm

(Bacteria became virulent when they transfer to the place that they are unfamiliar)

Escherichia coli

- Normally found in large intestines
- But when it reaches urinary tract, it can be virulent and can cause UTI

(3 Methods of Controlling Bacteria):

I. **Physical**

- Use of heat and radiation
  a. Heat -> moist -> pasteurization and boiling: disinfect
  b. Heat -> moist -> autoclave: sterilize
  c. **Radiation**: ionizing (gamma, x-ray) mutates DNA so bacteria is dead; non-ionizing: UV

II. **Chemical**

- **Alcohol**: most common disinfectant (75%)
- **Aldehyde**: sterilant (sporicidal)
  - **Formaldehyde**
  - **Glutaraldehyde**

- **Formalin**

- **Phenol**: Lysol
- **Chlorine**: water disinfectant (Halogen: Chlorine + Iodine)
- **Ethylene oxide (ETOX)**: sterilant

III. Mechanical

- Filtration -> HEPA & ULPA
- Filtration -> liquid -> sterilization (0.45 membrane filter)
- Handwashing

<u>Lesson 3: Micrococcaceae</u>

Micrococcaceae

- Members are gram-positive cocci, aerobic or facultative anaerobes
- Catalase positive (except Stomatococcus)
- Most members are indigenous microbiota:
    - *Staphylococcus*
    - *Micrococcus*
    - *Stomatococcus*

Vigorous bubbling (in an agar plate)

- Indicates positive bacteria

Staphylococcus

- Catalase positive
- Non-motile
- Facultative anaerobes
- Normal inhabitants of skin and mucous membranes
- Commonly cause human infections
- Species are differentiated by coagulase test, most important being the coagulase positive: *Staphylococcus aureus*
- Some animal species produce coagulase but rarely isolated from human sample

Micrococcus

- Opportunistic pathogen found only in immunocompromised patients of low pathogenic significance
- May be isolated as containment or as part of normal flora/normal microbiota

Stomatococcus

- Part of normal oral flora
- Rarely isolated from infections
- Colonies strongly adhere to agar surface

(Staphylococcus aureus infections):

1. **Skin and wound infections**

- Boils
- Furuncles
- Folliculitis

2. **Food poisoning**
- Staphylococcal enterotoxins A & D

3. **Scalded skin syndrome (Ritter's disease)**
- Skin condition caused by *S. aureus* that produce exfoliative (epidermolytic) toxins A & B

4. **TSS (Toxic Shock Syndrome)**
- caused by *S. aureus* that produces TSST-1 (formerly known as enterotoxin F.)
    - high fever
    - hypotension
    - diffuse, sunburn-like rash
    - skin peeling
    - vomiting and diarrhea

5. **Staphylococcal pneumonia**
- Commonly occurs secondary to influenza

6. **Osteomyelitis**
- Can occur secondary to bacteremia

(Virulence factors of Staphylococcus aureus):

- **I. Coagulase**
- **II. Protein A**
- **III. A, B Hemolysis**
- IV. **Hyaluronidase** – promotes spreading of infection
- V. **Lipase** – facultative colonization of bacteria in skin

(Methods of Identification of microorganism):

- A. **Phenotypic** – morphological; something that can observe
- B. **Immunological** – detects antigens of microorganism or antibodies produced by patient
- C. **Genotypic** – detects microorganism's DNA/RNA

(Laboratory Identification of Staphylococcus aureus):

- ➢ Microscopic examination
- ➢ Culture
- ➢ Biochemical identification by coagulase test
    - Cell bound coagulase (slide method)
    - Free coagulase (tube method)

- Use selective media from heavily contaminated specimen (MSA, DEA, SNA)

Staphylococcus aureus

- MSA positive

(Differential Characteristics):

- Bubbling = positive
- Coagulation of plasma = positive

Micrococcus

- Environmental organism
- Normal microbiota of the skin, respiratory tract
- Common contaminants
- Coagulase negative

(Laboratory Identification of Micrococcus):

- Modified oxidase test
    - Uses **6% Tetramethyl-p-phenylenediaminehydrochloride** in dimethyl sulfoxide
- Bacitracin susceptibility (0.04 Bacitracin)
- Furazolidone resistance (100 ug)

(Coagulase negative Staphylococcus):

- Found in normal flora in humans and animals
- Often are nosocomial infections
- Predisposing factors:
    - **Catheterization**
    - **Prosthetic device**
    - **Implants**
    - **Immunosuppressive**

