ANTIBIOTICS

Antibiotics are agents that are "selectively" toxic for bacteria (either killing them [bactericidal] or inhibiting their growth [bacteriostatic]) without harm to the patient.
They can thus be ingested. By definition, these compounds must act on structures found in bacteria, but not in the host. Antibiotics work most efficiently in conjunction with an active immune system to kill infecting bacteria in the host. After isolation of pure colonies, the susceptibility of bacterial isolates can be tested to a variety of antibiotics. The minimal inhibitory concentration (MIC) refers to the lowest concentration of an antibiotic that stops visible growth. More simply, the zone of inhibition around a disk impregnated with antibiotic (Kirby-Bauer) is another measure of antibiotic activity.

1. INHIBITORS OF CELL ENVELOPE SYNTHESIS

One major class of antibiotics inhibits the synthesis of **peptidoglycan**. Once cell wall synthesis (involving penicillin binding proteins) is inhibited, enzymatic autolysis of the cell wall can occur. Without the restraining influence of the cell wall the high osmotic pressure inside the cell bursts the inner and/or outer membranes of bacteria. Thus, these antibiotics are generally bactericidal.

1.1 PENICILLIN

Penicillin is made by the mold Penicillium chrysogenum. During fermentation the mold forms 6-aminopenicillanic acid which has a thiazolidine ring and a beta-lactam ring fused together.
Various chemical side chains have been **synthetically linked** to the ring structures producing a host of antibiotics with different properties in the host. Many penicillins display little activity against Gram negative bacteria, since they **do not penetrate the outer membrane**.

Cephalosporins and other newer penicillins are active against Gram negative bacteria, since they can penetrate the outer membrane. Other chemically modified penicillins have lower elimination rates from the patient; decreasing the frequency of administration of these drugs.

Penicillins can be destroyed by **beta lactamase** (penicillinase) produced by resistant bacterial strains. **Clavulinic acid** also has a beta lactam component which binds strongly to beta lactamases inhibiting their activity. It is used in conjunction with certain penicillins allowing their use against otherwise resistant bacteria.

1.2 POLYMYXIN B

Polymyxin B binds to the **lipid A** portion of **lipopolysaccharide** and also to phospholipids. However, it binds preferentially to lipid A. This disrupts the **outer membrane** of Gram negative bacteria. Since the cell membrane is not exposed in Gram positive bacteria polymyxin has little activity against them. This drug is toxic to human cells, since it can also lyse eukaryotic membranes; this explains its limited clinical use.

1.3 VANCOMYCIN

Vancomycin is a drug of last resort against Gram-positive bacteria. It is a glycopeptide made by an Acinobacter species. Vancomycin-resistance has arisen making this antibiotic less useful.

It is very hydrophilic and forms hydrogen binds with terminal **D-alanyl-D-alanine** moieties of the **NAM/NAG-subunits** and stops polymerization of the subunits to form long chains, to form finally **peptidoglycan**.

1.4 BACITRACIN

Bacitracin is a cyclic polypeptide produced by Bacillus subtilis var Tracy. Bacitracin inhibits **dephosphorylation** of **C55-isoprenyl pyrophosphate** which transports **peptidoglycan** components bacterial cell walls outside the inner membrane .

2. PROTEIN SYNTHESIS INHIBITORS

The selectivity of these agents is a result of differences in the **prokaryotic 70S ribosome** and the **80S eukaryotic ribosome**. Since mitochondrial ribosomes are similar to prokaryotic ribosomes, these antimetabolites can have some toxicity. They are mostly bacteriostatic.

2.1 ANTIMICROBIALS THAT BIND TO THE 30S RIBOSOMAL SUBUNIT

2.1.1. **Aminoglycosides** (bactericidal)

Streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and neomycin

Mode of action

The aminoglycosides irreversibly bind to the **30S ribosome** and freeze the 30S initiation complex (30S-mRNA-tRNA), so that no further initiation can occur. The aminoglycosides also slow down protein synthesis that has already initiated and induce misreading of the mRNA.

2.1.2 Tetracyclines (bacteriostatic(

Tetracycline, minocycline and doxycycline

a. Mode of action

The tetracyclines reversibly bind to the **30S ribosome** and inhibit binding of aminoacyl-**t-RNA** to the acceptor site on the 70S ribosome.

2.1.3 Spectinomycin (bacteriostatic)

a. Mode of action

Spectinomycin reversibly interferes **with mRNA interaction** with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA

2.2 ANTIMICROBIALS THAT BIND TO THE 50S RIBOSOMAL SUBUNIT

2.2.1 Chloramphenicol, lincomycin, clindamycin (bacteriostatic)

a. Mode of action

These antimicrobials bind to the 50S ribosome and inhibit **peptidyl transferase activity.**

2.2.2 Macrolides (bacteriostatic)

Erythromycin (also azithromycin, and clarithromycin (

a. Mode of action

The macrolides inhibit translocation of the **peptidyl tRNA** from the A to the P site on the ribosome.

2.3 ANTIMICROBIALS THAT INTERFERE WITH ELONGATION FACTORS

Fusidic acid (bacteriostatic)

a. Mode of action

Fusidic acid binds to **protein elongation factor** G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

3. INHIBITORS OF NUCLEIC ACID SYNTHESIS AND FUNCTION

The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

3.1 INHIBITORS OF RNA SYNTHESIS AND FUNCTION

Rifampin, rifamycin, rifampicin (bactericidal (

a. Mode of action

These antimicrobials bind to DNA-dependent **RNA polymerase** and inhibit initiation of RNA synthesis.

3.2 INHIBITORS OF DNA SYNTHESIS AND FUNCTION

Quinolones (nalidixic acid, ciprofloxacin, oxolinic acid) bactericidal

a. Mode of action

These antimicrobials bind to the A subunit of **DNA gyrase** (**topoisomerase**) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

4. ANTIMETABOLITE ANTIMICROBIALS (INHIBITORS OF FOLIC ACID SYNTHESI)

The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their own folic acid. In contrast, mammalian cells use folic acid obtained from food.

4.1 Sulfonamides, sulfones, sulfamethoxsazol (bacteriostatic)

a. Mode of action

These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

4.2 Trimethoprim, methotrexate, pyrimethamine (bacteriostatic)

a. Mode of action

These antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.

ANTIMICROBIAL DRUG RESISTANCE

PRINCIPLES AND DEFINITIONS

Clinical Resistance

Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety in vivo. Resistance to an antimicrobial can arise:

• By mutation in the gene that determines sensitivity/resistance to the agent

• By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene.

Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, i.e. the antibiotic selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

Cross Resistance

Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

MECHANISMS OF RESISTANCE

1- Altered permeability of the antimicrobial agent

Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

2- **Inactivation of the antimicrobial agent**

Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

3- **Altered target site**

Resistance can arise due to alteration of the target site for the antimicrobial agent.

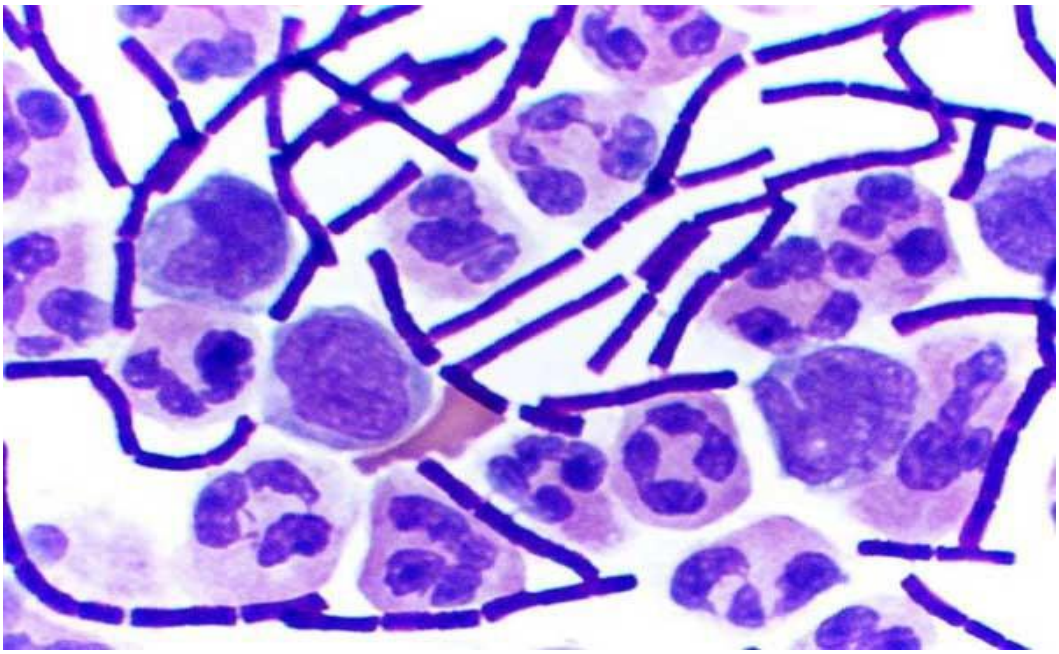
4- **Replacement of a sensitive pathway**

Resistance can result from the acquisition of a new enzyme to replace the sensitive one.

Bacillus - ANTHRAX

Morphology and physiology

Bacillus anthracis is the causative agent of anthrax. It is a Gram-positive, aerobic, spore-forming large bacillus. Spores are formed in culture, in the soil, and in the tissues and exudates of dead animals, but not in the blood or tissues of living animals. Spores remain viable in soil for decades.



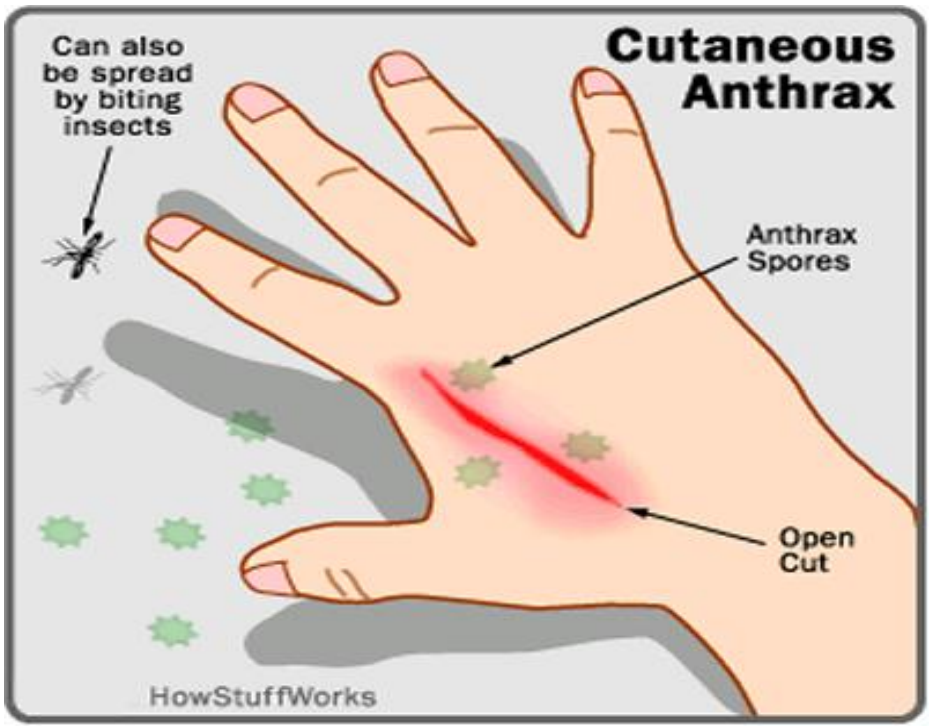
Epidemiology, transmission and symptoms

Anthrax is a major disease threat to herbivorous animals (cattle, sheep, and to a lesser extent horses, hogs, and goats). People become infected by the cutaneous route (direct contact with diseased animals, industrial work with hides, wool, brushes, or bone meal), by inhalation (Woolsorter's disease), or by ingestion (meat from diseased animals). It is not contagious.

Cutaneous anthrax

Accounts for more than 95% of human cases. Spores enter through small break in skin***, germinate into vegetative cells which rapidly proliferate at the portal of entry.*** Within a few days, a small papule emerges that becomes vesicular. The latter is filled with blue-black edema fluid.

Rupture of this lesion will reveal a black eschar at the base surrounded by a zone of induration. This lesion is called a malignant pustule; however, *no pus or pains are manifested*. The lesion is classically found on the hands, forearms or head. The invasion of the bloodstream will lead to systemic dissemination of bacteria.



Pulmonary anthrax

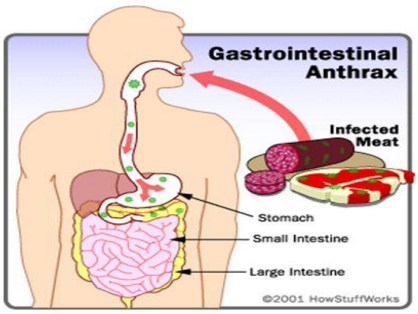
This results from inhalation of B. anthracis spores which are phagocytized by the alveolar macrophages where they germinate and replicate. The injured host cell and organisms infect the hilar lymph node where marked hemorrhagic necrosis may occur. The patient may manifest fever, malaise, myalgia, and a non-productive cough. Once in the hilar lymph node, infection may spread into the blood stream. Respiratory distress and cyanosis are manifestations of toxemia. Death results within 24 hours. This form of anthrax is of significance in biological.

• **Gastrointestinal anthrax**

This results from ingestion of meat-derived from an infected animal and leads to bacterial proliferation within the gastrointestinal tract, invasion of the epithelium and ulceration of the mucosa. The invasion spreads to the mesenteric lymph nodes and then to the bloodstream.

Initially, there is ***vomiting and diarrhea f***ollowed by blood in the feces. Invasion of the bloodstream is associated with profound prostration, shock and death. Because of strict control measures, this form of anthrax is not seen in the U.S. In addition without treatment of gastrointestinal anthrax, the majority of patients die but with antibiotic treatment, 60% or more survive.

Gastrointestinal Anthrax



- GI anthrax may follow after the consumption of contaminated, poorly cooked meat.
- There are 2 different forms of GI anthrax:
  1) Oral-pharyngeal
  2) Abdominal
- Abdominal anthrax is more common than the oral-pharyngeal form.

• Injection anthrax

It is not been seen in North America but has occurred in heroin users in northern Europe. Symptoms are similar to cutaneous anthrax but the infection may be deeper at the site of needle entry. The bacteria can spread more rapidly from site of infection to other parts of the body than is the case with cutaneous anthrax.

• Meningeal Anthrax.

All forms of anthrax above can progress to meningeal encephalitis with deep brain hemorrhagic lesions and infection of the cerebro-spinal fluid. It is almost always fatal.

Diagnosis

Clinical diagnosis of anthrax can be confirmed by direct examination or culture. Fresh smears of vesicular fluid, fluid from under the eschar, blood, or spleen or lymph node aspirates are stained with polychrome methylene blue and examined for the characteristic blunt ended, blue-black rods with a pink capsule. In case of a negative finding, the specimen can be cultured on blood agar plates. Cultured organisms stain as Gram-positive long thin rods.

Prevention and Treatment

Antibiotics

Penicillin and the quinolone, **Ciprofloxacin**, are the antibiotics of choice.

Anti-toxin

Antibody to the toxin complex is neutralizing and protective. This may be used in combination with antibiotics.

There are two vaccines available. One is for use for immunizing cattle and other herbivorous animals and the other for at-risk humans (certain laboratory workers, people who handle animals (veterinarians) and some military personnel.

Clostridium tetani (Tetanus)

These are Gram-positive rods. They are found in the environment (particularly soil) but also intestine of man and animals.

Clostridium tetani, a gram-positive rod that forms a terminal spore, is commonly found in the soil, dust and animal feces. Contamination of wounds, which provide anaerobic conditions, can lead to spore germination and tetanus, a relatively rare (in western countries) but frequently fatal disease.

Death occurs in about 11% of cases with most of these in the more elderly patients (over 60 years of age). Tetanus is also known as **lockjaw** because of the patient's inability to open the mouth as a result of muscle paralysis. The rarity of the disease results from an excellent vaccine and most cases that are now seen in the United States are in adults who never received the vaccine.

Infection usually occurs when spores (in dirt, feces or saliva) enter wounds and scratches where they germinate and produce tetanus toxin. Puncture wounds, such as by a needle or nail, other wounds and scratches and burns can all lead to C. tetani infections. More rarely, surgical procedures and dental extractions can lead to tetanus. Tetanus can also be contracted from the use of intravenous drugs.

Diagnosis

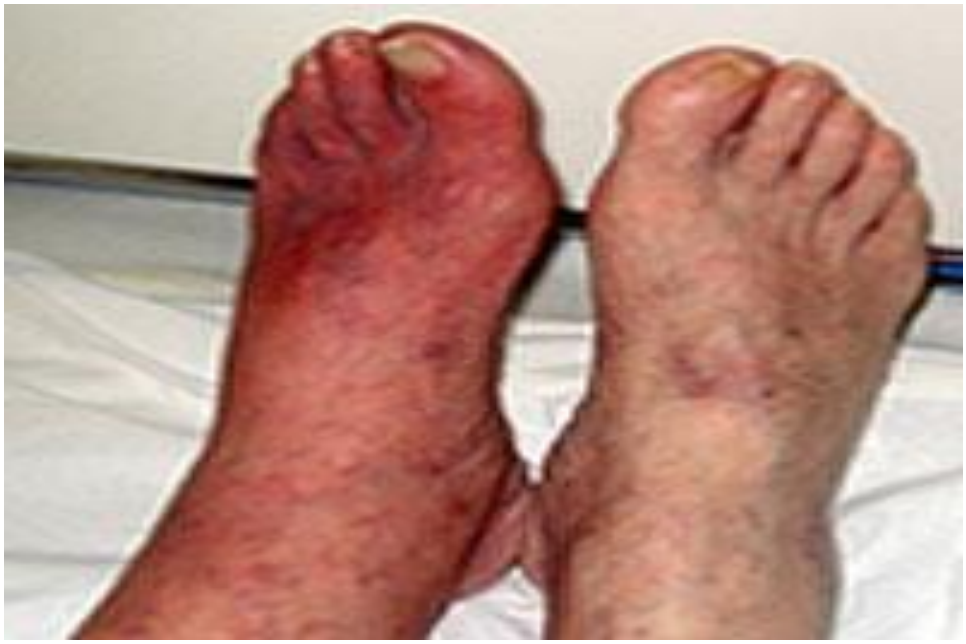
Diagnosis is clinical and bacteria are only derived from wounds in a minority of cases .

