Course Description:

This course will describe the mechanism of infection and pathogenicity of microorganism.

The following subjects will be covered:

bacterial pathogenesis, transmission, symptoms, diagnosis, control, Staphylococci, Streptococci, Enterococci, Corynebacterium, Bacillus, Clostridium. Listeria, Enteroacteriaceae, Pseudomonas, Brucella, Bordetella, and H. Influenza, Legionella, Campylobacter, Helicobacter, Neissria , Bacteriodes , Mycobacterium & branched bacteria, Spirochetes, Rickettsiae, Chlamydiae, and Mycoplasma.

Course Designation PHT 313:

- Course Name: Pharmaceutical Microbiology-II.
- No. of Credits hrs. 3
- Level 5
- Prerequisites :Pharmaceutical Microbiology-I .
- Credit Distribution (2+1).

Topics to be Covered		
List of Tonics ;	No of Weeks	Contact hours
Bacterial pathogenesis.	2	4
Staphylococci.	1	2
Streptococci, Enterococci.	1	2
Corynebacterium, Bacillus.	1	2
Clostridium/Listeria, Enterobacteriaceae:lactose fermetors. (E. coli, Klebsiella)	1	2
Enterobacter, and Citrobacter, Lactose non-fermentors (Proteus , Salmonella).	1	2
Shigella , Yersinia ,Vibrio.	1	2
Campylobacter/Helicobacter, Pseudomonas/ Bacteriodes.	1	2
Brucella, Bordetella, Legionella.	1	2
H. influenza, Neissria.	1	2
Neissria, Mycobacterium & branched bacteria.	1	2
Spirochaetes.	1	2
Rickettsiae, Chlamydiae.	1	2
Mycoplasma, and Antimicrobial Agents.	1	2
Total.	15	30

Tests and Exams:

- **Midterm 1 15**
- **Midterm 2 15**
- **Quizzes 5**
- **Practical 25 (10 final practical; and 15 through the semester).**
- **Final Exam 40**

Safety FIRST

- wearing Lab coat – only in the laboratory.
- No open shoes.
- use Permanent Marker.
- No eating or drinking in the lab.
- Hand washing before and after finishing the lab.

Request: Please be on time.

OBJECTIVES:

- Mechanism of infection &Bacterial Pathogenesis.
- Definition of Pathogens.
- Definition of Infection.
- The Infectious Process Stages.
- Host defenses.
- Bacterial Pathogenicity.
- Bacterial Virulence.

Mechanism of infection & Bacterial Pathogenesis:

Pathogen:

- A microorganism capable of causing disease.

Non-pathogen:

- A microorganism that does not cause disease .It may be part of normal flora.

Strict pathogens:

- Are more virulent and can cause diseases in a normal person.

Opportunistic pathogens :

- Are typically members of normal flora and cause diseases when they are introduced into unprotected sites, usually occur in people with underlying conditions.

Infection:

- The invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body.

Infection:

- may cause no symptoms and be subclinical, or it may cause symptoms and be clinically apparent.

Infection :

- may remain localized, or it may spread through the blood or lymphatic vessels to become systemic (body wide).