- Most common spp.:
    - ***S. epidermis***
    - ***S. saprophyticus*** – associated with UTI in young sexually active in young female
    - ***S. haemolyticus*** – may produce negative coagulase; occasionally cause wide range of infections
    - ***S. lugdunensis*** – may produce negative coagulase; occasionally cause wide range of infections
    - ***S. schleiferi*** – occasionally cause wide range of infections

(Laboratory Identification of Coagulase Negative Staphylococcus):

- Culture on Sheep's BAP
- Coagulase negative urine isolates are further tested to presumptively identify *S. saprophyticus* using Novobiocin Susceptibility
- Various commercial identification systems may be used

Staphylococcus aureus

- B-lactam antibiotics
- Chemical form has B-lactamase

MRSA (Methicillin-Resistant S. aureus)	ORSA (Oxacillin-Resistant S. aureus)
- Resistant to methicillin - Resistant to almost all B-lactam antibiotics - Most commonly used	- Resistant to oxacillin - Same as MRSA - Older but still seen in some books/labs

TEST	Micrococci	Staphylococci
Modified Oxidase Test	Oxidative	Fermentative
	Positive	Negative
Bacitracin Susceptibility	Sensitive	Resistant
Furazolidone Susceptibility	Resistant	Sensitive

Lesson 4: Streptococcaceae

Streptococcus, Enterococcus, and related genera

- Catalase negative, Gram-positive cocci, arranged in pairs or in chains
- Facultative anaerobes
- Used of enriched medium or blood is necessary for their isolation (Sheep's Blood Agar; not for MAC because it is for Gram-negative)
- Hemolysis patterns are helpful in identification
- Enterococci and some streptococci appear more elongated than spherical
- Streptococcal cells = in chains when grown in broth cultures

Negative Catalase Test

- Result differentiates streptococci and enterococci from staphylococci

Weak False-Positive Catalase

- Can be seen when growth is taken from media containing blood because of the peroxidase activity of hemoglobin

Hemolysis	Description
Alpha (α)	Partial lysis of RBCs around colony; Greenish discoloration of area around colony
Beta (β)	Complete lysis of RBCs around colony; Clear area around colony
Gamma (γ)	No lysis of RBCs around colony; No change in agar
Alpha-prime (α') or wide zone	Small area of intact RBCs around colony surrounded by a wider zone of complete hemolysis

S. pneumoniae

- α-hemolytic
- Part of the normal flora of urinary tract infection
- Key virulence factor is an antiphagocytic capsule
- There are approximately 80-90 antigenic types
- An important human pathogen causing:
    - Pneumonia
    - Sinusitis
    - Otitis media
    - Bacteremia

- Meningitis

- Direct smears often reveal leukocytes and numerous Gram-positive diplococci with end being slightly pointed, giving a lancet-shaped
- Enriched medium supplied with blood (CAP, BAP)
- Complex media such as BHIB or TSA with 5% blood or CAP are necessary for good growth
- Isolates may require increased CO_2
- Colonies are α-hemolytic
- Young cultures produce a round, glistening, wet, mucoid dome-shaped appearance (because of polysaccharides)
- Seen along with PMNs (in sputum)

(Laboratory ID of S. pneumoniae):

- Susceptibility of **optochin**
- **Bile solubility test**: S. pneumoniae will be lysed, forming a clear color
- **Quelling test/swelling reaction**
    - Relies swelling on capsule upon bonding homologous antibodies
    - Seen in OIO
- Most of Streptococcus are classified based on their carbohydrate antigen in their cell wall

Group A: Streptococcus pyogenes

- Cell wall contains Lancefield group A carbohydrate
- Group A streptococcus or BHS group A
- β-hemolytic

(Virulence factors):

I. **M-protein** – causes bacterial cell to resist phagocytosis by enabling it to adhere to mucosal cells
II. **Streptolysin O** – causes hemolysis of RBCs; can generate antibodies (Anti-ASO)
III. **Hyaluronidase** – spreading factor
IV. **DNAse** – all strains of pyogenes form 1 DNAse (DNAse B)
V. **Erythrogenic toxins** – responsible for red spreading rash in scarlet fever
VI. **Protein F (Fibronectin)** – binding protein facilitates bacterial adhesion to epithelials cells
VII. **Streptokinase**

(*S. pyogenes* Infection):