Treatment

Tetanus is an emergency situation and requires hospitalization. The patient is immediately treated with human tetanus immune globulin (or equine antitoxin). Drugs can control muscle spasms. The wound requires aggressive washing and treatment with antibiotics.

***Clostridium perfringens* (Gas Gangrene)**

Clostridium perfringens, a gram positive rod, causes wound colonization (gas gangrene) after soil, and to a lesser extent intestinal tract, contamination. It is primarily seen in time of war as a result on non-sterile field hospitals and projectile wounds. The term gas gangrene refers to swelling of tissues due to release of gas, as fermentation products, of clostridia. Progression to toxemia and shock is frequently very rapid.



Gas gangrene can easily be identified by the large, blackened sores and loud and distinctive sound (crepitus) caused by gas escaping from necrotic tissue. It is a moist gangrene, in contrast to dry gangrene which is not caused by a bacterial infection.

Clostridia

Clostridium botulinum (Botulism)

Botulism (a rare but fatal form of food poisoning) is caused by a potent nerve exotoxin (botulinum toxin). It is a serious paralytic illness caused by Clostridium botulinum and, more rarely, by strains of Clostridium butyricum and Clostridium baratii.

Symptoms

After eating contaminated food, the symptoms of botulism occur usually with a day or two but sometimes there may be a period of up to a week

before they appear. Vision and swallowing are affected and the patient may become nauseated and constipated. Muscle paralysis, usually starting at the head and, when the respiratory muscles are affected, death can result.

Treatment

Treatment for adults includes an enema to clear the gastro-intestinal tract of the toxin and injection of anti-toxin (antibodies produced in horses).

Clostridium difficile

C. difficile is frequently a nosocomial infection. The organism, a gram positive rod, can cause a variety of diseases. Those with immunocompromizing conditions and advanced age are also at high risk. However, C. difficile infection is rarely fatal.

Treatment

Therapy includes discontinuation of the implicated antibiotic (e.g. ampicillin). Severe cases require specific antibiotic therapy (e.g. with vancomycin).

INTRODUCTION

Microbiology is the science that studies organisms which ordinarily are too small to be seen without a microscope. These organisms are referred to as microorganisms or microbes. Among these microorganisms are bacteria, protozoa, viruses, yeast, molds, parasitic worms, arthropods, etc. Microorganisms are a heterogeneous group of several distinct classes of living beings. Based on the difference in cellular organization and biochemistry, the kingdom protista has been divided into two groups namely prokaryotes and eukaryotes. Bacteria and blue-green algae are prokaryotes, while fungi, other algae, slime moulds and protozoa are eukaryotes. Bacteria are prokaryotic microorganisms that do not contain chlorophyll. They are unicellular and do not show true branching, except in higher bacteria like actinomycetales. We come in contact with thousands of microorganisms in our everyday life, some are beneficial and a few pathogenic or able to cause disease.

1. Medicine: Infectious diseases are diseases cause by microorganisms. About 12 million people around the world die each year due to Infectious diseases which could be prevented or cured through the use of vaccines or medication (World Health Organization). Approximately half of all deaths caused by infectious diseases each year can be attributed to just three diseases: tuberculosis, malaria, and AIDS. The vast majority are beneficial by providing us with their by-products of metabolism such as antibiotics, etc
2. Research: Microorganisms have been used to study and clarify: Bio-principles , Evolution, Genetics , Cell physiology and Biochemistry
3. Ecology: Recycle nutrients. Some bacteria are able of converting nitrogen into its usable compounds to be used by plants, etc.
4. Industrial applications: • Production of beer, wine, bread. • Genetic recombination and Biotechnology. • New species to produce all kinds of proteins (*pseudomonas*: eat up pollutants) • *Acetobacter* produce cellulose fibers (strong fabric)

II. History of Microbiology:

Some ancient civilizations recognized that certain diseases were communicable (passed along) and they would isolate their sick.The study of microbes began with actual observation.

A. Antonie Van Leeuwenhoek: A linen merchant, Holland, around the 1600's . His hobby was grinding lenses to used in his simple microscopes. He attained magnifications of about 300 X (times) and his lenses were of amazingly good quality. All microscopes were simple (meaning one lens).In 1677. He sent a letter to Scientific Society of London with the **First description** of microorganisms like protozoa, Algae and even Simple bacteria. Meanwhile compound microscopes were being made and refined. **A simple microscope** has one lens **A compound microscope** has at least 2 lenses (one ocular and one objective lens). By 1767 Only 6 species were known, but by 1838, about 600 species were discovered.

III.Medical Microbiology History:

• Now seems simple and obvious that microorganisms cause infectious diseases, but people had no idea about this at that time in history.

• Experiments produced evidence for the Germ theory formulated by the French Microbiologist **Louis Pasteur. "Germ Theory of Disease" infectious diseases are caused by microorganisms , A specific microorganism causes a specific infectious disease.**

However, idea that diseases were caused by specific microbes was advanced mainly by two men:

• Louis Pasteur (1822 - 1895) in France and Robert Koch (1843 - 1910) in Germany.

- **Louis Pasteur:** Germ Theory completed some of the first studies showing that diseases could arise from infection. These studies (with the work of other scientists) became known as the "Germ Theory of Disease".
- **Heinrich Hermann Robert Koch** (1843 – 1910) provided remarkable contributions to the field of microbiology. He was a German general practitioner and a famous microbiologist. He is credited to be one of the founders of the specific field of modern bacteriology.
- As the founder, he identified the specific causative agents of tuberculosis, cholera, and anthrax and gave experimental support for the concept of infectious disease, which included experiments on humans and animals.
- For this he is also regarded as a pioneer of public health, aiding legislation and changing prevailing attitudes about hygiene to prevent the spread of various infectious diseases.
- For his work on tuberculosis, he was awarded the Nobel Prize in 1905 in Physiology or Medicine. Koch and his students isolated the bacteria causing:Tuberculosis – Cholera – Typhoid- Diphtheria – Pneumonia –Meningitis - Gonorrhea and Tetanus

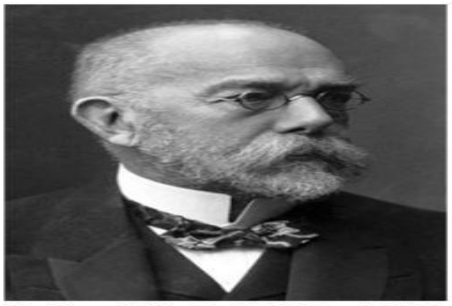
Koch's postulates: a series of proofs that verified the Germ Theory. They are the standard for identifying pathogens.

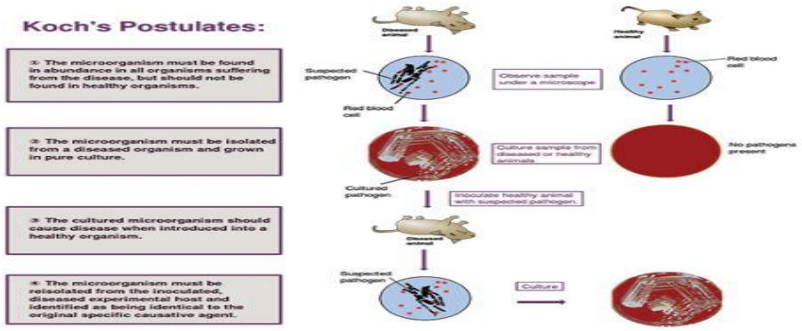
1. Observe similar looking microorganisms in all patients with similar disease (e.g. anthrax = rods in blood of all infected sheep).

2. Isolate the suspected microorganism in artificial media in the laboratory.

3. Inoculate a healthy experimental animal and observe the experimental animal come down or show signs and symptoms of disease.

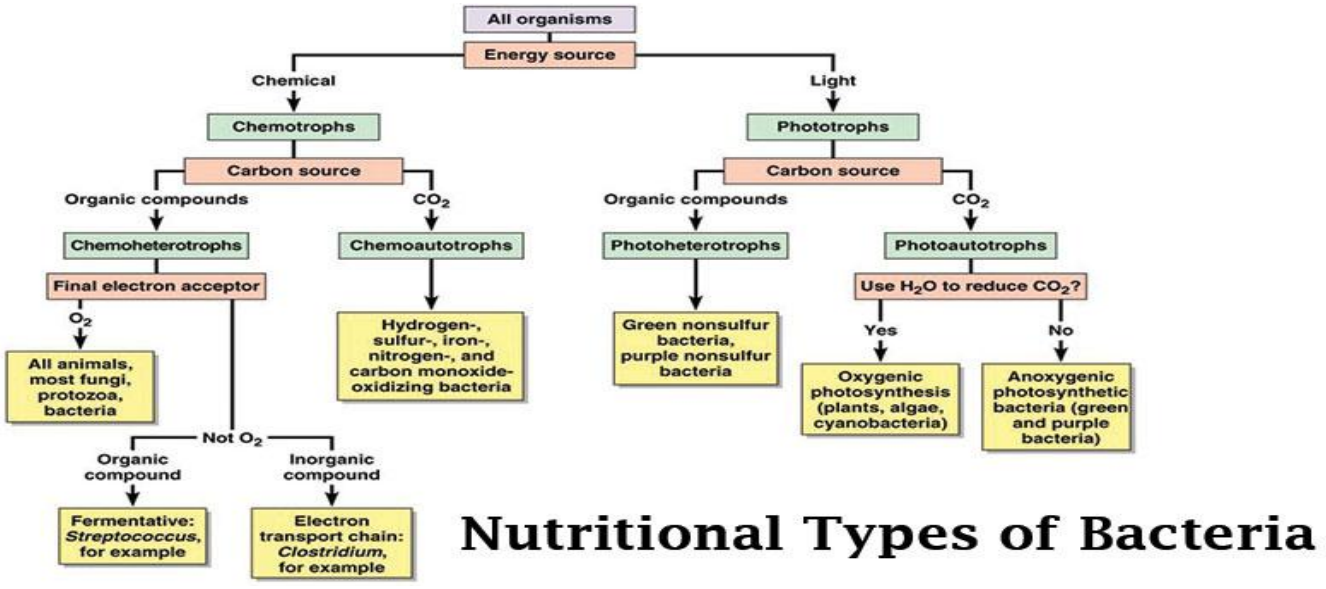
4. Re-isolate the organism from the experimental animal. If all these steps are followed and it confirms, it has been proven that this microorganism is the cause of the disease.





Classification of Bacteria

- Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists. The classification of bacteria serves a variety of different functions. Because of this variety, bacteria may be grouped using many different typing schemes it can be classified according to :-
- ***Classification of Bacteria on the basis of Nutrition***
- Nutrition is substances used in biosynthesis and energy production and therefore are required for all living things.
- Bacteria, like all living cells, require energy and nutrients to build proteins and structural membranes and drive biochemical processes.
- Bacteria require sources of carbon, nitrogen, phosphorous, iron and a large number of other molecules. The nutritional requirements for bacteria can be grouped according to the carbon source and the energy source.
- Some types of bacteria must consume pre-formed organic molecules to obtain energy, while other bacteria can generate their own energy from inorganic sources.



A- On the basis of **energy source** organisms are designated as:

1- **Phototrophs:** The organisms which can utilize light as an energy source are known as phototrophs. These bacteria gain energy from light.
2- **Chemotrophs:** These bacteria gain energy from chemical compounds. They cannot carry out photosynthesis.

B- On the basis of **electron source** organisms are designated as:

1- **Lithotrophs:** Some organisms can use reduced organic compounds as electron donors and are termed as Lithotrophs. They can be Chemolithotrophs and Photolithotrophs
2- **Organotrophs:** Some organisms can use organic compounds as electron donors and are termed as organotrophs. Some can be Chemoorganotrophs and Photoorganotrophs.

Thus, bacteria may be either:

- **Photo-lithotrops:** These bacteria gain energy from light and use reduced inorganic compounds such as H_2S as a source of electrons. eg: *Chromatium okeinii*.
- **Photo-organotrophs:** These bacteria gain energy from light an d use organic compounds such as Succinate as a source of electrons.eg; *Rhodospirillum*.
- **Chemo-lithotrophs:** These bacteria gain energy from reduced inorganic compounds such as NH3 as a source of electron eg; *Nitrosomonas*.
- **Chemo-organotrophs:** These bacteria gain energy from organic compounds such as glucose and ammino acids as a source of electrons.eg; *Pseudomonas pseudoflora*.
- Some bacteria can live ether chemo-lithotrophs or chemo-organotrophs like *Pseudomonas pseudoflora* as they can use either glucose or H2S as electron source.

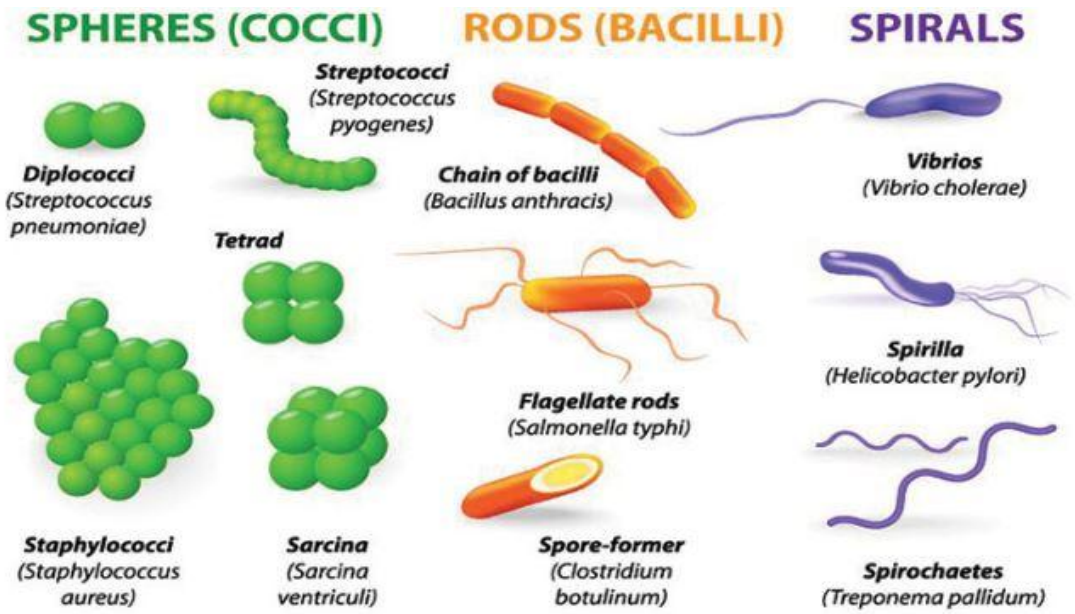
C- **Parasitic bacteria :-** These bacteria obtain their nutrition from the tissues of the hosts on which they grow. They may be harmless or may cause serious diseases. Parasitic bacteria which cause various diseases in plants and animals are known as pathogens, e.g., *Bacillus, B.* anthracis, *B.tetani. B.tuberculosis, B.* pneumoniae, *Vibrio* cholerae, *Pseudomonas citri* etc.

D- Symbiotic bacteria :- Symbiotic bacteria live in close association with other organisms as symbionts. The common examples are the nitrogen-fixing bacteria,*Rhizobium,Clostridium*, *Rhizobiumspp.*, *B.* radicicolaand *B.* azotobacter.

- These bacteria live inside the roots of leguminous plants. These bacteria fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria.

Classification of Bacteria on the Basis of Shape

In the year 1872 scientist Cohn classified bacteria to 4 major types depending on their shapes are as follows:



A) Cocci: These types of bacteria are unicellular, spherical or elliptical shape. Either they may remain as a single cell or may aggregate together for various configurations. They are as follows:

- **Monococcus:**– they are also called micrococcus and represented by single, discrete round Example: *Micrococcus flavus*.
- **Diplococcus:**– the cell of the Diplococcus divides ones in a particular plane and after division, the cells remain attached to each other. Example: *Diplococcus pneumonia*.
- **Streptococcus:** – here form chain of cells. Example: – *Streptococcus pyogenes*.
  - **Tetracoccus:** – this consists of four round cells, which defied in two planes at a right angles to one another. Example: – *Gaffkya tetragena.*
  - ***Staphylococcus***: –forming a structured like bunches of grapes giving and irregular configuration. Example: – *Staphylococcus aureus*.
- **Sarcina:** form a cube like configuration consisting of eight or sixteen cells but they have a regular shape. Example: –*Sarcina lutea*.

B) Bacilli: – These are rod shaped or cylindrical bacteria which either remain singly or in pairs. Example: –*Bacillus cereus*.

C) Vibro: – The vibro are the curved, comma shaped bacteria and represented by a single genus. Example: – *Vibro cholerae*.

