MICROBIOLOGY 2nd COURSE
Pathogenesis and Diagnosis

Assoc. Prof. Emrah Şefik Abamor

Pathogenicity

- Microorganisms capable of causing disease are called pathogens.
- Opportunistic (opportunistic) pathogens are often members of the normal body flora. However;

  -Does not cause disease or rarely causes disease in people with normal immunity,

  -Cause serious infections in immunocompromised or immunocompromised individuals

Virulence

- Virulence is a quantitative measure of pathogenicity and expresses the amount of pathogenic microorganisms required to cause disease.
- Virulence is determined by the following criteria:
- **The 50% lethal dose (LD50)** is the number of microorganisms required to kill half of the infected host;
- **The 50% infectious dose (ID50)** indicates the number of microorganisms required for the pathogen to infect half of the host.
- LD50 and ID50 values vary according to the type of pathogen and virulence factors.

Virulence factors of microorganisms:

- Have pilus (to adhere to mucous membranes)
- Produce exotoxins or endotoxins
- Have a capsule (to prevent phagocytosis)
- Surviving against host defenses (even in the most acidic environment eg stomach)

WHY DO PEOPLE GET INFECTIOUS DISEASES?

- Humans get infectious diseases when pathogens are stronger than host defenses.

- As a result of the increase in the amount of pathogens and their products (toxins, etc.) in the infected organism, various clinical symptoms such as fever and inflammation occur in the patient.

- Host defense occurs in two ways;

- Innate immunity

- Acquired immunity

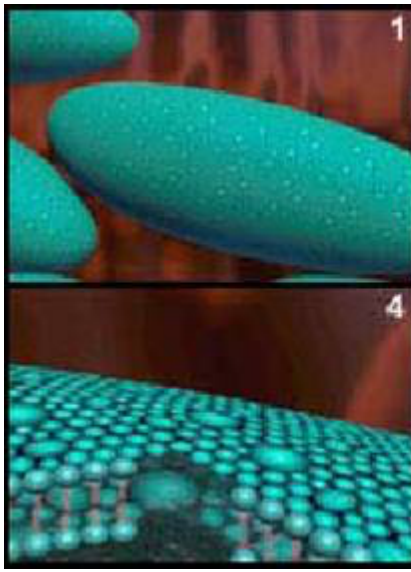
- As a result of the deterioration of any of these immunity, infection may develop.

EPIDEMIA AND PANDEMIC

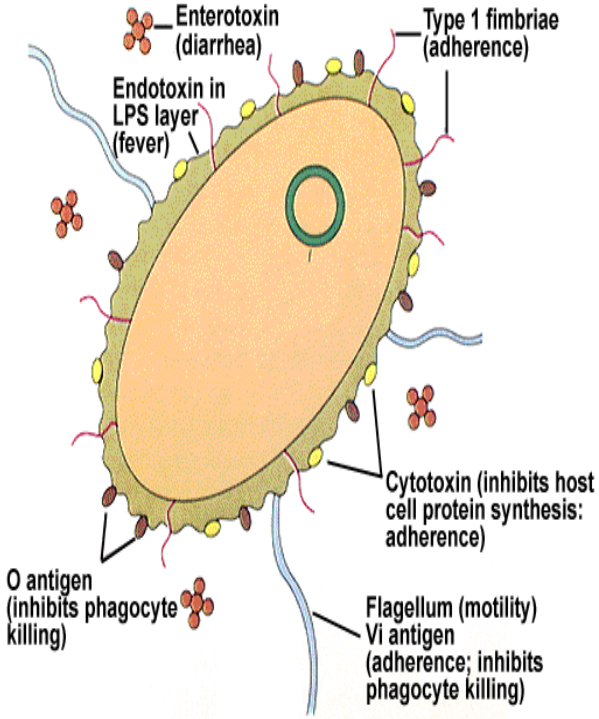
- An epidemic (epidemic) is an infection occurring more often than usual
- Pandemic is the spread of infection all over the world.
- Endemic is the **persistent low incidence** of an infection in a particular community or region.
- Most infections are silent or subclinical.
- In this case, the diagnosis can be made when an increase in antibody titer sometimes occurs.

INFECTION MECHANISM

- Bacteria cause disease by two major mechanisms:
  1. toxin production and
  2. invasion and inflammation.
- Toxins are divided into two general groups as exotoxins and endotoxins.
- While exotoxins are polypeptides released by the cell,
- Endotoxins are lipopolysaccharides that form an integral part of the cell wall.



- Endotoxins occur only in gram-negative bacteria;
- It is not released from the cell; causes fever, shock, and other general symptoms.
- Both exotoxins and endotoxins can themselves cause symptoms without the presence of bacteria in the host.



- Some infections may end in a latent state, with subsequent reactivation of the organism's reproduction and recurrence of symptoms.