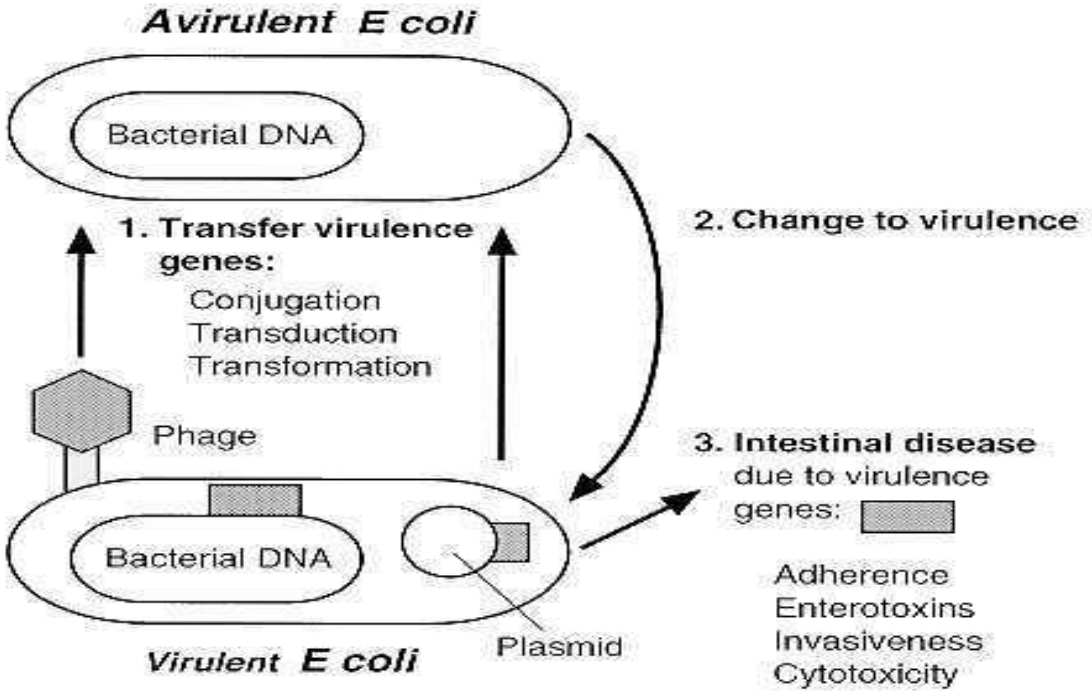
Pathogenicity:

- The ability of an infectious agent to cause disease.

Virulence:

- Relative ability of a microorganism to cause disease.

Mechanisms of Acquiring Bacterial Virulence Genes:



Quantification of virulence:

ID_{50} :

- The amount of organisms required to **cause disease** in fifty percent of those exposed to the pathogen (I=infectious , D=dose).

LD_{50} :

- The amount of organisms required **to kill** fifty percent of test animals (L=lethal).

Characteristic of Pathogenic Bacteria:

1. Transmissibility.
2. Adherence to host cells.
3. Invasion of host cells and tissue.
4. Evasion of the host immune system.
5. Toxigenicity.

Regulation of bacterial virulence factors:

Environmental factors:

- Often control the expression of the virulence genes.

Common factors:

- Temperature, iron availability, osmolality, growth phase, pH, specific ions, specific nutrient factors, bacterial cell-density, interaction with host cells.

The Infectious Process Stages:

- Entry into the host with evasion of the host primary defenses.
- Adhesion of the microorganism to host cells.
- Propagation of the organism.
- Damage to host cells by toxins or an inflammatory response.
- Evasion of host secondary defenses.

Entry into the human body:

The most frequent portals of entry:

1. Respiratory tract.
2. Gastrointestinal tract.
3. Urogenital tract.
4. Skin :cut ,punctured or burned.

Host defenses:

- Phagocytosis by phagocytic cell such as neutrophils and monocytes.
- Acidic environments of the stomach and urogenital tract.
- Hydrolytic and proteolytic enzymes found in the saliva , stomach and small intestine.

How bacteria causing diseases?

Bacterium may cause diseases by:

1. Destroying tissue (invasiveness).
2. Producing toxins (toxigenicity).
3. Stimulating overwhelming host immune responses.

Adherence to host cells:

Adherence of bacterium to epithelial or endothelial Cells allow them to colonize the tissue.

Adherence of bacteria to the host cell surface using:

1. pili (E.coli) and fimbriae.
2. Adhesion molecules.
3. Hydrophobic cell walls.

- Neisseria gonorrhoeae in which strains lack pili are not pathogenic.

Invasiveness:

Facilitated by several bacterial enzymes and followed by inflammation.

- **Invasins** is a protein in bacterial cell surface induce endocytosis by the host cells.

Damage to host cells:

1-Using host nutrients e.g. iron:

- Pathogen can produce siderophores to compete iron from host proteins (transferrin).