D) Spirilla: – These type of bacteria are spiral or spring like with multiple curvature and terminal flagella. Example: –*Spirillum volutans*. ***Other shapes of bacteria :-***

Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions

Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

Classification of Bacteria on the Basis of Temperature Requirement

Bacteria can be classified into the following major types on the basis of their temperatures response as indicated below:

1.Psychrophiles:

- Bacteria that can grow at 0°C or below but the optimum temperature of growth is 15 °C or below and maximum temperature is 20°C are called psychrophiles Psychrophiles have polyunsaturated fatty acids in their cell membrane which gives fluid nature to the cell membrane even at lower temperature such as *Psychroflexus.*

2. **Psychrotrops (facultative psychrophiles):**Those bacteria that can grow even at 0°C but optimum temperature for growth is (20-30)°C
3. **Mesophiles:** Those bacteria that can grow best between (25-40)^{o}C but optimum temperature for growth is 37C Most of the human pathogens are mesophilic in nature. Examples: *Staphylococci.*
4. **Thermophiles:** Those bacteria that can best grow above 45C. Thermophiles capable of growing in mesophilic range are called facultative thermophiles. contains saturated fatty acids in their cell membrane so their cell membrane does not become too fluid even at higher temperature. Examples: *Bacillus stearothermophilus.*

Classification of Bacteria on the Basis of Oxygen Requirement

1- Obligate Aerobes: require oxygen to live. Example: *Pseudomonas*, common nosocomial pathogen.

2- Facultative Anaerobes: Can use oxygen, but can grow in its absence. They have complex set of enzymes. Examples: *E. coli*, *Staphylococcus*, yeasts, and many intestinal bacteria.

3- Obligate Anaerobes: Cannot use oxygen and are harmed by the presence of toxic forms of oxygen. Examples: *Clostridium*

4- Aerotolerant Anaerobes: Cannot use oxygen, but tolerate its presence. Can break down toxic forms of oxygen. Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

5- Microaerophiles: Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen. Example: *Campylobacter*.

Classification of Bacteria on the Basis of pH of Growth

1. **Acidophiles:** These bacteria grow best at an acidic pH. The cytoplasm of these bacteria are acidic in nature. Some acidopiles are thermophilic in nature, such bacteria are called Thermoacidophiles.
2. **Alkaliphiles:** These bacteria grow best at an alkaline pH.
3. **Neutrophiles:** These bacteria grow best at neutral pH (6.5-7.5) Most of the bacteria grow at neutral pH.

Classification of Bacteria on the Basis of Osmotic Pressure Requirement

1- **Halophiles:** Require moderate to large salt concentrations. Cell membrane of halophilic bacteria is made up of glycoprotein with high content of negatively charged glutamic acid and aspartic acids. Such as *Halobacterium.*
2- **Extreme or Obligate Halophiles:** Require a very high salt concentrations (20 to 30%). Bacteria in Dead Sea, brine vats.
3- **Facultative Halophiles:** Do not require high salt concentrations for growth, but tolerate upto 2% salt or more.

Classification of Bacteria on the Basis of Number of Flagella

On the basis of flagella the bacteria can be classified as:

1. **Atrichos: –** These bacteria has no flagella. Example: *Corynebacterium diptherae*.
2. **Monotrichous:** – One flagellum is attached to one end of the bacteria cell. Example: – *Vibro cholerae*.
3. **Lophotrichous: –** Bunch of flagella is attached to one end of the bacteria cell. Example: *Pseudomonas*.
4. **Amphitrichous:** – Bunch of flagella arising from both end of the bacteria cell. Example: *Rhodospirillum rubrum.*
5. **Peritrichous :** – The flagella are evenly distributed surrounding the entire bacterial cell. Example: *Bacillus.*
6.

Classification of Bacteria on the basis of Spore Formation

1. **Spore forming bacteria:** Those bacteria that produce spore during unfavorable condition. These are further divided into two groups:

i) Endospore forming bacteria: Spore is produced within the bacterial cell.
Examples. *Bacillus, Clostridium, Sporosarcina* etc
ii) Exospore forming bacteria: Spore is produced outside the cell.Example. *Methylosinus*

2. **Non sporing bacteria:** Those bacteria which do not produce spores Eg. *E. coli, Salmonella.*

Anatomy of bacteria .

All bacteria, both pathogenic and saprophytic, are unicellular organisms that reproduce by binary fission. Most bacteria are capable of independent metabolic existence and growth, but species of Chlamyd ia and Rickettsia are obligate intracellular organisms. Bacterial cells are extremely small and are most conveniently measured in microns (10 -6 m). They range in size from large c ells such as Bacillus anthracis (1.0 to 1.3 µm X 3 to 10 µm) to very small cells such as Pasteurella tularensis (0.2 X 0.2 to 0.7 µm) , Mycoplasmas (atypical pneumonia group) are even smaller, measuring 0.1 to 0.2 µm in diameter. Bacteria have characterist ic shapes. Bacterial cells of other species grow separately. The microscopic appearance is therefore valuable in classification and diagnosis.

Prokaryotes have a nucleoid (nuclear body) rather than an enveloped nucleus and lack membrane-bound cytoplasmic organelles. The plasma membrane in prokaryotes performs many of the functions carried out by membranous organelles in eukaryotes. Multiplication is by binary fission

Structure of Bacteria:

Beginning from the outermost structure and moving inward, bacteria have some or all of the following structures:

1. The cell wall

As in other organism, the bacterial cell wall provides structural integrity to the cell. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other moleules inside the cell compared to its external environment.

The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycan (poly-N-acetyleglucosamine and N-acetylemuramic acid), which is located immediately outside of the cytoplasmic membrane.

Peptedoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. It is relatively porous and is not considered to be a permeability barrier for some substrates. While all bacterial cell walls (with a few exceptions e.g. extracellular parasites such as *Mycoplasma*) contain peptidoglycan, not all cell walls have the same overall structures.

Since the cell wall is required for bacterial survival, but is absent in some eukaryotes, several antibiotics (notably the penicillins and cephalosporins) stop bacterial infections by interfering with cell wall synthesis, while having no effects on human cells which have no cell wall, only a cell membrane.

There are two main types of bacterial cell walls, those of gram-positive bacteria and those of gram-negative bacteria, which are differentiated by their Gram staining characteristics. For both these types of bacteria, particles of approximately 2 nm can pass through the peptidoglycan.

The Gram Positive Cell Wall

In most of the gram-positive bacteria, the cell wall consist of many layers of peptidoglycan (also known as mucopeptide or **murein**) which forms a thick and rigid structure. The cell wall of the gram-positive also contains teichoic acids which is made up of alcohol (glycerol or orbitol) and phosphate.

Two types of teichoic acids are found in gram-positive bacteria: One is the lipoteichoic acid which span the peptidoglycan layer and is linked to the plasma membrane, and the other is teichoic wall acid, which is connected to the peptidoglycan layer. They have negative charge on them, and thus they can bind and regulate the movement of cations across the cell membrane.

The Gram Negative Cell Wall

The cell wall is made up of few layers of peptidoglycan and an outer membrane. The outer membrane is made up of Lipopolysaccharides (LPS), lipoproteins, and phospholipids.

The peptidoglycan remains bound to lipoproteins of the outer membrane. It is present in the periplasm which is a gel-like fluid between the outer membrane and the plasma membrane. The periplasma is filled with degrading enzymes and proteins aiding in the transportation of the molecules. The cell wall of gram-negative bacteria lacks the teichoic acid.

The cell is more susceptible to mechanical breakage as compared to the gram-positive bacteria as the cell wall has thin layer of peptidoglycan. However, due to the presence of outer membrane made up of lipoproteins and other components, the cell is not easily affected by antibodies, enzymes, metals, detergent, salts (bile salts), dyes, etc.

The outer membrane is permeable due to the presence of porins. The membrane is permeable to food, nutrition, H_2O, vitamin B12, iron, etc.

Gram positive bacteria and gram negative bacteria differ in several ways when looking at the cell wall:

a) Gram +ve bacteria have a thick, multi-layered peptidoglycan layer;
   Gram –ve bacteria have a thin, single layer one.
b) Gram +ve have techoic acids in the cell wall; Gram –ve do not.
c) Gram +ve do not have a periplasmic space; Gram –ve do.
d) Gram +ve bacteria have no outer membrane; Gram –ve have this feature.
e) Lipopolysaccharides are in high concentration only in Gram –ve bacteria.

f) Lipid concentration is higher in Gram –ve than in Gram +ve.

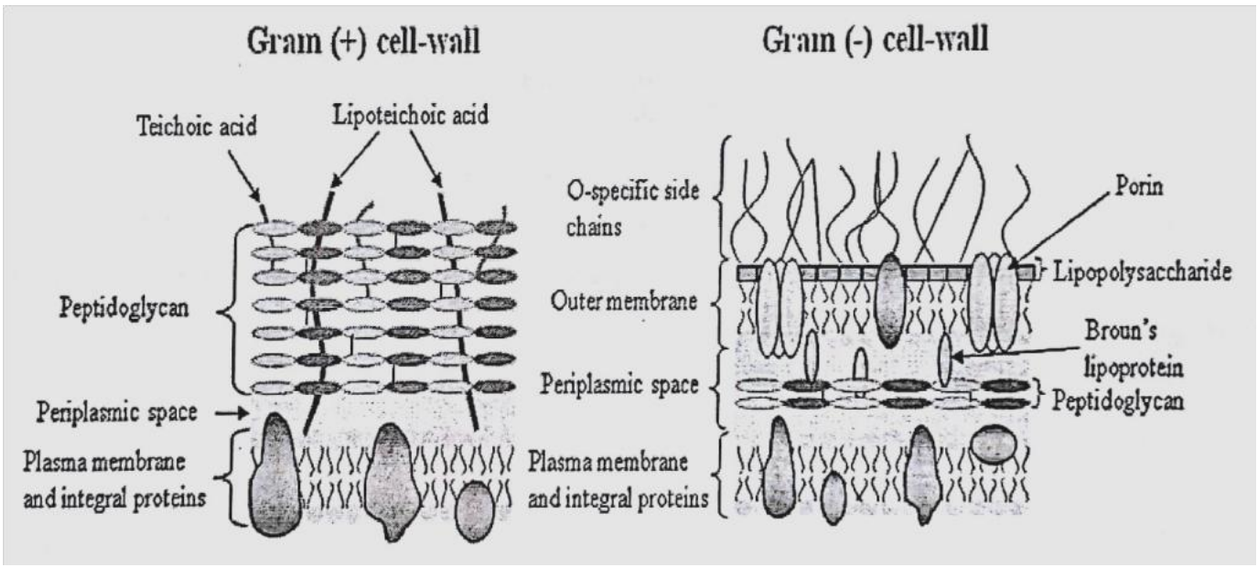
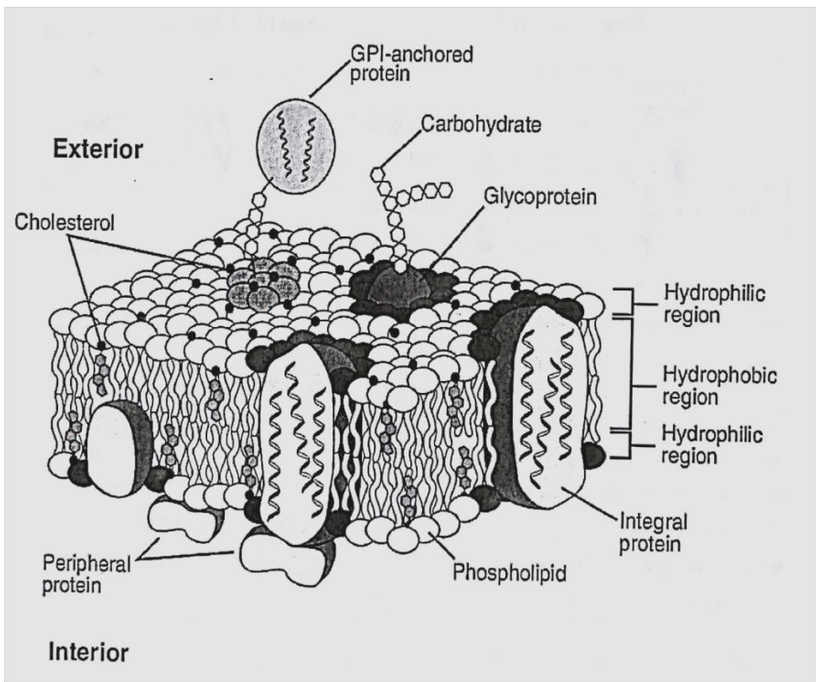


Fig.: Schematic structure of gram-positive and gram-negative cell wall. Gram-positive cell walls contain only one lipid plasma membrane and a thick peptidoglycan layer intrlinked with teichoic and lipotechoic acids, whereas Gram-negative bacteria have an inner and outer cell membrane and only a thin layer of peptidoglycan in the periplasmic space between the inner and outer membrane. There is a layer of lipopolysaccharide lining the outer membrane of Gram-negative.

2. The Bacterial cytoplasmic membrane:

The bacterial cytoplasmic membrane is composed of a phospholipid bilayer and thus has all of the general functions of a cell membrane such as acting as a permeability barrier for most molecules and serving as the location for the transport of molecules into the cell.

However, channel called porins are present in the outer membrane that allow for positive transport of many ions, sugars and amino acids across the outer membrane. These molecules are therefore present in the periplasm, the region between the cytoplasmic and outer membranes. The periplasm contains the peptidoglycan layer and many proteins responsible for substrate binding or hydrolysis and reception of extracellular signals.



3. Nucleoid

Prokaryotic chromosome is located in an irregularly shaped region called the nucleoid, composed of about 60% DNA, 30% RNA, and 10% protein by weight. Prokaryotes contain a single circle of double-stranded deoxyribonucleic acid (DNA), but some have a linear DNA chromosome.

Many bacteria possess plasmids in addition to their chromosome. There are double-stranded DNA molecules, usually circular, that can exist and replicate independently of chromosome or may be integrated with it (episomes). In both cases they are normally inherited or passed on to the progeny. Plasmid genes can render bacteria drug-resistant, give them new metabolic abilities, make them pathogenic, endow them with a number of other properties.

4. Ribosomes:

In most bacteria the most numerous intracellular structure is the ribosome, the site of protein synthesis in all living organisms. All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol.

The 70S ribosome is made up of a 50S and 30S subunits. These rRNA molecules differ in size in eukaryotes and are complexes with a large number of ribosomal proteins, the number and type of which can vary slightly between organisms.

5. Other bacterial surface structures:

A. Capsule:

Some bacteria have capsule. It is a gelatinous layer covering the entire bacterium, may be composed polysaccharide or polypeptide. Encapsulated bacteria grow as smooth colonies, whereas colonies of bacteria that have lost their capsules appear rough. Some bacteria produce slime to help them to stick to surfaces, usually made up from polysaccharides, produced by streptococcus mutants enables stick to the surface of teeth, where helps to form plaque, leading to dental carries.

Capsule importance:

1. Protection against deleterious agents (Lytic enzyme).
2. Contribute to virulence of many bacteria (inhibiting phagocytosis) and it play role in adherence of bacteria to human tissues, helping to prevent the bacterial cell from being killed.
3. Specific identification of microorganisms.

B. Fimbriae and Pili

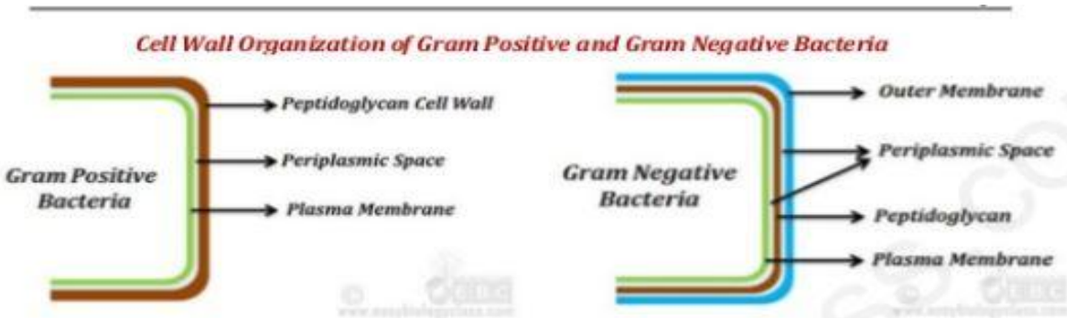
Fimbriae are short, hair-like structures that extend out from the outer membrane in many members of the very common in Gram-negative bacteria, but occur in some Gram-positive bacteria. They are generally ***short in length*** and present in ***high numbers about the entire bacterial cell surface***.

Fimbriae usually function to facilitate the attachment of bacterium to surface (e.g. to form a biofilm) or to other cells (e.g. animal cells during pathogenesis). A few organisms (e.g. *Myxococcus*) use fimbriae for motility to facilitate the assembly of multicellular structures such as fruiting bodies.

Pili are similar in structure to fimbriae but are ***much longer*** and present ***on the bacterial cell in low numbers***, they are composed of protein (pilin). Pili are involved in the process of bacterial conjugation. Non-sex pili also bacteria in gripping surfaces.