- Some other infections can lead to the development of a chronic carrier (portrait) where symptoms do not occur while the organisms continue to reproduce in the host.

- Chronic carriage, for example typhoid fever, is an important source of infection for other people and poses a serious threat to public health.

STAGES OF BACTERIAL PATHOGENESIS

1. Infection from an outside source to the host
2. Bypassing primary host defenses such as skin or stomach acid
3. Adherence to the cell to be infected through bacterial pili
4. Colonize by breeding at the sticking point
5. The appearance of disease symptoms as a result of producing toxins or invading cells
6. Both specific and nonspecific host responses (immune) during steps 3, 4 and 5
7. Disease progression or resolution

1. Contagion

- Although the transmission route of many infectious diseases is "human to human", infectious diseases can also be transmitted from sources such as soil, water and animals.
- Another factor that can cause infectious diseases is inanimate objects such as towels (fomites), which act as a source for microorganisms.

Transmission routes

1. Direct Transmission Route
2. Indirect Transmission Route

1. Direct Transmission Routes

*Direct contact (rabies, scabies, tetanus)

*Sexual contact (Syphilis, gonorrhea)

* Droplet infection (flu, measles, mumps)

2. Indirect Transmission Routes

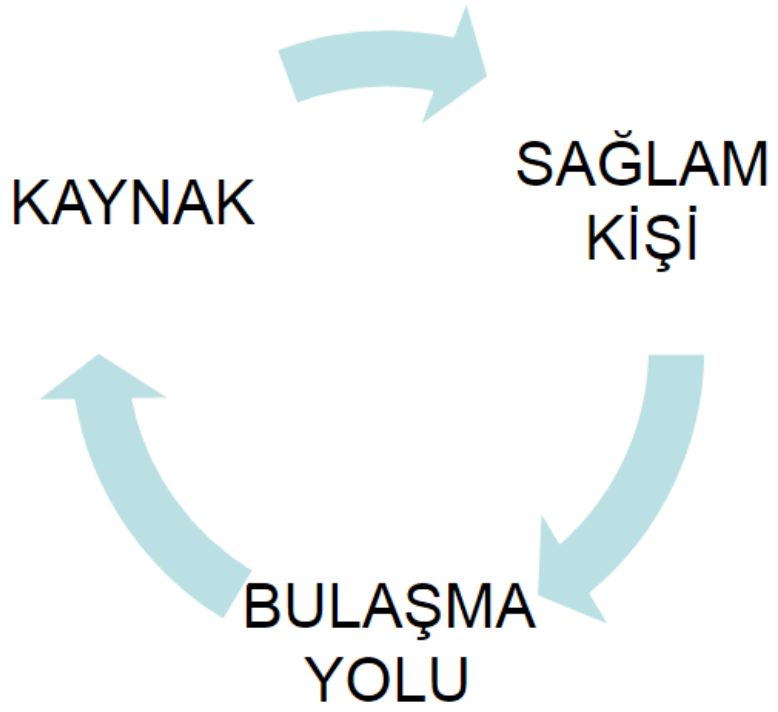
*Fomites (Cholera, measles)

*Vector (plague)

*Air and waterway (measles, typhoid)

ROUTES OF CONTACT

- Pathogens are transmitted from the infected patient to the healthy patient mostly through the respiratory and digestive tract.
- Transmission to the new host is generally by droplet or faecal contamination of food and water.
- Pathogens can also be transmitted through sexual contact, urine, skin contact, blood transfusions, infected needles or insect bites.



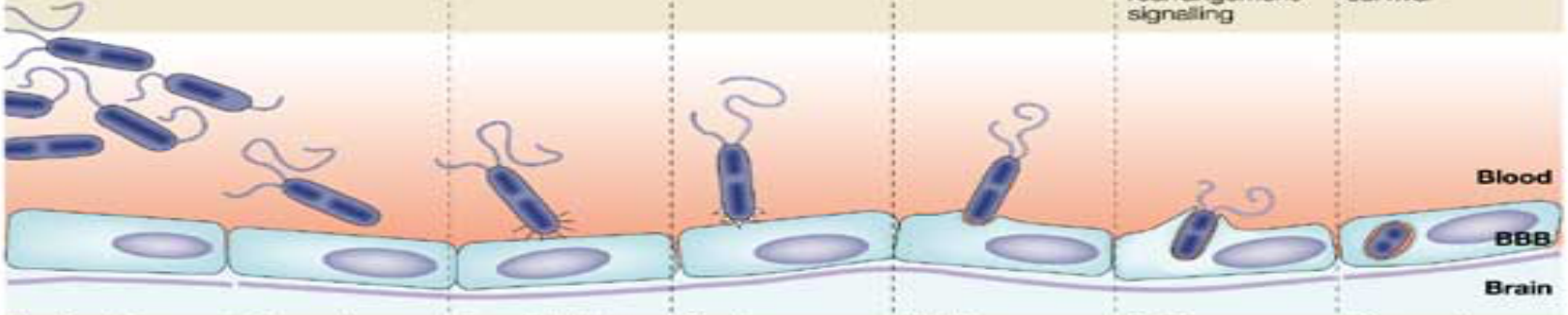
2. Adhesion to Cell Surfaces

- Bacteria have unique structures such as pili that allow them to adhere to the surface of human cells, thereby increasing their ability to cause disease.
- Apart from that, they also produce unique structures such as capsules or glycocalyx to adhere to cells.
- Bacteria lacking these mechanisms are mostly non-pathogenic.