- **Strep throat (pharyngitis)** – seen in children
- **Skin infections**
- **Scarlet fever** – begins on trunks and spreads to the extremities because of erythrogenic exotoxins
- **Rheumatic fever (heart problem) and glomerulonephritis (kidney problem)**
- **Streptococcal TSS**

(Laboratory ID of S. pyogenesis):

- **Culture**: β-hemolytic colonies on blood agar
- **Gram's stain:** Gram-positive cocci in pairs/chains
- **Bacitracin susceptibility**
- **Taxo A**: Positive (susceptible)
- **Resistant to SXT**
- **Hydrolyzes PYR (+):** red color indicates positive

Group B: Streptococcus agalactiae

- Cell wall contains lancefield group B carbohydrate
- GBS
- Found as normal microbiota of genitourinary tract

(Virulence factors):

I. **Capsule**
II. **Others (DNAse, Hyaluronidase)** have not been shown to be factors in infection

(S. agalactiae Infections):

- Contamination of vagina during birth (neonatal sepsis)
- Post partum fever and sepsis

(Laboratory ID of S. agalactiae):

- Also, **β-hemolytic** (but narrow hemolysis)
- When checked in Gram stain, chains or in pairs (Gram-positive)
- **Culture**
- **Gram's stain**
- **CAMP test positive**: done using sheep's BAP
- **Hippurate hydrolysis positive**
- **Resistant to SXT**
- **PYR (-) negative**

Group C and G

- Hemolytic species in lancefield group that occasionally isolate from clinical specimens
- Part of normal skin microbiota
  - **a. Group C**
    - *S. equi*
    - *S. zooepidemicus*
    - *S. equisimilis*
  - **b. Group G**
    - *S. anis*
    - *S. anginosus*
    - *S. milleri*

Group D

- Includes S. bovis and S. equinis
- Found as normal intestinal microbiota
- May be agents of:
  - Bacterial endocarditis
  - UTI
  - Abscesses
  - Wound infections
- Positive for Bile esculin
- Negative for PYR (-)
- α and γ-hemolytic pattern
- 6.5 NaCl (-)
- Serotyping
- Group D indicates agglutination

Enterococcus

- Found in the intestinal tract
- Most common:
  - E. faecalis (F. faecium, E. avium and E. durans)
- Share characteristics of group D, including antigen D
- Show resistance to several of commonly used antibiotics
- UTI is a common infection
- Gram-positive cocci

(Laboratory ID of Enterococcus):

- **Bile Esculin (+), 6.5%. PYR (+)**
- **SXT resistance**
- Should be screened for **HLAR** (High Level Aminoglycoside resistant enterococcus): growth resistance test
- **VRE** (Vancomycin-resistant enterocci) is a major concern
    - Van A gene: most common resistance mechanism
- Common species:
    - *E. faecalis*
    - *E. faecium*
- Synergistic approach:
    - Aminoglycoside + cell wall active agent
    - **Aminoglycoside**: Gentamicin, Tobramycin, Amikacin, Streptomycin

Viridans Streptococcus

- Include those α-hemolytic streptococci that lack Lancefield group antigens
- Do not meet criteria for *S. pneumoniae*
- Part of normal microbiota of the upper respiratory tract, female genital tract, and gastrointestinal tract
- Fastidious (Needs a lot of nutrients) and require increased CO_2
- Frequent cause of **BSE (Bovine Spongiform Encephalopathy)**
- ***S. mutans*** (α/γ-hemolytic), ***S. salivarus*** (α-hemolytic), ***S. sanguis*** (α-hemolytic), ***S. mitis*** (α-hemolytic), ***S. milleri*** (variable, can be β-hemolytic)
- **NVS (Nutritionally Variant Streptococcus)** have been isolated from patients who have endocarditis and otitis media; nutritionally deficient
- Requires **vitamin B6**

(Treatments):

- Most species are susceptible to Penicillin
- S. agalactiae – less susceptible to some antibiotics than Group A and may require a combination of Ampicillin + Aminoglycoside
- Pneumococcal isolates are treated with erythromycin in case of penicillin resistance
- Linezolid is being prescribed to VRE infections

<u>Lesson 5: Neisseria Species and Moraxella</u>

Neisseria

- Gram-negative cocci
- Kidney-bean shaped
- Usually arranged as diplococci
- Oxidase positive
- Catalase positive
- Non-motile
- Non-spore-forming
- Aerobic/Capnophilic
- Often fastidious
- Grow best under increases CO_2
- Require enriched media for optimal growth

(Two (2) Major Human Pathogens):