C. Flagella

Flagella are whip-like structures protruding from the bacterial cell wall and are responsible for bacterial motility (i.e. movement). The arrangement of flagella about the bacterial cell is unique to the species observed. A flagellum consists of three parts: (1) the long filament, which li es external to the cell surface. (2) the hook structure at the end of the filament. (3) the basal body, to which the hook is anchored and which imparts motion to the flagellum The basal body traverses the outer wall and membrane structures. It consists of a rod and one or two pairs of discs ; the rotation of bacterial flagellum is due to the b asal body. The ability of bacteria to swim flagella provides them with the mechanical means to perform chemotaxis (movement in response to a attachments and repellence substances in the environment). Chemically, flagella are constructed of a class of proteins called flagellins Flagellins are immunogenic and constitute a group of protein antigens called the H antigens, which are characteristic of a given species, strain, or variant of an organism. The species specificity of the flagellins reflects differences in the primary structures of the proteins. Antigenic changes of the flagella known as the phase variation of H1 and H2 occurs in *Salmonella typhimurium*



Similarities between Gram Positive and Gram Negative Bacteria

- Both are bacterial cells
- Both groups are prokaryotic
- Both lack membrane bounded organelles
- Both groups have covalently closed circular DNA as the genetic material
- Both groups contain extra-chromosomal genetic materials (plasmids)
- Both groups poses capsule
- In both groups, cell wall is made up of peptidoglycan
- In both groups, cytoplasm is surrounded by lipid bilayer with many membrane spanning proteins.
- Both gram-positive and gram-negative bacteria commonly have a surface layer called an S-layer
- Both groups of bacteria undergo genetic recombination through transformation, transduction and conjugation
- Both groups undergo binary fission as a mode of asexual reproduction
- Both groups contain many flagellated and non-flagellated species
- Both gram positive and gram negative bacteria are inhibited by antibiotics (their sensitivity varies)
- Both groups includes flagellated (motile) and non-flagellated (non-motile) forms

Difference between Gram Positive Bacteria and Gram Negative Bacteria

Sl. No.	Gram Positive Bacteria	Gram Negative bacteria
1	Appears as dark violet or purple coloured under microscope after Gram staining	Appears as pink or red coloured after Gram staining
		

3	Examples: *Bacillus subtilis, Streptococcus, Lactobacillus, Leuconostoc, Clostridium*	Examples: *Escherichia coli, Rhizobium, Vibrio, Acetobacter*
4	Cell wall single layered, straight and even	Cell wall two layered and uneven (wavy)
5	Cell wall is very rigid and less elastic	Cell wall is less rigid and more elastic
6	The rigidity of cell wall is due to the high proportion of peptidoglycans (80%)	The elasticity of cell wall is due to the less amount of peptidoglycan (2 – 12 %)
7	Thickness of cell wall varies from 15-20 nm, sometimes up to 80 nm	Thickness of cell wall varies from 7.5 to 12 nm
8	Muramic acid content of cell wall is more, 16 – 20% of dry weight	Muramic content of cellwall is less, 2 – 5% of dry weight
9	Cell wall is resistant to alkalis and insoluble in 1% KOH solution	Cell wall is sensitive to alkalis and soluble in 1% KOH solution
10	Cell is highly resistant of physical disruptions	Cell is highly susceptible to physical disruptions
11	S-layer is attached to the peptidoglycan layer	S-layer is attached to the outer membrane
12	Teichoic acid present in the cell wall	Teichoic acid absent in the cell wall
13	Periplasmic space absent	Periplasmic space present
14	Outer membrane absent	Outer membrane present

17	Lipopolysaccharides usually absent	Lipopolysaccharides present
18	Mesosomes are more prominent	Mesosomes are less prominent
19	Produce endospores spores during unfavourable conditions	Usually do not produce endospores
20	Flagella with 2 rings in the basal body	Flagella with four rings in the basal body
21	Usually produce exotoxins	Usually produce endotoxins
22	Gram positive bacteria are more susceptible to antibiotics	Gram negative bacteria are more resistant to antibiotics
23	Can be killed by vancomycin antibiotic	Cannot be killed by vancomycin antibiotic
24	Shows high resistance to sodium azide solution	Shows low resistance to sodium azide solution
25	Cells shows high susceptibility towards penicillins and sulfonamide antibiotics	Cells shows low susceptibility towards penicillins and sulfonamide antibiotics
26	Cells show low susceptibility towards streptomycin, chloramphenicol and tetracyclines	Cells shows high susceptibility towards streptomycin, chloramphenicol and tetracyclines
27	Cell wall is highly susceptible to degradation by lysozyme enzyme	Cell wall is less susceptible to degradation by lysozyme enzyme
28	Shows high tolerance towards dryness	Shows low tolerance towards dryness

Biochemical tests investigate the enzymatic activities of cells are powerful tests in the identification of bacteria. In the research lab a specific series of tests, defined by a specific flow chart, would be performed to aid in the identification of the genus and species of the bacterium. As many as 50 - 100 tests may have to be performed In order to identify the unknown bacteria, examination of seven characteristics of the unknown bacteria is necessary. These seven characteristics are: 1. colony morphology, 2. cell morphology, 3. Gram stain reaction, 4. Oxygen requirements for growth, 5. carbon source utilization, 6. presence of endospores in a culture

7. Motility

- Carbohydrate fermentation test (Sugar fermentation test).

Aim: To determine the ability of microbes to ferment carbohydrates with the production of an acid and/or gas.

Principle: Sugars are metabolized through different metabolic pathways depending on types of microbial species and aerobic or anaerobic environment .If fermenting bacteria are grown in a liquid culture medium containing the carbohydrate, they may produce organic acids as by-products of the fermentation. These acids are released into medium and lower pH of medium. If a pH indicator such as phenol red or bromocresol blue is included in the medium, the acid production will change the medium from its original color to yellow. Gases produced during the fermentation process can be detected by using a small, inverted tube, called a Durham tube, within the liquid culture medium. If gas is produced, the liquid medium inside the Durham tube will be replaced; by the gas in the form of a bubble.

- Indole production test

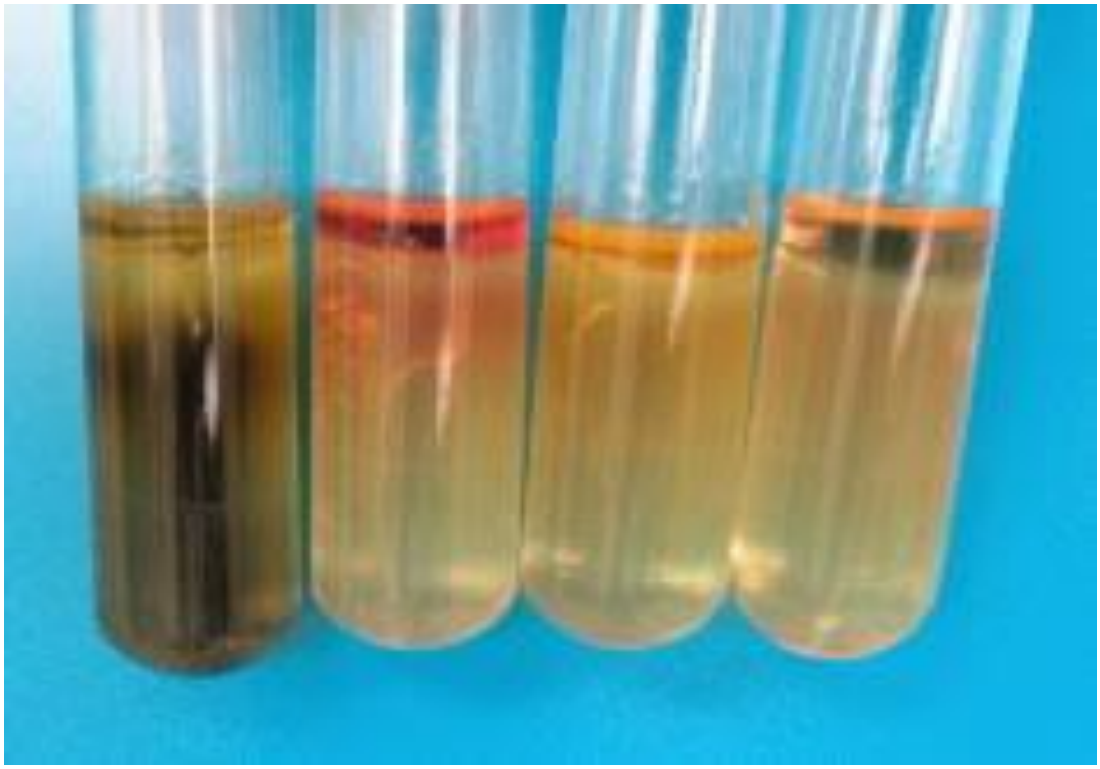
Aim: To determine the ability of microbe to degrade the amino acid tryptophan.

Principle :

Development of cherry red colour at the interface of the reagent and the broth, within seconds after adding the Kovacs' reagent indicates the presence of indole and the test is positive .

- Methyl red test (MR)

Aim: To differentiate E.coli and E.aerogen and to determine the ability of microbes to oxidize glucose with production and stabilization of high content of acid end product.

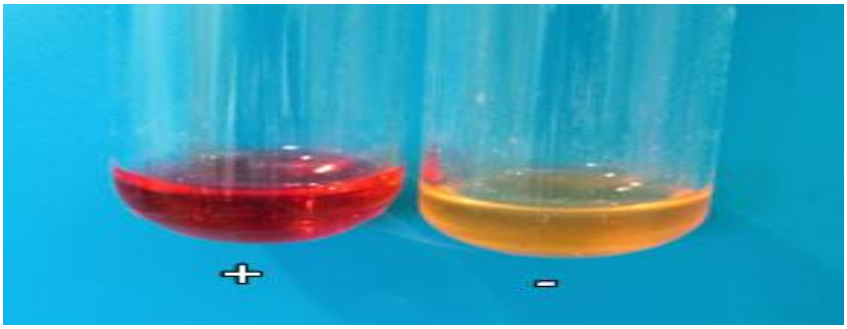


Voges – Proskauer test (VP)

Aim: To differentiate the E.coli and E.aerogen by the production of 2,3 – butanediol and acetoin via glucose fermentation .

Principle :- This test determines the capability of some organisms to produce non-acidic or neutral end products, such as acetyl methyl corbinol (acetoin), from the organic .acid that results from glucose metabolism

Interpretation: Development of crimson red colour indicates positive test for E.aerogen. And no colour change indicates negative test.



- Citrate utilization test

Aim: To determine the ability of the microbes to ferment citrate as sole carbon source.

Principle: Citrate as a sole carbon source for their energy needs, Presence of a citrate permease that facilitates transport of citrate into the bacterium. pH indicator - bromothymol blue. This test is done on slants since O2 is necessary for citrate utilization.

This raises pH, turns the pH indicator to a blue color, and represents a positive citrate test; absence of a color change is a negative citrate test. Citrate-negative cultures will also show no growth in the medium and the medium remains Green

- Nitrate Reduction test

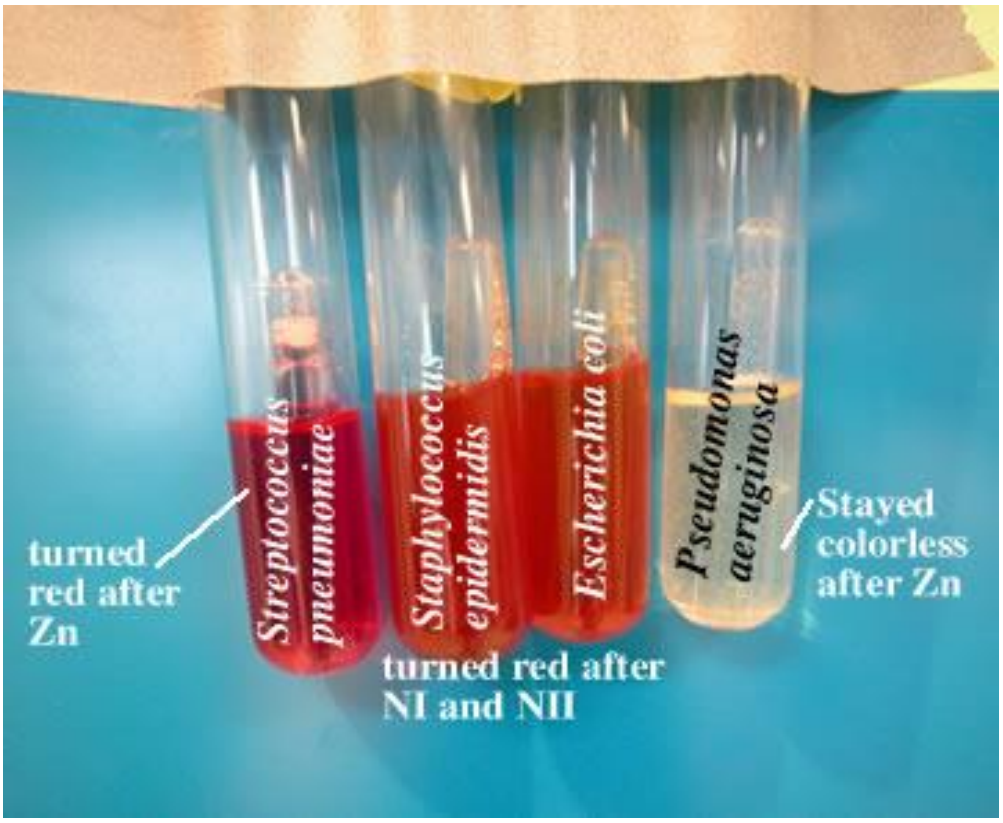
Aim: To determine the ability of some microbes to reduce nitrate (NO3 -) to nitrites (NO2 -) or beyond the nitrite stage.

This test is important in the identification of both Gram-positive and Gram-negativespecies.

After incubation, these tubes are first inspected for the presence of gas in the Durham tube. In the case of no fermenters, this is indicative of reduction of nitrate to nitrogen gas. However, in many cases gas is produced by fermentation and further testing is necessary to determine if reduction of nitrate has occurred. This further testing includes

the addition of sulfanilic acid (often called nitrate I) and dimethyl-alpha-napthalamine (nitrate II). If nitrite is present in the media, then it will react with nitrate I and nitrate II to form a red compound.

elemental zinc is added to the broth. Zinc will convert any remaining NO_3^- to NO_2^- thus allowing nitrate I and nitrate II to react with the NO_2^- and form the red pigment (a verified negative result). If no color change occurs upon addition of zinc then this means that the NO_3^- was converted to NO_2^- and then was converted to some other undetectable form of nitrogen (a positive result).



- Urease test

.Aim: To determine the ability of microbes to degrade urea by urease

Principle: Urea is diamide carbonic acid often referred as carbamide , The hydrolysis of urea is catalysed by specific enzyme urease to yield 2 moles of ammonia. Urease attacks the nitrogen and carbon bond in urea and forms ammonia, Medium containing the pH

indicator phenol red. Splitting of urea creates the alkaline condition which turns phenol red to deep pink in colour. Mainly used for identification of Proteus spp.

TSI(Triple sugar Iron) Agar test Kliger's Iron Agar (KIA)

Aim: To differentiate among and between the members of Enterobacteraceae family.

Principle: It is also used to distinguish the Enterobacteriaceae from other gram negative intestinal bacilli (by their ability to catabolize glucose, lactose, or sucrose, and to liberate sulfides from ferrous ammonium sulfate or sodium thiosulfate.) TSI agar slants contain a 1% concentration of lactose and sucrose, and 0.1% glucose

•The pH indicator, phenol red, is also incorporated into the medium to detect acid production from carbohydrate fermentation. Sodium thiosulfate and ferrous sulfate make H2S indicator system. Thiosulfate is reduced to H2S by several species of bacteria and H2S combines with and form insoluble black precipitates. FeSO4 present in the medium Blackening usually occurs in butt of tube.

- Oxidase tes

Aim: To determine the ability of microbes to produce Oxidase enzyme

Principle: Cytochrome oxidase transfers electrons from the electron transport chain to oxygen (the final electron acceptor) and reduces it to water. In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns a dark purple. This is considered a positive result

- Catalase test

Aim: To determine the ability of an organism to produce catalase.

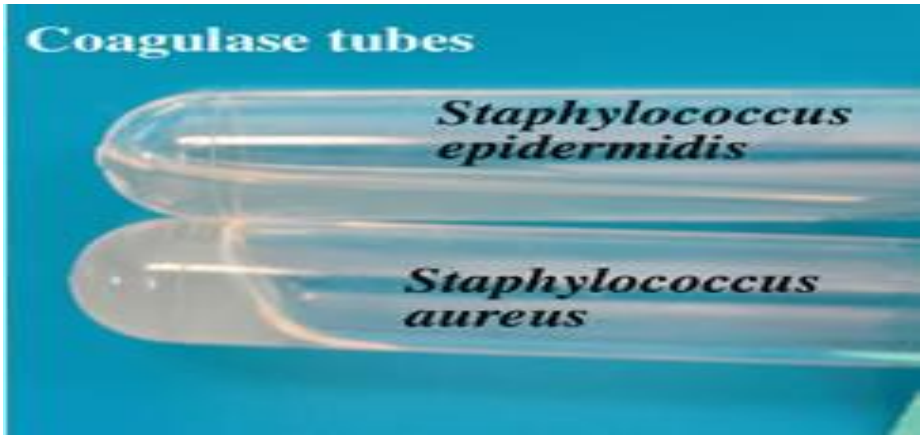
Principle: Certain organisms produce hydrogen peroxide during aerobic respiration and sometimes extremely toxic superoxide radicals. Bubbles of O2 represent a positive catalase test; the absenceof bubble formation is a negative catalase test (Staphylococcus spp.)

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

- Coagulase test

Aims :- Coagulase is an enzyme that clots blood plasma .

Principle :- This test is performed on Gram-positive, catalase positive species to identify the coagulase positive Staphylococcus aureus. Coagulase is a virulence factor of S. aureus. The formation of clot around an infection caused by this bacteria likely protects it from phagocytosis



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- Motility agar

is a differential medium used to determine whether an organism is equipped with flagella and thus capable of swimming away from a stab mark. The results of motility agar are often difficult to interpret. Generally, if the entire tube is turbid, this indicates that the bacteria have moved away from the stab mark (are motile).