3. Invasion, Inflammation, and Cell Life

- Disease-causing bacteria invade tissue and cause inflammation

- Many enzymes secreted by bacteria play an important role in pathogenesis, especially tissue invasion.



Steps	Bacteraemia	Binding I	Binding II	Invasion	Cytoskeletal rearrangement/ signalling	Intracellular survival
Bacterial determinants	K1 capsule O-LPS	Type 1 fimbriae (FimH) S fimbriae (?)	OmpA	CNF1 Ibe proteins AslA TraJ	CNF1 OmpA Ibe proteins (?)	K1 capsule
Host factors	Serum Neutrophils	CD48 sialoglycoprotein/ glycolipid (?)	gp96	37LRP / 67LR 45-kDa receptor (IbeA)	FAK PI3K Rho cPLA2	Rab7
References	12,26	28,30–32	27,29,38	33–37,39	37,42–44	24,47,48

Enzymes involved in pathogenesis

1. Collagenase and hyaluronidase break down collagen and hyaluronic acid, respectively, and in this way allow the bacteria to spread in the subcutaneous tissue.

2. Coagulase is produced by Staphylococcus aureus and accelerates the formation of a fibrin clot from the fibrinogen precursor (this clot can protect the bacteria from phagocytosis by enveloping organisms with a fibrin layer and sealing the infected area).

3. Immunoglobulin A (IgA) protease breaks down IgA, allowing the organism to adhere to the mucous membranes.

4. Leucocidins can destroy both neutrophilic white blood cells and macrophages.

- In addition to enzymes involved in pathogenesis, many virulence factors (capsule and bacterial cell wall proteins) contribute to the ability to invade by limiting the effective functioning of host defense mechanisms, especially phagocytosis.

Inflammation

- Inflammation, inflammation, inflammation or as it is known among the people, is a series of responses of living tissue to all kinds of living and non-living foreign factors or internal / external tissue damage.

1. regional temperature increase,
2. pain,
3. redness,
4. swelling and
5. It progresses with symptoms such as loss of function.

Survival inside the cell

Bacteria use many different mechanisms to survive and reproduce inside the cell.

These mechanisms

1. Preventing the phagosome from fusing with the lysosome, thereby preventing the organism from encountering the destructive enzymes in the lysosome;
2. Inhibition of acidification of the phagosome and thereby reducing the activity of lysosomal destructive enzymes;
3. Escape from the phagosome into the cytoplasm, in which there are no destructive enzymes.

4. Toxin Production

- The second main mechanism used by bacteria to cause disease is toxin production.

A. Exotoxins

- Unlike endotoxins, which are only found in gram-negative bacteria, exotoxins are produced by many gram-positive and gram-negative bacteria.

- The main feature of exotoxins is that although they are secreted by bacteria, endotoxin is a building block of the cell wall.

- Exotoxins are among the most toxic substances known.
- For example, the lethal dose of tetanus toxin in humans has been calculated to be less than 1 mg.

A. Gram-positive bacterial exotoxins

1. Diphtheria toxin produced by Corynebacterium diphtheriae
2. Tetanus toxin produced by Clostridium tetani
3. Botulinum toxin produced by Closiridium botulinum
4. Clostridium difficile toxin
5. Clostridium perfringens gas gangrene toxin
6. Anthrax toxin produced by Bacillus anthracis
7. Toxic shock syndrome toxin synthesized by Staphylococcus aureus
8. Staphylococcal enterotoxins
9. Exfoliatin
10. Erythrogenic toxin produced by S. pyogenes.

B. Gram-negative bacteria exotoxins

1. Thermolabile enterotoxin produced by E.coli
2. Verotoxin
3. Vibrio cholera enterotoxin (cholera agent)
4. Bacillus cereus enterotoxin (diarrheal agent)

B. ENDOTOXINS

- Endotoxins form the cell wall structure of gram-negative bacteria (rods and cocci).
- Endotoxins - lipopolysaccharide (LPS)
- It is exotoxins-polypeptide;
- The genes encoding the enzymes that produce the endotoxin are located on the bacterial chromosome.
- Genes encoding exotoxins are located in plasmid or bacteriophage DNA.