2-Direct damage to colonized area:

- Growth and replication in host cells causes damage.
- Penetration through host cells.
- Lysis of host cells to obtain nutrients.

3-Production of toxins:

- **Toxins** : poisonous substances produced by microbes. Some bacteria cause disease by producing toxins.

Toxins:

- **Exotoxins** :proteins secreted by both gram **negative** and gram **positive** bacteria .
- **Endotoxin:** integral components of **gram negative bacteria** (lipopolysaccharides),often liberated when bacteria lyse.

Exotoxins:

- An exotoxin can cause damage to the host by:
  - **destroying cells. OR**
  - **disrupting normal cellular metabolism**.

- They are highly potent and can cause major damage to the host.
- Exotoxins may be **secreted**, or, similar to endotoxins, may be **released during lysis** of the cell.

Types of Exotoxins:

Type I: cell surface-active:

- Type I toxins bind to a receptor on the cell surface and stimulate intracellular signaling pathways.
- **Superantigens:** produced by several bacteria e.g. *Staphylococcus aureus* & *Streptococcus pyogenes* that cause toxic shock syndrome.
- **Heat-stable enterotoxin:**Some strains of *E. coli* produce heat-stable enterotoxins , which are small peptides that are able to withstand heat treatment at 100 °C.

Type II: membrane damaging toxins:

- **Leukocidin**s :make protein channels in phagocytic leukocytes.
- **Hemolysin**s : make protein channels in the RBCs e.g. (β-hemolysis: streptococcus pyogenes).

Type III: intracellular:

- Type III exotoxins can be classified by their mode of entry into the cell, or by their mechanism once inside.
- Some bacteria **deliver toxins directly** from their cytoplasm to the cytoplasm of the target cell through **a needle-like structure.**
- Another group of intracellular toxins is **the AB toxins**. **The 'B'subunit** (binding) attaches to target regions on cell **membranes**, the **'A'subunit (active**) enters through the **membrane** and possesses enzymatic function that affects internal cellular bio-mechanisms.

Gram negative rods and cocci:

Endotoxin:

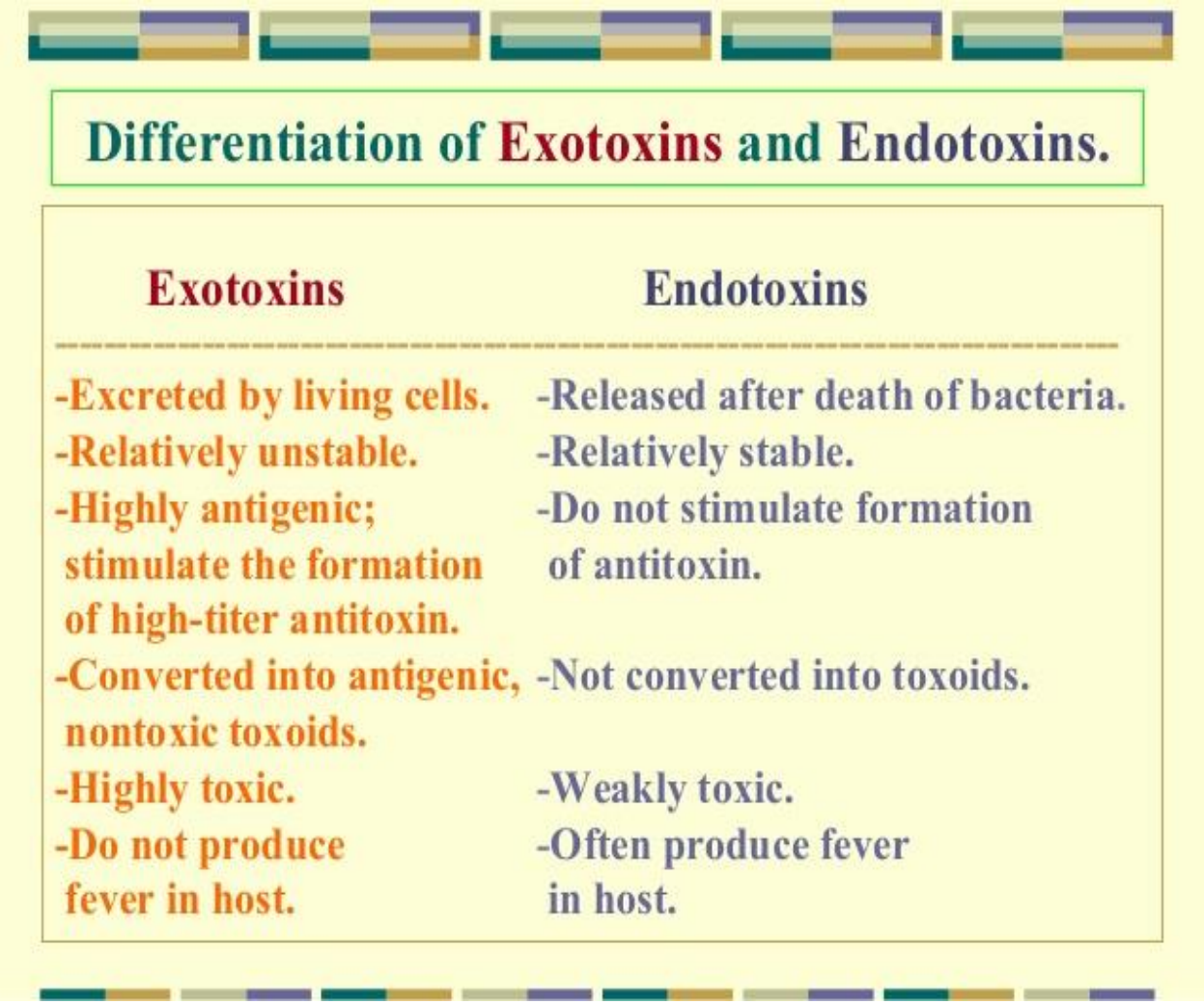
Lipid A, the superantigen part of lipopolysaccharide (LPS) of gram negative outer membrane .

Causes an over stimulation of macrophages with production of various cytokines.

Fever, vasodilation ,inflammation ,shock, and disseminated intravascular coagulation.

While Gram negative pathogens can have other virulence factors (capsules , fimbriae , exotoxins) all have endotoxin and are thus dangerous.

Bacterial toxins:



VIRULENCE FACTORS:

1-Capsules:

- Organized glycocalyx layer (carbohydrates) outside cell wall , Impairs phagocytosis: prevents engulfment and destruction by leukocytes.

2-Biofilm:

- formed on a surface by the bacteria that are bound together within a sticky web of polysaccharide, It can protect the bacteria from host defenses and antibiotics.

3-Cell wall:

- **M protein of *streptococcus pyogenes:***
  -Mediated attachment of bacterium to epithelial cells.
  -Resist phagocytosis by lecukocytes.
- **Mycolic acid (waxy) of *Mycobacterium tuberculosis:***
  Resist digestion by phagocytes then *mycobacteria* grows inside phagocytes.

3-Enzymes:

Coagulase:

- Clot fibrin in blood to create protective barrier against host defenses.

Kinases: dissolve clots(fibrinolysis) e.g.

-Streptokinase (*streptococcus pyogenes*)

-Staphylokinase (*staphylococcus aureus*)

Hyaluronidase :

- hydrolyze hyaluronic acid ('gloe' hold together connective tissue **Clostridium species** and epithelium barriers) allowing deeper invasion e.g causing gangrene

Collagenase:

- Breaks down collagen(fibrous part of connective tissue) for invasion into muscles and organs e.g. *Clostridium* species.

IgA proteases:

- Destroy host **IgA** e.g. Nisseria sepecies that infect CNS .

4-Antigenic variation:

Pathogen alters its surface antigens to escape attack by antibodies and immune cells , e.g. Nisseria gonorrhoeae.