BORDETELLA

Bordetella pertussis is the only organism of major clinical significance within this genus; it causes whooping cough in infants and young children. However, a closely related organism, B. parapertussis can also cause a milder form of bronchitis. B. bronchosepticus, another member of the genus Bordetella, is the causative agent of respiratory diseases in cats and swine, but can cause Broncho-pulmonary symptoms in severely immunosuppressed individuals.

Bordetella pertussis

Morphology and physiology

B. pertussis is an extremely small, strictly aerobic, Gram negative, non-motile cocobacillus (short rod). Compared to other *Bortdetella* species, B. pertussis does not grow on common laboratory media and can be distinguished from *B. parapertussis* in that *B. pertussis* is oxidase positive but urease negative, while *B. parapertussis* is oxidase negative and urease positive. *B. bronchosepticus* is positive for both enzymes.

Pathogenesis

The symptoms following the infection are due to many factors. In addition to the attachment to and growth on ciliated cells, the organism produces a number of exotoxins, which contribute to these symptoms. Including Pertussis toxin (pertussigen), Adenylate cyclase toxin, Tracheal cytotoxin, Dermonecrotic (heat-labile) toxin, Filamentous haemagglutinins (agglutinogens) Lipopolysaccharide (LPS).

Diagnosis

Symptoms are characteristic. Laboratory diagnosis is made by obtaining a nasopharyngeal aspirate and primary culture on Bordet-Gengou medium (potato-glycerol-blood agar). Growth is inhibited by peptones, unsaturated fatty acids, sulphides, etc. found in ordinary media. The organism grows as small transparent hemolytic colonies. It can be serologically distinguished from B. parapertussis and B. bronchosepticus.

Prevention and treatment

A killed whole bacterial vaccine is normally administered as DPT combination. An acellular vaccine consisting of filamentous hemagglutinins and detoxified pertussigen is also available and is recommended for booster shots. Erythromycin is the current drug of choice.

HAEMOPHILUS

The genus Haemophilus contains many species but H. influenzae is the most common pathogen. Other species of Haemophilus that are of clinical importance to immuno-competent humans are H. ducreyi (causes chancroid: an STD), H. influenzae aegyptius (associated with conjunctivitis and Brazilian purpuric fever) and H. parainfluenzae (a rare cause of pneumonia and endocarditis). There are several species of Hemophilus that are normal flora, but may be pathogenic in immuno-compromised hosts. The capsulated strain of H. influenzae (type b) is most virulent, although some non-encapsulated (non typable) strains are also pathogenic.

Haemophilus influenzae

Morphology and physiology

H. influenzae is a small Gram negative bacillus which can be grown on chocolate agar (heated blood) and requires hemin (factor X) and nicotinamide adenine dinucleotide (NAD+: factor V) for growth which is enhanced by high CO2 concentration (5%). It does not grow on normal blood agar. The factor V and factor X requirement can be used to distinguish between H. influenzae which requires both, H. parainfluenzae which requires factor V only and H. ducreyi which requires factor X only. H. influenzae are divided into several strains on the basis of capsular polysaccharides (a-f) or the absence of a capsule (non-typable).

Epidemiology and symptoms

H. influenzae causes a variety of clinical symptoms some of which may depend on the presence of the bacterial capsule. Until the availability of the Hib vaccine, the type-b H. influenzae was the main cause of meningitis in children between 6 months and 5 years, although older children, adolescents and adults can also be infected. The infection initially causes a runny nose, low grade fever and headache (1-3 days). Due to its invasive nature the organism enters the circulation and crosses the blood-brain barrier, resulting in a rapidly progressing meningitis (stiff neck), convulsions, coma and death. Timely treatment may prevent coma and death, but the patient may still suffer from deafness and mental retardation. Type-b H. influenzae may also cause septic arthritis conjunctivitis,cellulitis, and epiglottitis, the latter results in the obstruction of the upper airway and suffocation. H. influenzae of other types may rarely cause some of the symptoms listed above. Non-typable strains of H. influenzae are the second commonest cause of otitis media in young children (second to Streptococcus pneumoniae). In adults, these organisms cause pneumonia, particularly in individuals with other underlying

pulmonary infections. These organisms also cause acute or chronic sinusitis in individuals of all ages.

Pathogenesis

The exact mechanism of pathogenesis is not known but the presence of capsule, which is anti-phagocytic, is a major factor in virulence. Type-b H. influenzae are more invasive and pathogenic than other strains. The lipopolysaccharide is responsible for the inflammatory process. The organisms also produce IgA1-specific protease which may aid their mucosal colonization.

Diagnosis

Presumptive diagnosis is based on history, physical examination and symptoms. Blood cultures are positive in more than 50% of symptomatic patients, except those with conjunctivitis. Polyribitol phosphate (PRP), a component of the capsular polysaccharide is present in the serum, cerebrospinal fluid (CSF) and concentrated urine of more than 95% of H. influenzae-b meningitis cases. Gram-negative cocobacilli can be found in the CSF in more than 80% of meningitis cases. Some Gram-stained preparations may be useful in rapid diagnosis of septic arthritis and lower respiratory diseases.

Treatment and prevention

Unless prompt treatment is initiated, H. influenzae-b meningitis and epiglotitis are almost 100% fatal. Due to common resistance to ampicillin and some resistance to chloramphenicol, cephalosporin, which penetrates the blood brain barrier, is the antibiotic of choice in these cases. Other diseases caused by this organism can be treated with ampicillin (if

susceptible) or choice of trimethoprim-sulphamethoxazol, tetracyclin and cefaclor.

Hib-C vaccine which consists of capsular PRP conjugated to tetanus toxoid has been used successfully to provide protection and is a part of the recommended routine vaccination schedule.

Cell Physiology

The increase in the cell size and cell mass during the development of an organism is termed as growth.

The growth of the organisms is affected by both physical and nutritional factors. **The physical factors** include the PH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. **The nutritional factors** include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium.

Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in ***an actively growing phase***.

To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilizing the components of the media and it will increase in its size and cellular mass.

The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

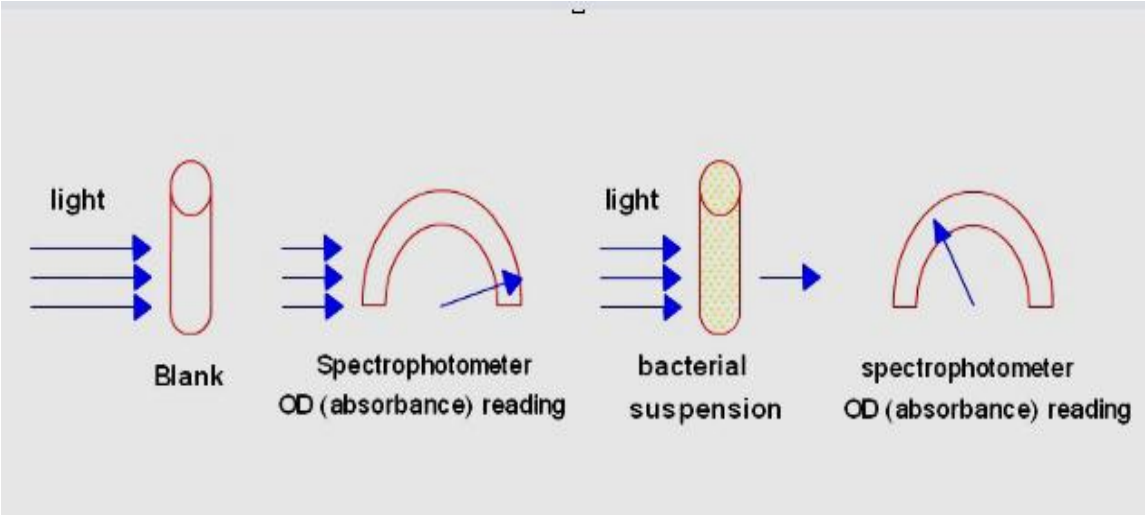


Fig.1: Absorbance reading of bacterial suspension

The biochemical reactions that together enable bacteria to live, grow, and reproduce.
metabolism describes the total chemical reactions that take place in a cell, while physiology describes the role of metabolic reactions in the life processes of a bacterium.

Cell Metabolism

Cell metabolism describes all of the chemical reaction that are happening in the cell. Some reactions, called **anabolic reactions**, create needed products. Other reactions, called **catabolic reactions**, break
down products.

• **Catabolism:** The energy releasing process, in which a chemical or food is used (broken down) by degradation or decomposition, into smaller pieces.

• **Anabolism:** Anabolism is just the opposite of catabolism. In this portion of metabolism, the cell consumes energy to produce larger molecules via smaller ones.

Bacterial Growth Curve:

The growth curve has four distinct phases.

1- Lag phase: The first phase of growth is the **lag phase**, a period of slow growth when the cells are adapting to the high-nutrient environment and preparing for fast growth. The lag phase has high biosynthesis rates, as proteins necessary for rapid growth are produced.

2- The second phase of growth is the **logarithmic phase**, also known as the **exponential phase**. The log phase is marked by rapid exponential growth. The rate at which cells grow during this phase is known as the growth rate (k), and the time it takes the cells to double is known as the generation time (g). During log phase, nutrients are metabolized at maximum speed until one of the nutrients is depleted and starts limiting growth.

3- The third phase of growth is the **stationary phase** and is caused by depleted nutrients. The cells reduce their metabolic activity and consume nonessential cellular proteins. The stationary phase is a transition from rapid growth to a stress response state and there is increased expression of genes involved in DNA repair, antioxidant metabolism and nutrient transport.

4- The final phase is the **death phase** where the bacteria run out of nutrients and die.

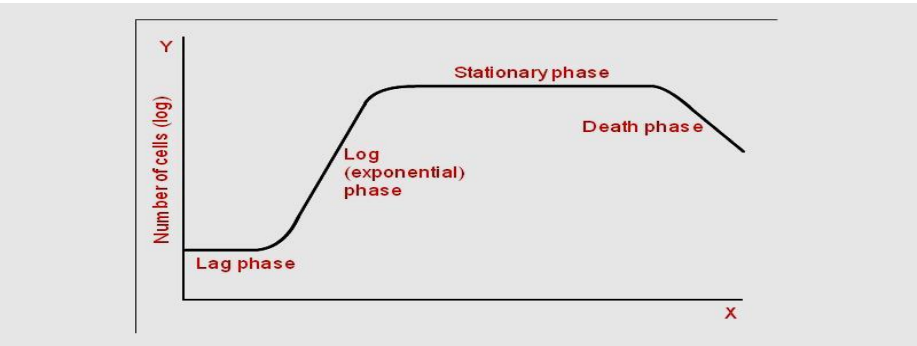


Fig 2: Different phases of growth of a bacteria

The Influence of Some Environmental Factors on Bacterial Growth and Control of Microbial Growth

The growth of microorganisms also is greatly affected by the chemical and physical nature of their surroundings.

The four main requirements for bacterial growth are **nutrition, moisture, warmth and time**.

A\ Nutritional Requirements

1-Basic nutritional requirements for growth :

a- Carbon - building blocks of cell components.
b- Nitrogen - production of proteins, nucleic acids.
c- Hydrogen - occur in organic compounds.
d- Oxygen - involved in the production of energy.
e- Minerals, Trace Elements - required in small amount.

2- Special metabolites (growth factors)

a- Substances required for growth that the cell cannot produce using the basic requirements already listed
(Ex. : vitamins, amino acids, carbohydrates, blood factors)
b- Organisms may be described as being fastidious.

B\ Moisture

Most foods naturally contain sufficient moisture to provide bacteria with the water they need in order to grow. Where moisture has been deliberately removed (e.g. in dehydrated foods such as milk powder, soup mixes, etc.), then bacteria will not grow whilst the food remains dry, but once water is added then bacterial growth may occur once more.

C\ Warmth / Temperature

Bacteria have varying requirements in terms of the range of temperatures in which they will grow. Those which grow at low temperatures (usually below 20°C) are called **psychrophiles** and at high temperatures (above 45°C) are **thermophiles**. Most pathogens, however, like warmth and are known as **mesophiles**. They will grow at temperatures between 20°C and 45°C, and have an optimum temperature for growth of about 37°C.

D\ Time

In ideal conditions (i.e. in moist foods at 37°C) bacteria will grow and multiply by dividing into two every 20 minutes.

Other Factors Affecting Growth

E\ PH Level

The acidity or alkalinity of foods will affect bacterial growth. Most bacteria like neutral conditions (pH value of 7).

F\ Oxygen

Pathogens vary in their oxygen requirements. Those which require oxygen are called **aerobes**, e.g. *Bacillus cereus*. Those which do not need oxygen are called **anaerobes**, e.g. *Clostridium perfringens*. Those which will grow or survive with or without oxygen are known as **facultative anaerobes** and include *Salmonella species* and *Staphylococcus aureus*. **Microaerophilic** - require the presence of small amounts of oxygen (2% - 10%).

G\ Osmotic pressure

a- Exerted by solutes in water
b- Increase o.p. outside cell - water leaves cell (very high o.p. - dehydrates cell)
c- Decreased o.p. outside cell - water enters cell (very low o.p. - lysis of cell)
d- Halophiles - require the presence of 3 % NaCl (extreme halophiles, 20 to 30 % NaCl)

H\ Light (Radiation)

1-Very small group photosynthetic bacteria (cyanobacteria) - require UV light.
2- Nonphotosynthetic bacteria (eubacteria) - UV light is lethal (causes mutations).

****

Sterilization

There are three different processes (cleaning, disinfection, and sterilization) are commonly referred to as “disinfection,” so it would be useful to start by defining our terms.

Cleaning simply reduces the number of contaminants present and, in doing so, removes a proportion of organisms present.

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction on number of contaminating organisms to a level that cannot cause infection. Some organisms and bacterial spore may survive.

Sterilization is the killing or removal of all microorganisms including bacterial spores which are highly resistant. Sterilization is an absolute term, mean the absence of all microorganisms.

Disinfectants are Chemical that are used for disinfection. Disinfectant should be used only on non-living objects.

Antiseptics are mild forms of disinfectants that are used externally on the living tissues to kill microorganisms, on the surface of skin and mucus membranes.

How can microorganisms be killed ?

1. Denaturation of proteins (ex: wet heat , ethylene oxide).

2. Oxidation (ex: dry heat , hydrogen peroxide).
3. Filtration
4. Interruption of DNA synthesis (ex: radiation).
5. Interference with protein synthesis (ex: bleach).
6. Disruption of cell membranes (ex: phenols).

Classification of Sterilization Methods

The sterilization done by:

A- Physical Method: (Heat, Radiation, Filtaration)

1- Heat

a) Sterilization by dry heat

Mechanisms of dry heat:
(1) Protein denaturation .
(2) Oxidative damage .
(3) Toxic effect .

It is unsuitable for clothing which may be spoiled.

1] Flaming and Incineration: The object is passed through flame without allowing it to become red, hot flaming is used for metallic devices like needles, scalpels, scissors, wire loops used in microbiology laboratory are sterilized heating to red in bunsen burner.

2] Alcohol-flamed: Flame and alcohol are used together where the scalpels or tweezers are immersed in alcohol and then passed through the flame. This results in the combustion of alcohol and thus the sterilization process occur.

3] Hot Air Oven: It is one of the most common methods used for sterilization. Glass wares, all glass syringe, petri dishes, pipettes are sterilized in hot air oven. For sterilization, a temperature for 160°C is maintained (holding) for 1 hour. Spore are killed at this temperature. It lead to sterilization.

b) Sterilization by moist heat

Moist heat act by

- denaturation of protein,
- breaking of DNA strands,
- loss of functional integrity of cell membrane.

- **Sterilization at 100°C**

1- Boiling: Boiling at 100°C for 30 minutes is done in water path. Surgical instruments may be sterilized by this method. All bacteria and certain spore are killed.

2- Tyndallization (fractional Sterilization): Heat Labile media like those containing sugar, milk, gelatin can be sterilized by this method. Steaming at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight. This procedure is repeated for another 2 successive days. That is ‘steaming’ is done 3

successive days. Spore, if any, germinate to vegetative bacteria during incubation and are destroyed during steaming on second and third day. It leads to sterilization.

- **Sterilization above 100°C: Autoclaving**
  Autoclaving: is one of the most common methods of sterilization, that done by steam under pressure. The temperature of boiling depends on the surrounding atmospheric pressure. Steaming at temperature higher than 100°C is used by autoclave (autoclaving) . When the autoclave is closed and made air-tight, and water starts boiling, the inside pressures increases and now the water boils above 100°C. at pressure 15 pound per square inch, 121°C temperatures is obtained. This is kept for 15 minutes for sterilization to kill spores.
- **Sterilization below 100°C**

1- **Pasteurization :** Pasteurization is heating of milk (72°C for 15 minutes) or (63°C and 66°C for 30 minutes) so as to kill pathogenic bacteria that may be present in milk without changing color, flavour and nutritive value of the milk.

2- **Inspissation**: is the process used when heating high-protein containing media, and it is done between 75°C to 80°C. It means stiffening of protein without coagulation as the temperature is below coagulation temperature. Media containing serum or egg is sterilized by heating for 3 successive days.

2. Sterilization by Radiation

Two type of radiation are used :

1. Ionizing radiations.
2. Non- ionizing radiations.
   - Type of ionizing radiations: X-ray , Gamma ray , Cosmic ray .
   - Nonionizing radiation: suitable for disinfecting air, transparent fluids and surfaces of objects. Two types of non-ionizing radiation:

Infrared rays : used for rapid mass sterilization of syringes and catheters.

ultraviolet rays: used for disinfecting enclosed areas such as bacterial laboratory, nurseries, cafeterias, inoculation hood, laminar flow and theatres. Damages DNA by producing thymine dimers, which cause mutations.