Septic Shock

- Microorganisms settled in the body as a result of sepsis (blood poisoning) and toxins formed in the tissues it infects damage the walls of blood vessels and cause leakage. As a result, the blood volume in the veins decreases and the contractile function of the veins is lost. This causes “septic shock” to occur.
- Causes of septic shock:
  - Endotoxins of Gram-negative bacteria
  - Surface molecules of gram-positive bacteria

- Symptoms of septic shock
  - Fever and hypotension
  - Mortality is 30-50%.

Toxic Shock

- **Toxic Shock** Syndrome is a serious infection that is caused by toxins secreted by a bacterium called Staphylococcus Aureus, which can reach life-threatening dimensions.

- Although they are also present in the normal flora, as a result of this overgrowth, these bacteria secrete their toxins into the blood, creating a kind of poisoning.

- The most common symptoms of toxic shock syndrome are:
  - ✓Sudden rising fever
  - ✓Nausea, vomiting
  - ✓Diarrhea
  - ✓Widespread aches in the body
  - ✓Feeling faint

Septic Shock

- In septic shock, bacteria are found in the bloodstream.
- Blood cultures are positive in septic shock.

Toxic Shock

- In toxic shock, the substance found in the blood is the toxin itself.
- Blood cultures are negative in toxic shock.

In addition, even if the bacteria in the blood of the person are killed in septic shock, the shock continues because the toxins previously secreted by the bacteria will continue to stimulate cytokines such as TNF and interleukin-1.

5. Immunopathogenesis

- Immunopathogenesis is the event that the disease occurs as a result of the immune response of this microorganism in the living host organism instead of the pathogenic microorganism.

- For example, in febrile rheumatism, antibodies against the M protein of S. pyogenes are formed and these antibodies cross-react with joint, heart and brain tissue.

- As a result, the characteristic findings of this disease are arthritis and inflammation, resulting in carditis.

The immune system is divided into:

1. **Innate (non-specific) immunity:** while providing general protection against all microorganisms

2. **Acquired (specific) immunity:** provides protection against a particular organism.

Innate defense is divided into three main groups:

- **Physical Barriers:** intact skin and mucous membranes
- **Phagocytic Cells:** neutrophil, macrophage and natural killer cells
- **Proteins:** complement, lysozyme and interferon

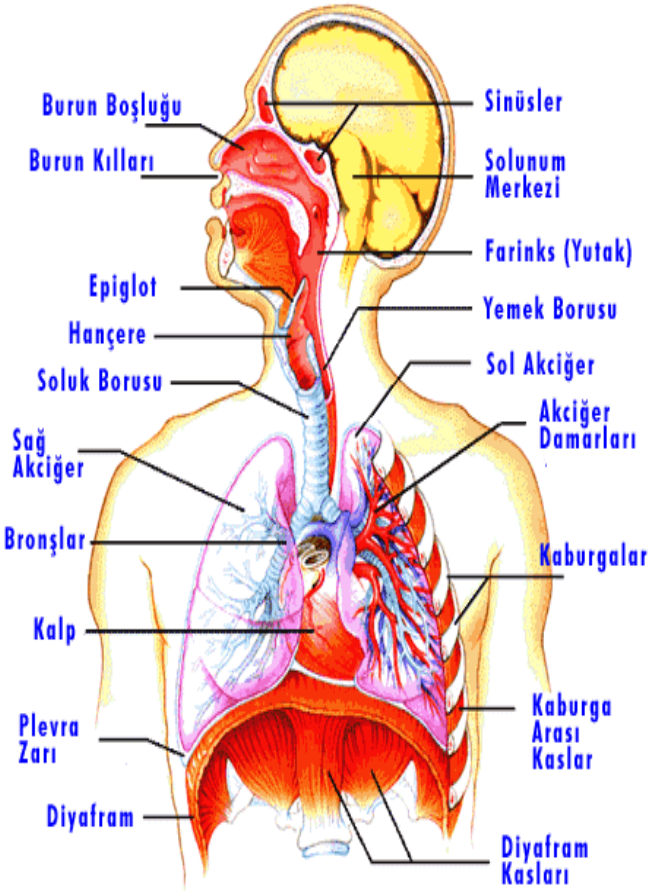
CONGENITAL (NON-SPECIAL) DEFENSE

1. Skin and Mucosa

- The skin layer is the first line of defense against many organisms.
- In addition to the physical barrier it creates, the skin also shows antibacterial and antifungal activity with the **fatty acids** it secretes from the sebaceous glands.
- Due to these fatty acids, the **low pH of the skin (between 3 and 5)** also has an antimicrobial effect.

- A second important defense is the mucous layer of the respiratory tract, which is covered with cilia and mucus.
- The simultaneous movement of the cilia directs the mucus to the nose and throat, and in this way the bacteria trapped in the mucus can be expelled.
- Other protective mechanisms of the respiratory tract:

alveolar macrophages, lysozyme in tears and mucus, nasal hair, and cough reflex that prevents aspiration into the lungs



Non-specific protective mechanisms of the digestive tract:

- **Hydrolitic enzymes in saliva, acid in the stomach and various destructive enzymes and macrophages in the small intestine.**
- In the adult woman, the vagina is protected by the low pH produced by lactobacilli, which are part of the normal flora.