1. **Neisseria gonorrhoeae**
- Gonorrhea
- Primarily a mucosal pathogen
- Genital tract infection

2. **Neisseria meningitidis**
- Meningitis
- Meningococcemia/septicemia
- Can cause rapidly progressive invasive disease

Commensal Neisseria

- Normally inhabit the upper respiratory tract
- Non-pathogenic
- Some can occasionally cause opportunistic infections
    - *N. lactamica*
    - *N. sicca*
    - *N. subflava*
    - *N. mucosa*
    - *N. flavescens*
    - *N. cinerea*
    - *N. elongata*
    - *N. weaver*

(Neisseria gonorrhoeae vs. Neisseria meningitidis):

Feature	N. gonorrhoeae	N. meningitidis
Gram Stain	**Gram negative diplococci**	**Gram negative diplococci**
Main site	**Genital tract**	**Nasopharynx**
Major disease	**Gonorrhea**	**Meningitis/septicemia**
Capsule	**Can be capsulated/not capsulated**	**Present**
Glucose	**Present**	**Present**
Maltose	**Absent**	**Present**
Transmission	**Sexual/perinatal**	**Respiratory droplets/close contact**
Major Virulence Feature	**Pili, Opa, Por, LOS**	**Capsule**
Important Specimens	**Genital specimens/urine**	**CSF/blood**
Classic laboratory clue	**Intracellular Gram-negative diplococci in PMNs**	**Intracellular/extracellular Gram-negative diplococci in CSF**

(Growth Requirements):

- Pathogenic Neisseria are fastidious organisms
- They require:
    - Enriched nutrients
    - Increased CO_2
    - Moisture
    - Appropriate temperature
    - Proper specimen transport
    - Prompt processing

(Culture Media):

1. **Chocolate Agar**

- Enriched
- Supports the growth of fastidious organisms
- Pathogenic Neisseria can grow here

2. **Modified-Thayer-Martin (MTM)**

- Enriched and selective
- Contains antimicrobial agents that suppress competing organisms while allowing pathogenic Neisseria to grow

(Colony Morphology):

- Pathogenic Neisseria generally produces colonies that may be:
    - Small
    - Smooth
    - Moist
    - Gray to translucent

Oxidase Test

- Oxidase test detects Cytochrome C oxidase
- Neisseria is oxidase positive
- A positive reaction produces a purple/dark purple color
- A negative reaction produces a colorless color
- Reagent used is Tetramethyl-p-phenylenediamine dihydrochloride (TMPD_

Catalase Test

- Neisseria are known to be catalase positive
- Catalase breaks down hydrogen peroxide
- H_2O_2 -> $H_2O + O_2$
- Formation of "bubbles" indicate a positive result

Carbohydrate Utilization

- *N. gonorrhoeae* produces glucose only
- *N. meningitidis* produces glucose and maltose

(Virulence factors of N. gonorrhoeae):

1. **Pili**

- Responsible for initial attachment
- Colonization
- Microcolony formation
- Resistance to mechanical removal
- Antigenic variation

2. **Opa proteins**

- Close attachment to host cells
- Interaction with host receptors
- Invasion

3. Por proteins

- Major outer membrane that affects membrane permeability
- Contribute to resistance against host defenses
- May undergo antigenic variation

4. LOS (Lipooligosaccharide)

- Functions similarly to endotoxin
- Does not have the long O-polysaccharide side chain
- Contributes to:
    - Inflammation
    - Tissue injury
    - Endotoxic effects

5. IgA protease

- Breaks down secretory IgA on mucosal surfaces
- IgA degradation -> better survival on mucosal surfaces

6. Antigenic variation

- Neisseria can alter surface structures such as:
    - Pili
    - Opa
    - Por proteins

7. Iron Acquisition

- Neisseria need iron for growth and metabolism
- Because free iron is limited in human body, they have systems that obtain iron from host proteins such as:
    - Transferrin
    - Lactoferrin

(Virulence factors of N. meningitidis):

1. Capsule

- Protects against phagocytosis
- Helps resist complement-mediated killing
- Allows survival in the bloodstream
- Promotes invasive disease
- Forms the basis for serogrouping

(Diseases caused by N. gonorrhoeae):

- Urethritis
- Epididymitis
- Prostatitis
- Other urethral complications
- Cervicitis
- Endometritis
- Salpingitis
- Pelvic inflammatory disease