3.Sterilization by filtration

It is used for sterilize solutions that may be damaged or denatured by high temperatures or chemical agents . The filtering depends on pore size .

This method is commonly :

1. Used for sensitive pharmaceutical and protein solutions in biological research.
2. Useful for substances which get damaged by heat.
3. To sterilize sera, vaccins, sugars, proteins and antibiotic solutions.
4. To obtain bacteria free filtrates of clinical sampels.
5. Purification of water.

A filter with pore size in the range (0.22 ─ 0.45 μm) will effectively remove bacteria, fungi, yeast , while if viruses must also be removed, a much smaller pore size around 20 nm is needed .

B- Chemical Methods:

The chemical materials used to disinfect the rooms and the floors, the degree of the effect of these chemical materials depend on many factors:

1. **Concentration of chemical agent** : Higher concentration bactericidal except alcohol 70 % .
2. **Time** : Longer time of exposure, better killing action.
3. **Temperature** : High temperature speeds up rate of chemical reaction.
4. **Nature of surrounding medium** : pH of medium, presence of extraneous materials like blood or pus.
5. **Nature of organism** : Ability to produce spores , number and size of inoculum.

Chemical material that used in disinfection:

1- **The Halogens:** These are an anti-oxidant materials like chlorine (Cl) also Iodine and it is compounds can be used in the disinfection of wounds.
2- **Heavy metal compounds:** like mercury that used in the form of mercuric chloride HgCl, they act by inactivating cellular protein and it is cytotoxic.
3- **Phenol compounds:** Like Lysol, they act by a number of mechanisms such as disrubtion of cells and inhibit the activity of enzymes and proteins.
4- **Alcohol:** Like Ethanol and it is used at concentration 70%.
5- **Synthetic detergent:** like sodium lauryl sulfate, they help in mechanical removal of microorganism.
6- **Gases:** like formaldehyde with a concentration 80%.

Bacterial Genetics

When any living organism reproduces, it pass on genetic information to its offspring. This information takes the form of genes, linear sequences of DNA that can be thought of as the basic units of heredity. The total complement of an organism's genetic material is called its genome.

Principle of bacterial genetic:

Bacterial genetics deals with the study of heredity and gene variation seen in bacteria. All hereditary characteristic of the bacteria are encoded in their DNA (deoxyribonucleic acid). Bacterial DNA is present in chromosome as well in extra-chromosomal genetic material as plasmid.

Bacterial DNA

Bacteria possess a single haploid chromosome, comprising of super coiled circular double stranded DNA of 1 mm length .The bacterial DNA lacks basic proteins .however, some bacteria have a linear DNA chromosome and some have two chromosomes (e.g.*Vibrio cholerae*). Bacteria do not have a true nucleus; but the genetic material is located in an irregularly shaped region called the nucleoid. There is no nuclear membrane or nucleolus.

DNA replication in bacteria is semi-conservative that mean each strand of DNA is conserved intact during replication and becomes one of the tow strands of the new daughter molecules.

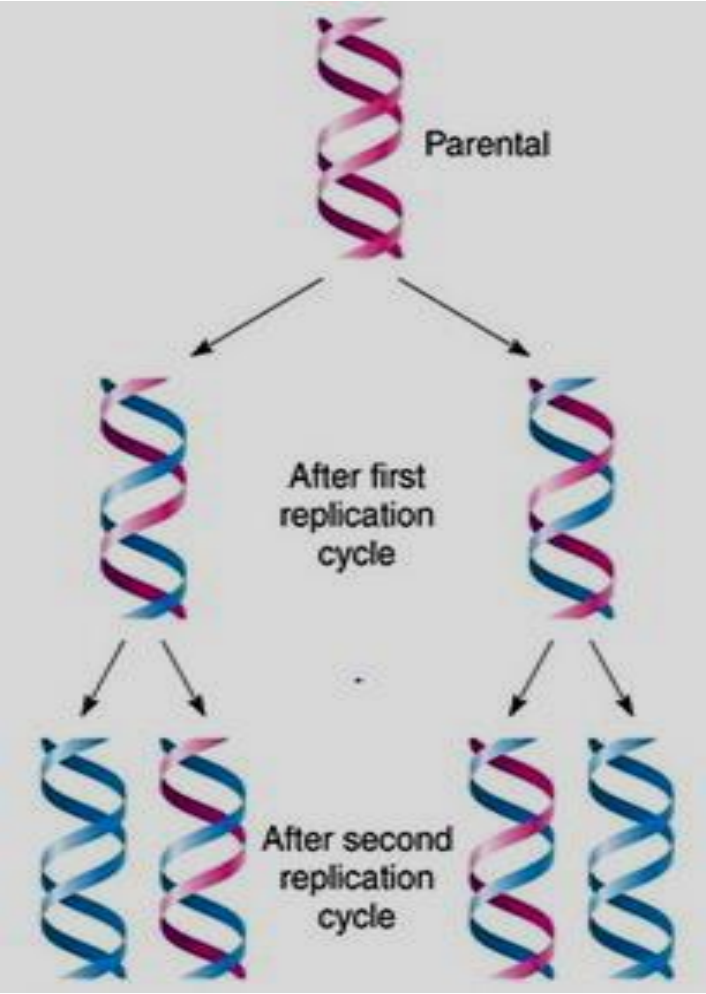


Fig (1): Semiconservative DNA replication

Plasmids

Most cells contain at least one chromosome but some cells also contain an additional DNA element or elements called plasmids.

Plasmids are self-replicating extra chromosomal DNA molecules , Usually they are circular, but some linear plasmids also exist,. They take advantage of the cellular environment of the cell but can also carry a rich diversity of genes which can be beneficial for the cell.

Some plasmids confer the ability to degrade organic compounds and to fix nitrogen. Other plasmids carry antibiotic resistance genes and their spread in pathogenic bacteria is of great medical significance.

Plasmids are used in molecular studies of various organisms and are important in many branches of biology, medicine, ecology and evolution as well as basic research in microbiology, molecular biology and structural biology

Specific Types of Plasmids

There are five main types of plasmids: fertility F-plasmids, resistance plasmids, virulence plasmids, degradative plasmids, and Col plasmids.

Fertility F-plasmids

Fertility plasmids, also known as F-plasmids, contain transfer genes that allow genes to be transferred from one bacteria to another through ***conjugation***. These make up the broad category of conjugative plasmids. F-plasmids are ***episomes***, which are plasmids that can be inserted into chromosomal DNA.

It is found in the bacterium *E. coli*. Bacteria that have the F-plasmid are known as F positive (F+), and bacteria without it are F negative (F–).

Resistance Plasmids

Resistance or R plasmids contain genes that help a bacterial cell defend against environmental factors such as poisons or antibiotics. Some resistance plasmids can transfer themselves through conjugation. When this happens, a strain of bacteria can become resistant to antibiotics.

Virulence Plasmids

When a virulence plasmid is inside a bacterium, it turns that bacterium into a pathogen, which is an agent of disease. Bacteria that cause disease can

be easily spread and replicated among affected individuals. The bacterium *Escherichia coli* has several virulence plasmids.

Degradative Plasmids

Degradative plasmids help the host bacterium to digest compounds that are not commonly found in nature, such as camphor, xylene, toluene, and salicylic acid.

Col Plasmids

Col plasmids contain genes that code for extracellular toxin Bacteriocin (also known as colicin), which are proteins that kill other bacteria and thus defend the host bacterium. Bacteriocins are found in many types of bacteria including *E. coli*, which gets them from the plasmid ColE1.

Genetic variation in bacteria

Genetic variation is a term used to describe the variation in the DNA sequence in each of our genomes. Genetic variation is brought about, fundamentally, by mutation, and Gene transfer.

1.Mutation

A change that occurs in DNA sequence. It could be spontaneous or induced by chemical and physical means mutants are variants in which one or more bases in their DNA are altered ; which are heritable and irreversible.

Types of mutation

1-Substitution: A substitution is a mutation that exchanges one base for another (i.e., a change in a single "chemical letter" such as switching an A to a G). Such as change an amino-acid-coding codon to a single "stop" codon and cause an incomplete protein. This can have serious effects since the incomplete protein probably won't function.

2-Deletion: Deletions are mutations in which part of a chromosome or a sequence of DNA is lost during DNA replication. Any number of nucleotides can be deleted, from a single base to an entire piece of chromosome.

3-Insertion: Mutations in which extra base pairs are inserted into a new place in the DNA. Insertions can be anywhere in size from one base pair incorrectly inserted into a DNA sequence to a section of one chromosome inserted into another.

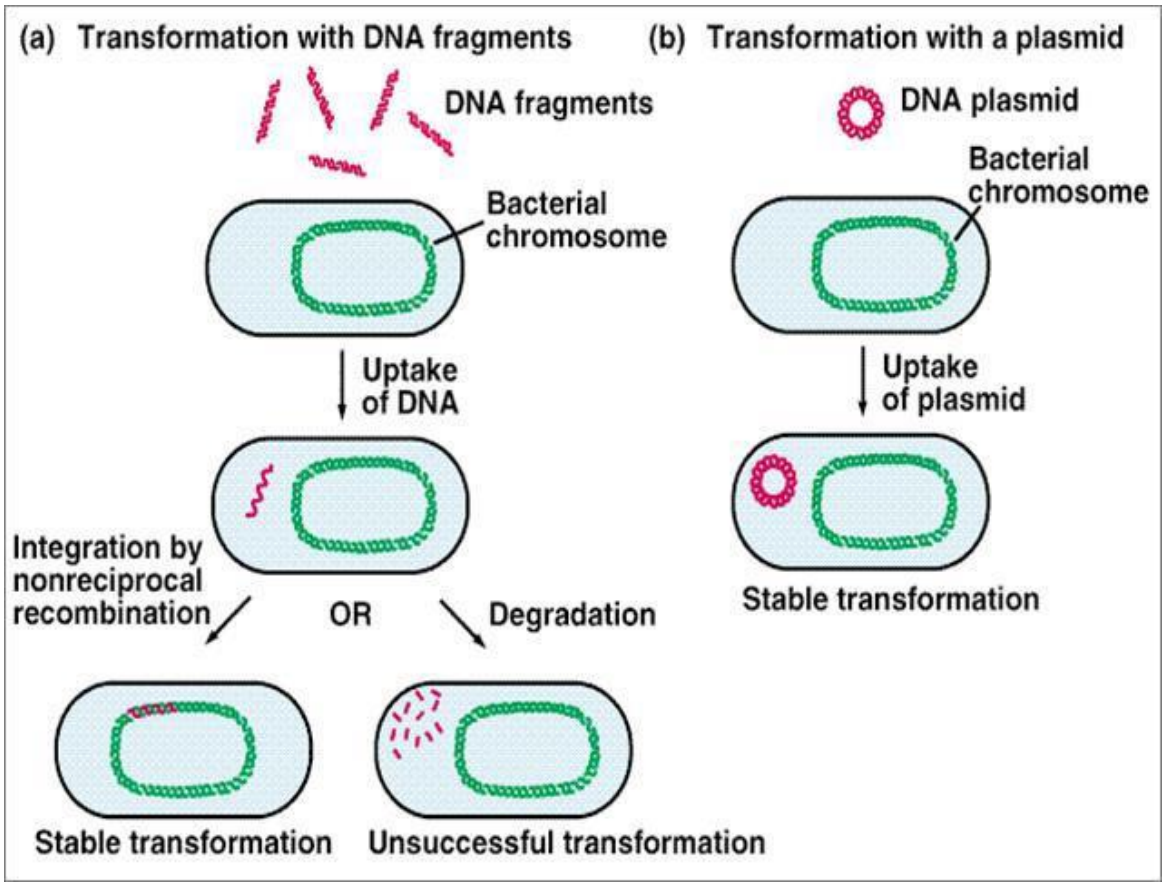
2. Gene transfer

Bacterial variation can also occur by gene transfer of genetic material from one cell to another. Consider two cells from different populations: bacterium B has features distinct from those of bacterium A.

There are three possible mechanisms for transferring a trait from B to A: (1) transformation, (2) transduction and (3) conjugation. For all three process, the transferred DNA must be stably incorporated into the genetic material of the recipient bacterium

Transformation

Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. *Bacillus, Haemophilus, Neisseria, Pneumococcus*) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome and this will lead to change in pathogenicity and antibiotic sensitivity pattern of bacterium



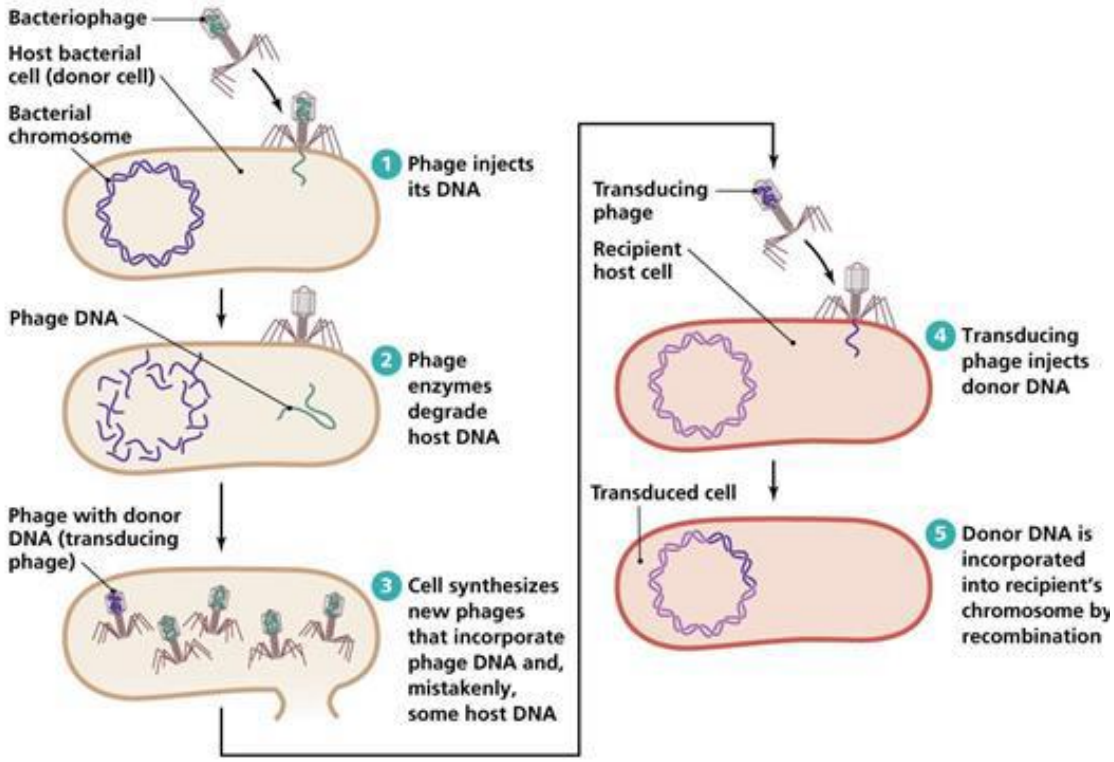
Fig(2):Gene transfer by (Transformation)

Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction.

In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer

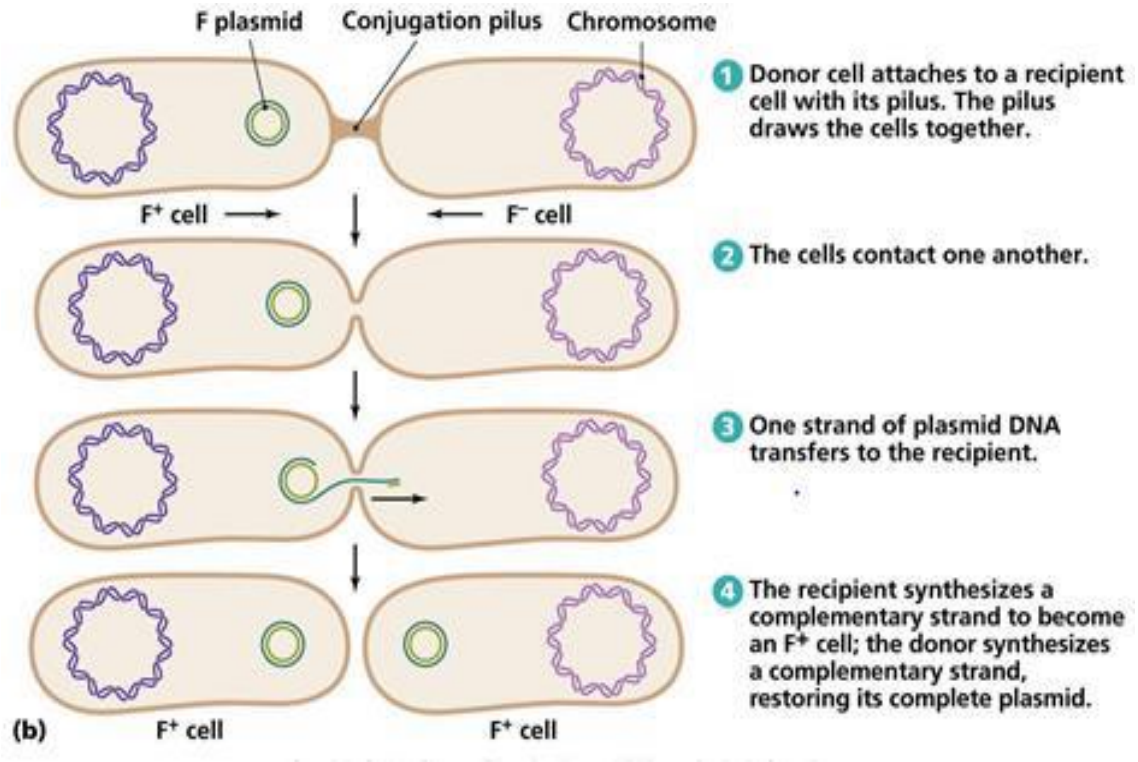
between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.