2. Inflammation and Phagocytosis

Pathogenic bacteria cause inflammation in the body.

In the inflamed area

- **blushing,**
- **swelling,**
- **fire and**
- **pain occurs.**

These symptoms are caused by:

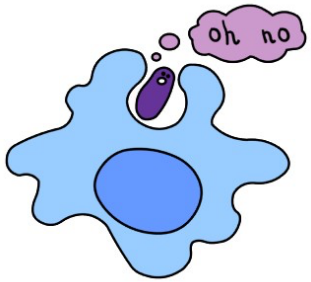
- increased blood flow,
- increased capillary permeability
- is the escape of fluid and cells into interstitial spaces.

Phagocytosis

- **Carried out by macrophages and neutrophils**
- It has 3 steps

1. migration,
2. swallowing
3. kill

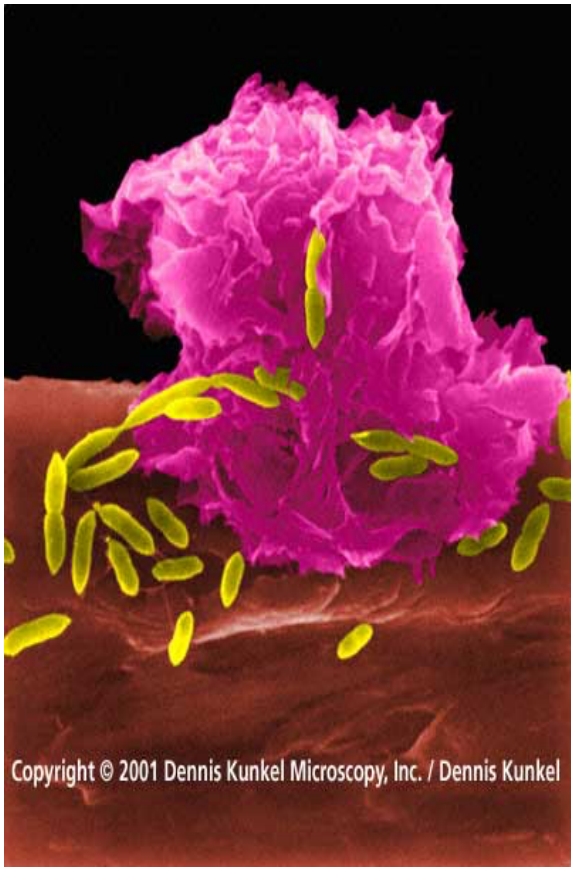
SWALLOWING



- Phagocytic cells form a vacuole (phagosome) in their cell membrane
- Bacteria are engulfed in the vacuole.

- IgG antibodies –
- Binds to bacterial surface
- Increases bacterial engulfment – opsonization

- C3b component of complement - exacerbates opsonization



KILL

- **Respiratory burst - Begins during the act of swallowing**
- Two microbicidal agents are produced

$$O_2 + 1e^- \rightarrow O_2^- \qquad \text{(superoxide radical)}$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad \text{(hydrogen peroxide)}$$

- Hydrogen peroxide is more toxic than superoxide
- However, it is not effective against catalase producing organisms (such as staphylococci).

- **Nitric Oxide (NO)**
- a microbicidal agent - free radical
- oxidatively kills ingested microbes
- Produced in response to endotoxins
- Overproduction of NO,
  - vasodilation in peripheral blood vessels
  - → HYPOTENSION
- NO production stops respiratory burst

- **Degranulation (Phagolysosome)**

Bacteria inside the phagosome are killed by degranulation and hypochlorite ions

- Degranulation-
- With two types of granules in the neutrophil cytoplasm
- **Phagosome fuses, -phagolysosome is formed**
- Granules are lysosomes containing various enzymes required for killing
- Degradation occurs within the phagolysosome.

LYSOSOMES

15% - coarse lysosome particles

- myeloperoxidase
- transports lysozyme and other destructive enzymes

85% - small granules,

- lactoferrin
- proteases,
- nucleases
- Contains additional destructive enzymes such as lipases.

- killing the microorganism
  - oxygen-dependent
  - provided by oxygen-independent mechanisms.