Ophthalmia Neonatorum

- An infected mother can transmit N. gonorrhoeae to the newborn during delivery
- Organism infects the conjunctiva and causes severe purulent conjunctivitis
- Untreated infection can cause serious ocular complications, including blindness

Disseminated Gonococcal Infection (DGI)

- Disease when N. gonorrhoeae enters the bloodstream
- Classic manifestations include: dermatitis + tenosynovitis + migratory polyarthritis
- Other findings may include:
    - Fever
    - Joint pain
    - Skin lesions
    - Arthritis

(Diseases caused by N. meningitidis):

- Meningitis
- Meningococcemia/septicemia: when *N. meningitidis* enters bloodstream
- Pneumonia
- Arthritis
- Other invasive infections

Meningococcemia

- Survive thru capsule mediated invasion
- Can cross blood-brain-barrier
- Happens when N. meningitidis enters bloodstream

Waterhouse-Friderichsen Syndrome

- Massive adrenal hemorrhage
- Endotoxin mediated vascular injury
- Can cause hemorrhage and shock
- Can cause DIC
- Occurs in meningococcemia

(Laboratory diagnosis of Gonorrhea):

Specimen

- Urethral specimen
- Endocervical specimen
- Vaginal specimen
- Rectal
- Pharyngeal
- Conjunctival
- First-catch urine

NAAT

- Preferred for routine diagnosis of uncomplicated gonorrhea
- Detects nucleic acid of N. gonorrhoeae

Direct Gram Stain

- Useful in a symptomatic male with urethritis
- Finding: Intracellular Gram-negative diplococci within PMNs
- Female genital specimens contain normal flora and other organism that may resemble Neisseria which makes direct gram stain less useful

Culture of N. gonorrhoeae

- Chocolate Agar or MTM

(NAAT vs. Culture):

NAAT	CULTURE
Detects nucleic acid	Detects viable organisms
Highly sensitive	Useful for susceptibility testing
Rapid	More demanding
Commonly preferred for uncomplicated gonorrhea	Important when resistance testing is needed

(Laboratory diagnosis of N. meningitidis):

- **Meningitis**: CSF and Blood
- **Meningococcemia**: Blood cultures
- Organism may be identified through:
    - Gram stain
    - Culture
    - Biochemical testing
    - Serologic methods
    - Molecular methods
    - MALDI-TOF MS

MALDI-TOF MS

- Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
- Identified bacteria by analyzing characteristic protein patterns
- Rapid
- Accurate
- Useful for cultured isolates
- Can differentiate bacterial species

(Commensal Species):

N. cinerea	- misidentified as N. gonorrhoeae - susceptible to colistin
N. flavescens	- yellow pigmented Neisseria spp. - growth at approximately 22 degrees
N. lactamica	- resemble N. meningitidis - found in the nasopharynx of infants and children - uses glucose, maltose, lactose - associated with lactose utilization
N. mucosa	- large colonies - Often adherent to agar - very mucoid - commonly isolated from nasopharynx - non pathogenic
N. sicca	- dry, wrinkled, adherent, breadcrumb-like
N. subflava	- less yellow - part of upper respiratory microbiota
N. elongata	- rod shaped - associated with endocarditis

N. weaver	- normal oral microbiota in dogs - can occur in human infections following dog bites - catalase positive - gram-negative rods - does not produce acid from carbohydrate traditionally used for Neisseria identification

Moraxella catarrhalis

- Gram-negative diplococcus
- Oxidase positive
- Catalase positive
- Asaccharolytic
- DNAse positive
- Butyrate esterase positive
- Commensal of the upper respiratory tract
- Opportunistic pathogen

(Clinical Infections of M. catarrhalis):

- Otitis media (acute otitis media in children)
- Sinusitis
- Bronchitis
- Pneumonia

(Laboratory Diagnosis):

- Grows on CAP, and Sheep Blood Agar
- Possible specimen:
  - Middle-ear effusion
  - Nasopharyngeal specimen
  - Sinus aspirate
  - Sputum
  - Bronchial aspirate

(Biochemical Tests of M. catarrhalis):

Test	Result
Oxidase	Positive
Catalase	Positive
Carbohydrate utilization	Negative/asaccharolytic
DNAse	Positive
Butyrate esterase	Positive

- Most isolates produce β-lactamase, therefore, they are generally resistant to:
    - Ampicillin
    - Amoxicillin

(Treatment options for M. catarrhalis):

- Amoxicillin-clavulanic acid
- Extended-spectrum cephalosporins
- Azithromycin
- Trimethoprim-sulfamethoxazole