Fig(3):Gene transfer by (Transduction)

Conjugation

Transfer of DNA from a donor to a recipient by direct physical contact between the cells. In bacteria there are two mating types a donor (male) and a recipient (female) and the direction of transfer of genetic material is one way; DNA is transferred from a donor to a recipient.



Fig(4):Gene transfer by (Conjugation)

Mycobacteria

The mycobacteriaare rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, after being stained, they resist decolorization by acid or alcohol and are therefore called “acid-fast” bacilli. Mycobacterium tuberculosis causes tuberculosis and is a very important pathogen of humans (table-1).

table-1

Species	**Host**	**Significance**
M. leprosy	human	leprosy
M.Tuberculosis	human	pulmonary and disseminated tuberculosis
M. bovis	human , bovis	bovine tuberculosis

MYCOBACTERIUM TUBERCULOSIS

Morphology and identification:

On artificial media, coccoid and filamentous forms are seen with variable morphology from one species to another. Mycobacteria cannot be classified as either gram positive or gram negative. When stained by basic dyes, they cannot be decolorized by alcohol, regardless of treatment with iodine. True tubercle bacilli are characterized by "acid fastness"—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria. Acid fastness depends on the integrity of the waxy envelope. The Ziehl-Neelsen technique of

staining is used for identification of acid-fast bacteria.

Culture

The media for primary culture of mycobacteria should include a nonselective medium and a selective medium. Selective media contain antibiotics to prevent the overgrowth of contaminating bacteria and fungi. There are three general formulations that can be used for both the nonselective and selective media.

1.Semi synthetic agar media These media (eg, Middle brook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol; the 7H11 medium also contains casein hydrolysate.

2. Inspissated egg media—These media (eg, Lwenstein-Jensen) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks,

potato flour, and other ingredients in various combinations). Malachite green is included to inhibit other bacteria.

3. Broth media—Broth media (eg, Middle brook 7H9 and 7H12) support the proliferation of small inocula.

Growth Characteristics

Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Increased CO_2 tension enhances growth.

Reaction to Physical and Chemical Agents

Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth. Dyes (eg, malachite green) or antibacterial agents (eg, penicillin) that are bacteriostatic to other bacteria can be

incorporated into media without inhibiting the growth of tubercle bacilli.

Constituents of Tubercle Bacilli

A. Lipids

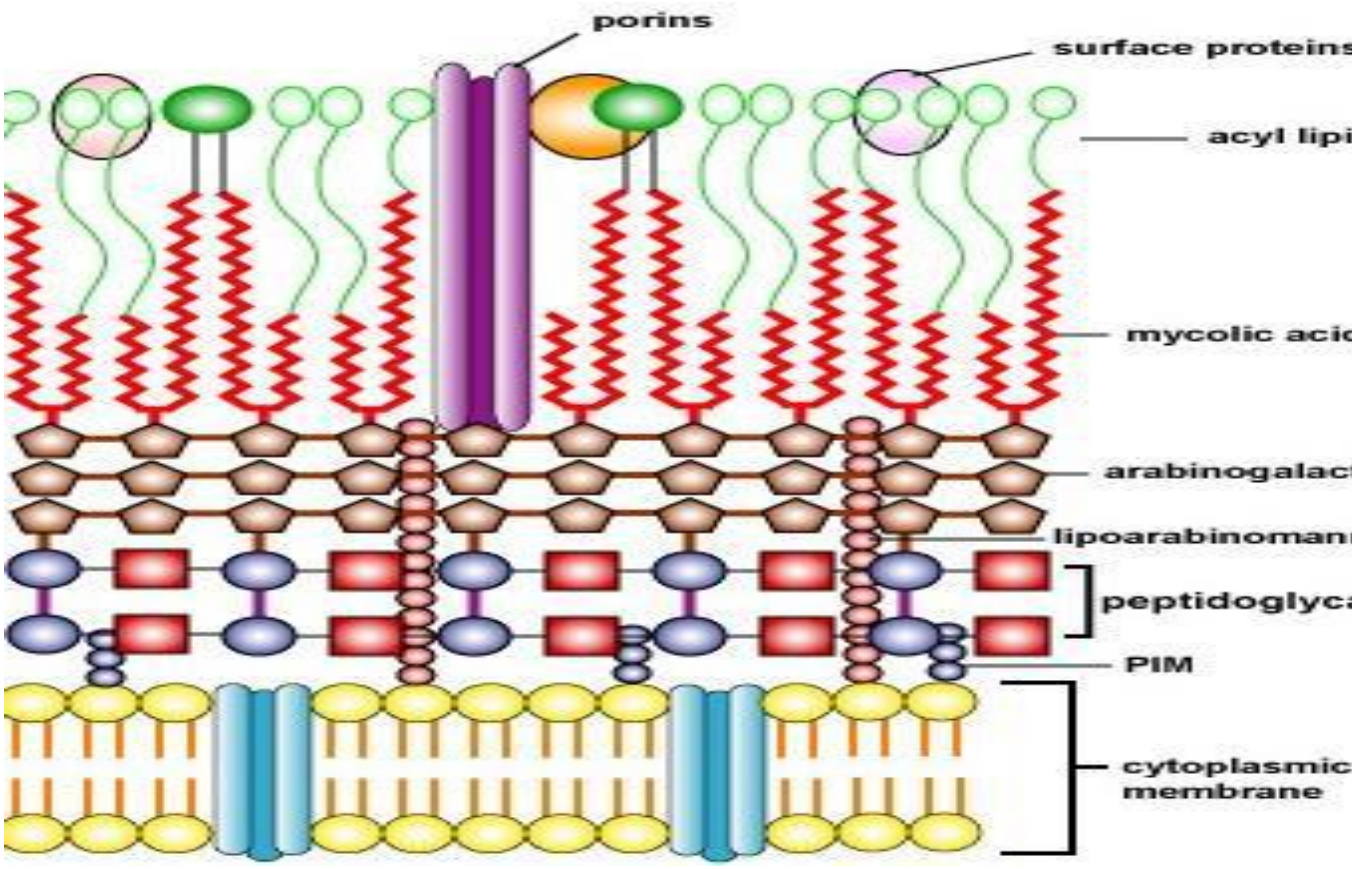
Mycobacteria are rich in lipids. These include mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides. In the cell, the lipids are largely bound to proteins and polysaccharides. Lipids are to some extent responsible for acid fastness. Their removal with hot acid destroys acid fastness, which depends on both the integrity of the cell wall and the presence of certain lipids.

B. Proteins

Each type of mycobacterium contains several proteins that elicit the tuberculin reaction. Proteins bound to a wax fraction can, upon injection, induce tuberculin sensitivity. They can also elicit the formation of a variety of antibodies.

C. Polysaccharides

Mycobacteria contain a variety of polysaccharides. Their role in the pathogenesis of disease is uncertain. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with sera of infected persons.



Pathogenesis

Mycobacteria are emitted in droplets smaller than 25 μmin diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. One to 2 months after exposure, pathogenic lesions associated with infection appear in the lung.

Spread of Organisms in the Host

Tubercle bacilli spread in the host by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastrointestinal tract. In the first infection, tubercle bacilli always spread from the initial site via the lymphatics to the regional lymph nodes. The bacilli may spread farther and reach the bloodstream, which in turn distributes bacilli to all organs (miliarydistribution).

Intracellular Site of Growth

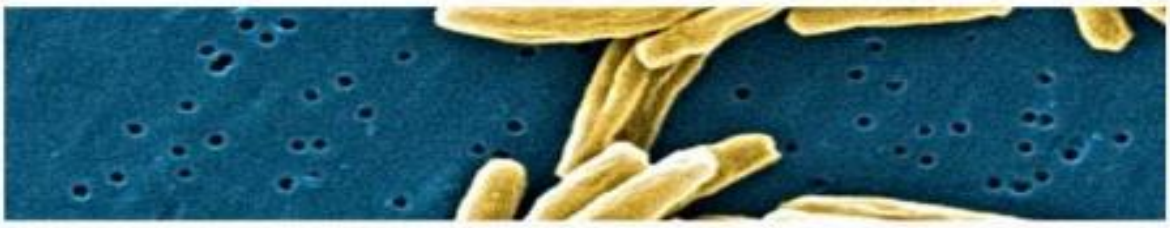
When mycobacteria establish themselves in tissue, they reside principally intracellularly in monocytes, reticuloendothelial cells, and giant cells. The intracellular location is one of the features that makes chemotherapy difficult and favors microbial persistence.

Primary Infection and Reactivation Types of Tuberculosis

When a host has first contact with tubercle bacilli, the following features are usually observed:

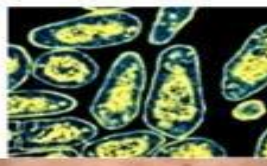
(1) An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly.

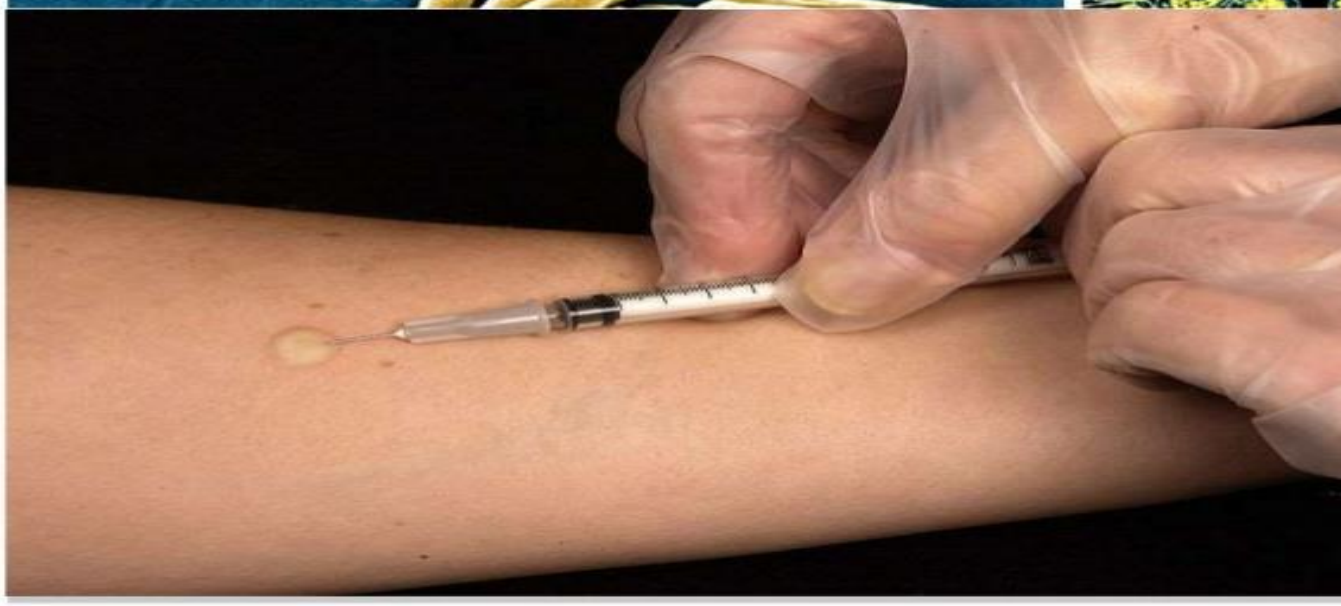
(2) The lymph node undergoes massive caseation, which usually calcifies (Ghonlesion).



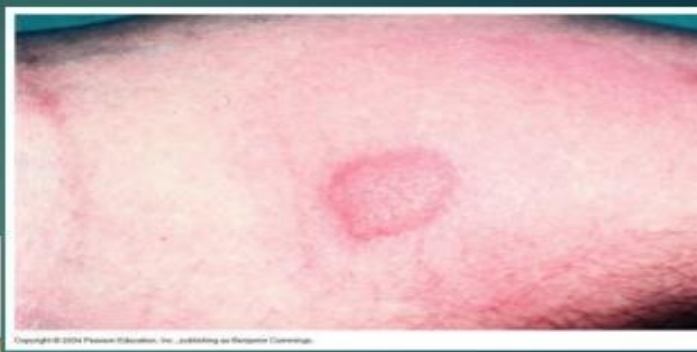
TUBERCULIN TESTS

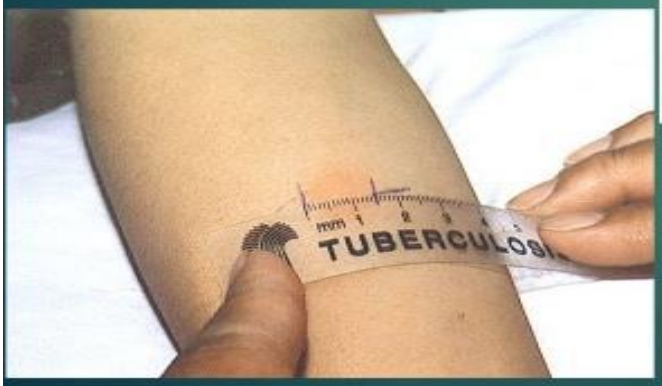
- In an individual who has not had contact with mycobacteria, the reaction.
- An individual who has had a primary infection with tubercle bac develops induration, edema, erythema in 24–48 hours, and, with intense reactions, even central necrosis.
- The skin test should be read in 48 or 72 hours.
- It is considered positive if the injection of 5 TU[Tuberculin units followed by induration 10 mm or more in diameter.
- Positive tests tend to persist for several days. Weak reactions ma more rapidly.

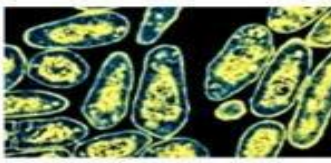




- ✓ *Induration of 10 mm or more is considered positive.*
- ✓ *positive test leads to red area at injection site*







Interpretation of Tuberculin Test

- A positive tuberculin test indicates that an individual has been infected in the past.
- It does not imply that active disease or immunity to disease is present.
- Tuberculin-positive persons are at risk of developing disease from reactivation of the primary infection, whereas tuberculin-negative persons who have never been infected are not subject to that risk, though they may become infected from an external source.

Immunity and Hypersensitivity

During the first infection with tubercle bacilli, a certain resistance is acquired, and there is an increased capacity to localize tubercle bacilli, retard their multiplication, limit their spread, and reduce lymphatic dissemination. This can be attributed to the development of cellular immunity, with evident ability of mononuclear phagocytes to limit the multiplication of ingested organisms and even to destroy them.

Diagnostic Laboratory Tests

A positive tuberculin test result does not prove the presence of active disease caused by tubercle bacilli. Isolation of tubercle bacilli provides such proof.

A. Specimens

Specimens consist of fresh sputum, gastric washings, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy material, blood, or other suspected material.

TREATMENT

- Anti-tuberculous drugs
  - INAH
  - Rifampicin
  - Ethambutol
  - Pyrazinamide
- Multi-drug resistant tuberculosis

لا تتسلق الجبال ليراك العالم ولكن تسلقها لترى انت العالم

Lec 9

Dr.Azal Alaa

Microbial Pathogenesis

Introduction

❖A **pathogen** is a microorganism that is able to cause disease in a plant, animal or insect.

❖**Pathogenicity** is the ability to produce disease in a host organism.

❖Microbes express their pathogenicity by means of their **virulence**, a term which refers to the degree of pathogenicity of the microbe.

❖**Determinants of virulence** of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host.

❖The relationship between a host and a pathogen is dynamic, since each modifies the activities and functions of the other.

❖The outcome of such a relationship depends on:
- ❖ the virulence of the pathogen and
- ❖the relative degree of resistance or susceptibility of the host, mainly due to the effectiveness of the host defense mechanisms.