- oxygen-dependent mechanism-
  - by myeloperoxidase
  - hypochlorite ion is produced:

$$Cl^- + H_2O_2 \rightarrow ClO^- + H_2O$$

- hypochlorite
  - destroys cell walls
  - It breaks down cells by reacting with double bonds in fatty acids of membrane lipids

- Oxygen-independent mechanism - important in anaerobic conditions

- Important factors in this mechanism are:

- lactoferrin - chelates with bacterial iron
- lysozyme - breaks down peptidoglycan in the bacterial cell wall
- cationic proteins - destroy bacterial membranes
- low pH

ACQUIRED (SPECIFIC) IMMUNITY

- Acquired immunity,
- Encounter with the microorganism - **effective acquired immunity**,
- Provided by the use of previously formed antibody in another host - **passive acquired immunity**
  - Passage of immunoglobulins from mother to child through the placenta (IgG) or milk (IgA)
  - The use of preformed antibodies can be lifesaving in some diseases, such as botulism and tetanus, which are caused by potent exotoxins.

- Effective acquired immunity,
  - Subclinical infection or
  - Based on encountering a vaccine.
- The advantage of active immunity is the anamnestic (secondary) response - the immune system responds quickly to an antigen it has encountered before.
- Mediators of active immunity
  - **antibodies (immunoglobulins)**
  - **T cells**

- **Functions of antibodies**
  - neutralization of toxins,
  - lysis of bacteria in the presence of complement,
  - opsonization of bacteria
  - inhibition of the adhesion of bacteria and viruses to cell surfaces.
- **Tasks of T cells**
  - cytotoxic destruction of virus-infected cells and bacteria,
  - activation of macrophages
  - mediates reactions such as delayed hypersensitivity.
  - It helps B cells to produce antibodies.

Laboratory Diagnosis

- Methods used in the laboratory diagnosis of infectious diseases

1. Bacteriological method
   - Staining of the microorganism and
   - Culture
2. Immunological (serological) method
   - Detection of antibodies to the microorganism in the patient's serum
3. Molecular methods

Points to be considered in bacteriological methods:

- Taking the appropriate sample
- The sample is quickly sent to the laboratory and stored correctly
- Providing basic information to properly guide laboratory staff

Approaches used in laboratory diagnosis:

STAINING- microscopic examination of the microorganism after staining

CULTURE - obtaining a pure culture of the microorganism by cultivation in a bacteriological medium

TESTS - microorganisms are diagnosed on selective media or using specific antibodies

Serological tests

If the microorganism cannot be cultured, the use of other methods becomes mandatory.

- In this case, serological tests are used.
- In these tests, the presence of antibodies specific to microorganisms is detected.

Culture Method

- The obtained samples are cultured by inoculation on **solid medium (agar).**

  The most commonly used agars (General Media):**Blood Agar**

  - Supports the growth of many bacteria
  - Allows differentiation of microorganism types
  - It is used primarily for diagnosis.

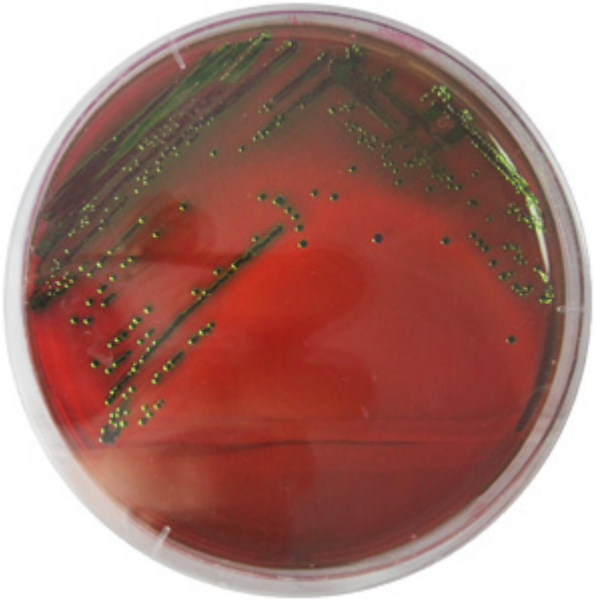
- **Chocolate Agar**

  - Obtained by cooking blood agar
  - Contains inhibitors for bacteria

- Saboraud Agar

  - Used in the culture of all fungi

- **Selective media**
  - They contain substances that selectively allow the growth of certain bacteria. Thus, the growth of undesirable bacteria is prevented.
  - They allow bacteria to be distinguished from others based on certain biochemical reactions.



Example: Mac Conkey agar allows growth of Gram-negative bacteria only

BACTERIOLOGICAL METHODS

- **Blood Culture**
- **Throat Culture**
- **Salivary Culture**
- **Stool Culture**
- **Urine Culture**
- **Reproductive Channel Culture**
- **Wound and Abscess Culture**

Blood Culture

- Blood culture is most often done when sepsis, endocarditis, osteomyelitis, meningitis or pneumonia is suspected.
- Microorganisms most commonly isolated from blood cultures
    - Staphylococcus aureus
    - Streptococcus pneumoniae
    - Escherichia coli,
    - Klebsiella pneumoniae
    - Pseudomonas aeruginosa

- blood cultures
    - Incubated for 7 days or longer
    - turbidity or CO_2 production is inspected daily.
- If reproduction
    - gram stain,
    - passage
    - antibiotic susceptibility tests are performed,
- Cultures should be incubated for 14 days if an infection by slow-growing bacteria such as Brucella is suspected.

Throat Culture

- throat cultures,
  - Angina,
  - diphtheria,
  - gonococcal pharyngitis
  - It is done when diseases such as thrush (Candida) are suspected.
- Swab while sampling
  - to the posterior pharynx
  - to both tonsils
  - the tonsillar fossa should be rubbed.
- The sample on the swab is cultured on blood agar.

Salivary Culture

- sputum cultures
  - pneumonia,
  - tuberculosis
  - It is done when a lung abscess is suspected.
- Most common cause of pneumonia
  - Streptococcus pneumoniae
  - Klebsiella pneumoniae
  - Gram-negative rods such as Pseudomonas aeruginosa.

- The smear made from the sample is stained with gram stain and examined under a microscope.

- Sputum culture on blood agar
  - gives typical colonies
  - typified by various serological or biochemical tests

- If Legionella pneumonia is suspected
  - It is cultured on charcoal-yeast agar containing high concentrations of iron and sulfur.
- If tuberculosis is suspected
  - acid resistant painting is applied
  - sputum culture is incubated for at least 6 weeks
- Anaerobic cultures are important in the diagnosis of aspiration pneumonia and lung abscesses.

CSF (Cerebrospinal Fluid) Culture

- CSF cultures are mainly done when meningitis is suspected.
- In acute meningitis
  - Cultures prepared on blood agar and chocolate agar are incubated at 35°C in an oven containing 5% CO_2.
  - Smears prepared from culture are stained with Gram-stain and examined under a microscope.

- CSF samples taken from encephalitis, brain abscess and subdural hematoma cases generally give negative culture results.

- subacute meningitis
  - *Mycobacterium tuberculosis*
  - Caused by *Cryptococcus neoformans* species

- The two most commonly used tests are
- latex particle agglutination
- counter-immune electrophoresis

Stool Culture

- Stool cultures are mainly used in cases of enterocolitis.
- Stool is examined directly with a microscope
- The stool sample is usually stained with methylene blue.
- The presence of a large number of white blood cells in the post-staining preparation indicates the presence of an invading organism.
- Stool with Gram stain
  - staphylococcus,
  - clostridium
  - The presence of campylobacteria can be demonstrated.
  - However, gram staining is not generally used in stool culture because it also stains the normal flora of the large intestine.

- *For Salmonella and Shigella cultures*
  - MacConkey agar
  - A selective medium such as eosin-methylene blue agar is used.

- *To distinguish Salmonella from Shigella*
  - Using slanted culture on three sugar iron (TSI) agar
  - Agglutination test can be done (cell wall O antigen)
- *Campylobacter jejuni*
  - In an atmosphere of 5% and 10% CO_2
  - At 42 °C
  - It is cultured on an antibiotic-containing medium such as Skirrow agar.

Urine Cultures

- Urine cultures are done when pyelonephritis or cystitis is suspected.
- The most common bacteria in urinary tract infections;
  - E. coli,
  - Enterobacter,
  - proteus
  - It is Enterococcus faecalis.
- Culture done within 1 hour of sampling, or
- It should be stored at 4 °C for not more than 18 hours.

Reproductive Channel Cultures

- Reproductive tract cultures are primarily made from samples taken from people with a sexually transmitted disease.
- One of the most important pathogens of the reproductive tract is *Neisseria gonorrhoeae*.
    - Samples for culture are taken by rubbing with a swab from the urethral canal (male), cervix (female), and anal canal (male and female). Smearing is done from the samples.

Wound and Abscess Cultures

- Brain, lung and abdominal abscesses
  - Bacteroides fragilis
  - aureus
  - S. pyogenes
- In traumatic open wound infections → *Clostridium perfringens*
- In surgical wound infections → It depends on *S.aureus.*

IMMUNOLOGICAL METHODS

Immunological methods using antiserum for the diagnosis of a microorganism

1. Capsule Swelling (Quellung) Reaction
2. Slide Agglutination Test
3. Latex Agglutination Test
4. Counter-Immunoelectrophoresis Test
5. Enzyme-Linked Immunosorbent Assay (ELISA)
6. Fluorescent-Antibody Tests

1. CAPSULE Swelling (QUELLUNG) REACTION)

- In the presence of homologous antiserum, swelling of the capsule is observed under the microscope.

- Many bacteria can be directly recognized in clinical samples by this reaction.

2. SLIDE AGLUTINATION TEST

- Antisera that cause agglutination (clustering) of unknown organism can be used for typing *Salmonella* and *Shigella*.

3. LATEX AGLUTINATION TEST

- Latex beads coated with specific antibody cluster in the presence of homologous bacteria or antigen.
- this test
  - yeast Candida neoformans,
  - many streptococcal species,
  - Neisseria meningitidis and
  - It is used to detect the capsule antigen of Hemophilus influenza.

4.OPPOSITE- IMMUNOELECTROPHORES TEST

In this test

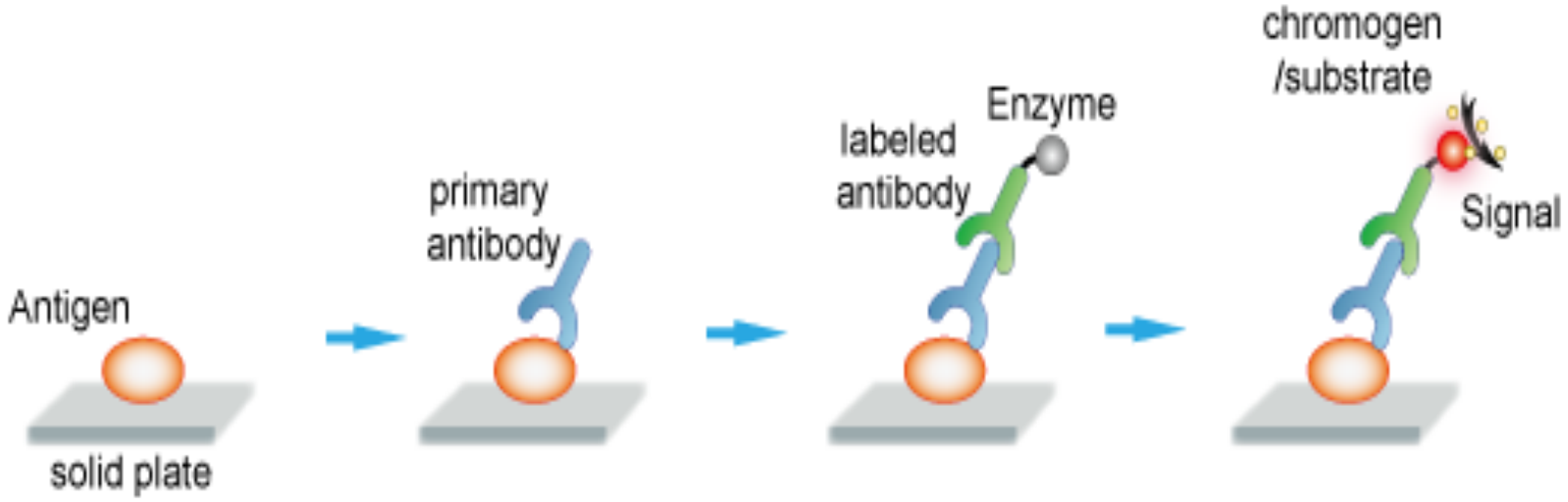
- with unknown bacterial antigen
- a known specific antibody
- are moved towards each other in the electric field.

- If these two are homologous, a precipitate forms on the agar base.
- Only negatively charged antigens can be tested, as antibodies are positively charged at test pH.
- This test is in BOS
  - group B *streptococcus*,
  - S. *pneumoniae*,
  - N. *meningitidis* and
  - It can be used to detect the presence of capsule antigens of H. *influenzae*.

5. ENZYME-LINKED IMMUNOSORBENT TEST

- This test uses a specific antibody to which an enzyme has been attached to detect the presence of the homologous antigen.
- This test is useful in diagnosing a wide variety of bacterial, viral and fungal infections.

Indirect ELISA



1. Antigen is coated onto wells by passive adsroption and incubation.
2. Primary antibody is added and incubated with
3. Anti-species antibody conjugated with enzyme is added and incubated.antigen.
4. Substrate / chromophore is dded and colour develops.

6. FLUORESCENT-ANTIBODY TESTS

- Various bacteria can be typed under a fluorescent microscope by comparing them to an antibody labeled with a fluorescent dye.

- There are various methods that can be used in this field, such as direct and indirect methods.

NUCLEIC ACID BASED METHODS

- There are three types of nucleic acid-based tests used in the diagnosis of bacterial diseases.
  - nucleic acid amplification tests,
  - nucleic acid probes and
  - nucleic acid sequence analysis.
- Nucleic acid-based tests
  - highly specific,
  - quite sensitive
  - It is much faster than the culture method.
- These tests are particularly useful for bacteria that are difficult to culture, such as *Chlamydia* and *Mycobacterium*.

- In nucleic acid amplification tests
  - PCR (polymerase chain reaction) method is used
  - Therefore, the sensitivity of the test is quite high.

- Many bacteria can be identified using these tests

- Tests using nucleic acid probes,
  - Using a labeled DNA or RNA probe
  - Designed for direct detection of bacterial DNA or RNA.

- These tests are less sensitive.

- Nucleic acid sequence analysis is based on the identification of bacteria by determination of the ribosomal RNA base sequence.