- ❖ Normal flora (beneficial or ignored):
  - ❑ GI track, skin, upper respiratory track
- ❖ Virulent bacteria (actively cause disease):
  - ❑ pathogenic islands
- ❖ Opportunistic bacteria (when host with underline problem)
  - ❑ *Pseudomonas aeruginosa:* cystic fibrosis/ burn
  - ❑ TB, Kaposi's sarcoma (herpesvirus): AIDS

Microbial Pathogenesis

Entry into the Host

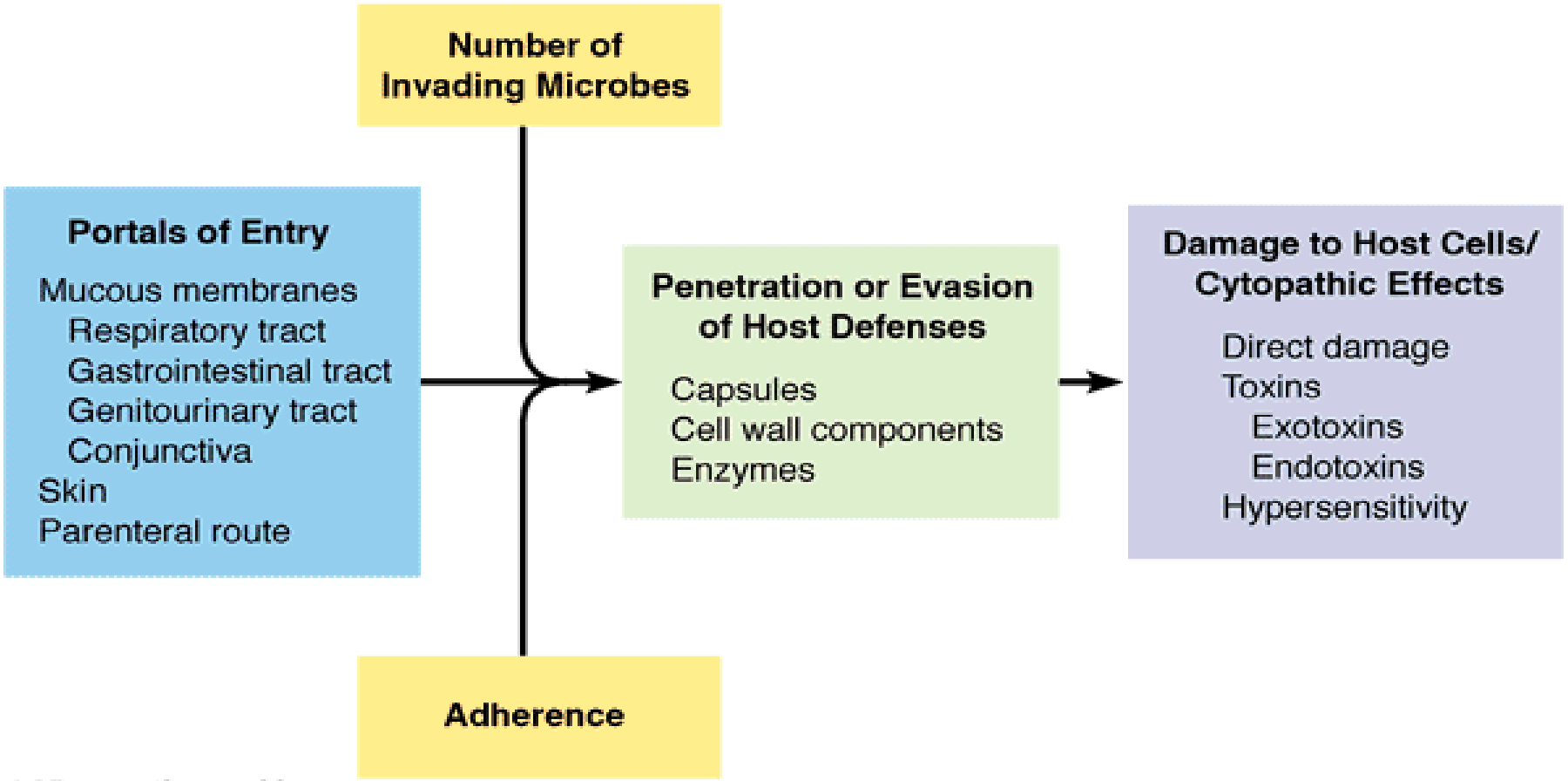
Must access and adhere to host tissues, penetrate or evade host defenses, and damage tissue to cause disease.

Portals of Entry

The three main portals of entry are:

- Mucous membranes
- Skin
- Parenteral

MICROBIAL MECHANISMS OF PATHOGENICITY: HOW MICROORGANISMS CAUSE DISEASE



I. Mucous Membranes

Epithelial tissue lining the:

- **Respiratory tract**: Easiest and most frequently used entry site for microbes.
- **Gastrointestinal tract**: Another common entry site. Enter through water, food, contaminated fingers and fomites. Must survive stomach HCl, enzymes, and bile.
- **Genitourinary tract**: Entry site for most sexually transmitted diseases (STDs).
- **Conjunctiva**: Membrane covering eyes and eyelids.

II. Skin

- Unbroken skin is impenetrable by most microbes.
- Some microbes gain access through hair follicles and sweat glands.
- *Nectator americanus* (hookworm) can bore through intact skin.
- Certain fungi (dermatophytes) grow on skin and produce enzymes that break down keratin.

III. Parenteral Route

Microbes are deposited directly into the tissues beneath the skin or mucous membranes.

- **Examples: Injections, bites, cuts, wounds, surgery, punctures, and splitting due to swelling or drying.**

Preferred Portal of Entry

Many microbes have a preferred portal of entry which is a prerequisite to cause disease.

- **Example: *Streptococcus pneumoniae* that are inhaled can cause pneumonia; if swallowed generally don't cause disease.**

Number of Invading Microbes

- Higher number of pathogens increase the likelihood of developing disease.
- LD_{50}: Lethal dose for 50% of hosts. Number of microbes that will kill 50% of inoculated test animals.
- ID_{50}: Infectious dose for 50% of hosts. Number of microbes that will cause a demonstrable infection in 50% of inoculated test animals.

Adherence

Attachment between of microbe to host tissue requires:

Adhesins or Ligands: Surface molecules on pathogen that bind specifically to host cell surface molecules. May be located on glycocalyx, fimbriae, viral capsid, or other surface structure.

Receptors: Surface molecules on host tissues to which pathogen adhesins bind.

Cell Wall Components

M protein: Found on cell surface and fimbriae of *Streptococcus pyogenes*. Mediates attachment an dhelps resist phagocytosis.

Waxes: In cell wall of *Mycobacterium tuberculosis* helps resist digestion after phagocytosis.

How Bacterial Pathogens Penetrate Host Defenses

Capsules

- **Increase the virulence of many pathogens.**
- **Examples: *Streptococcus pneumoniae, Klebsiella pneumoniae, Hemophilus influenzae, Bacillus anthracis,* and *Yersinia pestis*.**
- **Resist host defenses by impairing phagocytosis.**
- **Host can produce antibodies to capsule, which attach to microbe and allow phagocytosis.**

Cell Wall Components

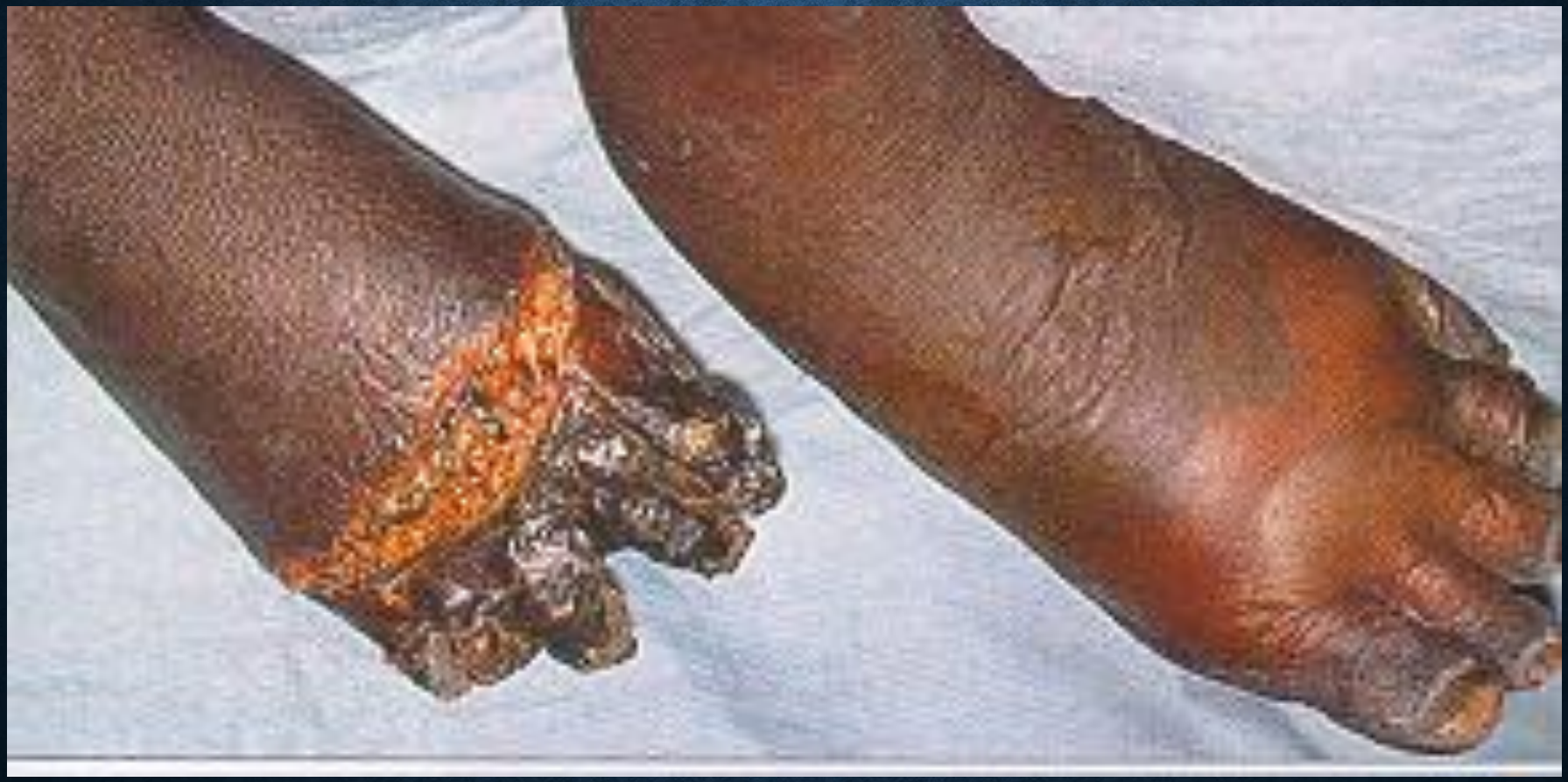
- **M protein: Found on cell surface and fimbriae of *Streptococcus pyogenes*. Mediates attachment and helps resist phagocytosis.**
- **Waxes: Cell wall of *Mycobacterium tuberculosis* helps resist digestion after phagocytosis.**

Microbial Enzymes

Extracellular enzymes (exoenzymes) lyse cells, form or dissolve clots, and dissolve materials in tissue.

- **Leukocidins**: Destroy white blood cells that are phagocytes. Produced by staphylococci and streptococci.
- **Hemolysins**: Destroy red blood cells. Produced by *clostridium perfringens* (gangrene) and streptococci.
- **Coagulases**: Produce clots in blood, which may wall off site of infection from immune response. Produced by some staphylococci.
- **Bacterial Kinases**: Break down clots produced by body to isolate infection. Made by streptococci and staphylococci.
- **Hyaluronidase**: Breaks down hyaluronic acid which holds cells together in connective tissue. Made by some streptococci and gangrene causing clostridia.

Tissue Damage Caused by Microbial Enzymes of *Clostridium perfringens*



Severe gangrene caused by *Clostridium perfringens*.
Source: Tropical Medicine and Parasitology, 1997

Microbial Enzymes (Continued)

- **Collagenase**: Breaks down collagen which forms connective tissue of muscles, skin, and other organs. Produced by several clostridia.
- **Necrotizing Factors**: Kill body cells.
- **Hypothermic factors**: Decrease body temperature.
- **Lecithinase**: Destroys plasma membrane of cells.
- **Proteases**: Break down proteins in tissue.

TISSUE DAMAGE CAUSED BY ENZYMES OF FLESH-EATING *STREPTOCOCCUS PYOGENES*

Necrotizing fasciitis with blood filled vesicles.
Source: Perspectives in Microbiology, 1995

Penetration into Host Cells

Invasins: Surface proteins that alter actin filaments of host cell cytoskeleton, allowing microbes to enter cells.

- **Examples:** *Salmonella typhinurium* and *E. coli.*

Cadherin: A glycoprotein that bridges junctions between cells, allowing microbes to move from one cell to another.

How Bacterial Cells Damage Host Cells

Three mechanisms:

- **Direct Damage**
- **Toxins***
- **Hypersensitivity Reactions**

*** Most bacterial damage is carried out by toxins.**

1. Direct Damage

- **Some bacteria can induce cells to engulf them (*E. coli*, *Shigella*, *Salmonella*, and *Neisseria gonorrhoeae*).**
- **Microbial metabolism and multiplication kills host cells.**
- **Other microbes enter the cell by excreting enzymes or through their own motility.**

2. Toxin Production

Toxins: Poisonous substances produced by microbes. ◆

Frequently toxins are the main pathogenic factor. ◆

Toxigenicity: Ability of a microbe to produce toxins. ◆

Toxemia: Presence of toxins in the blood. ◆

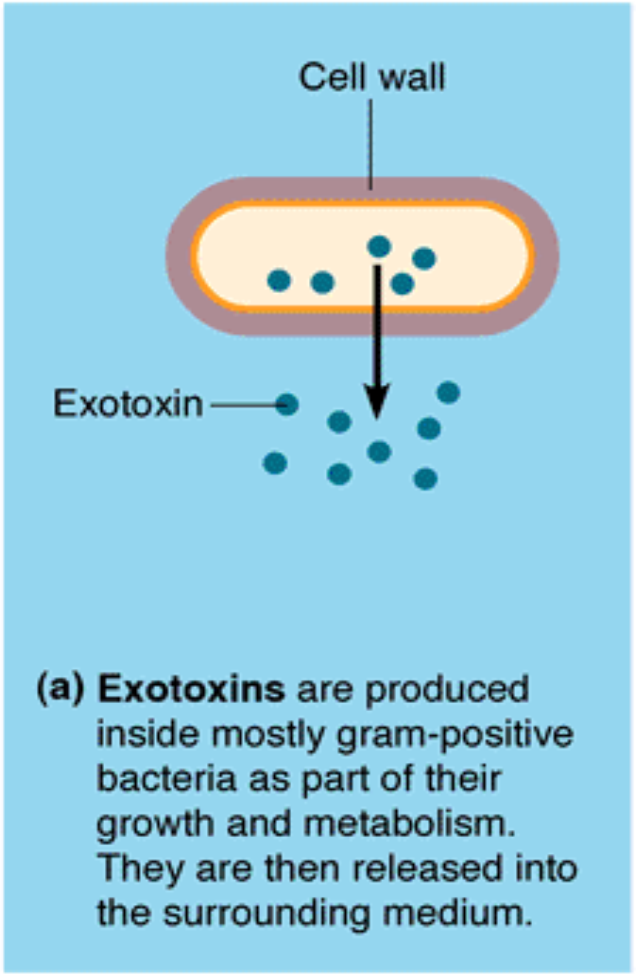
Toxin effects: May include fever, cardiovascular problems, diarrhea, shock, destruction of red blood cells and blood vessels, and nervous system disruptions. ◆

Of 220 known bacterial toxins, 40% damage eucaryotic cell membranes. ◆

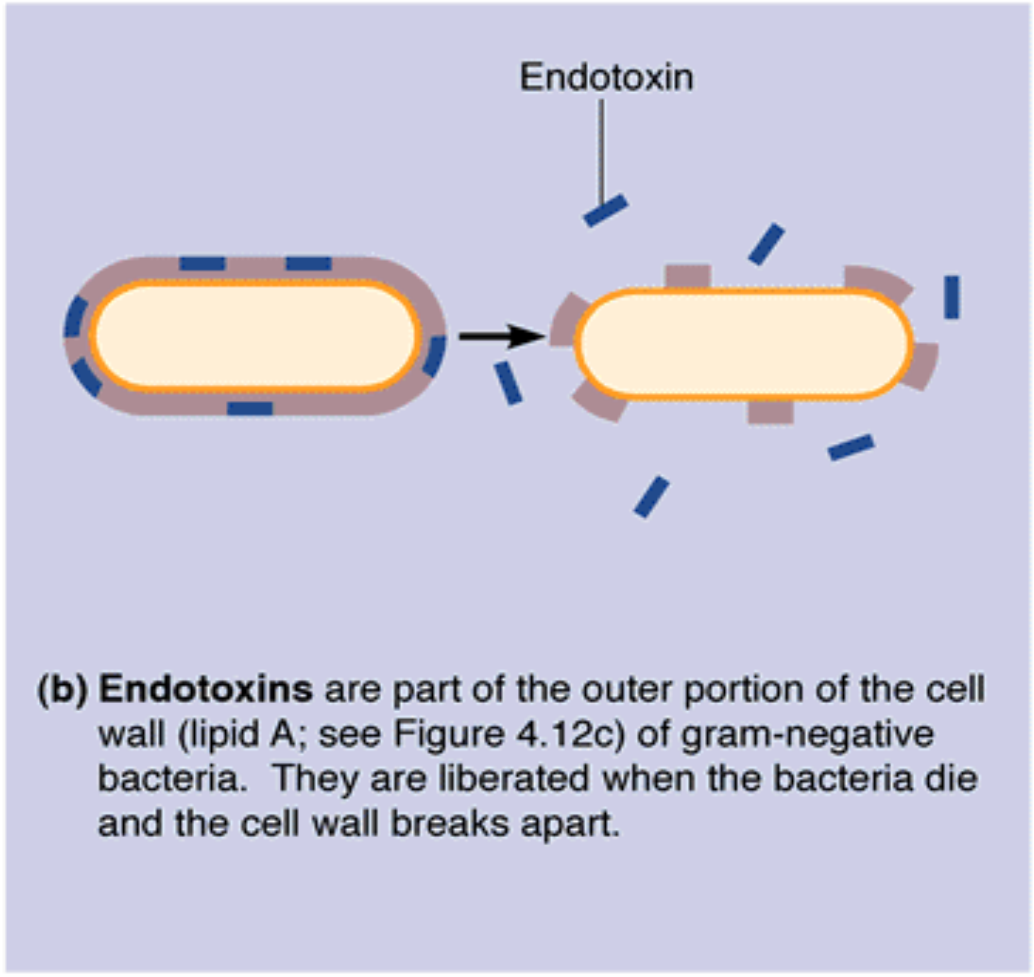
Two types of toxins: ◆

- **Exotoxins**
- **Endotoxins**

EXOTOXINS VERSUS ENDOTOXINS



(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then released into the surrounding medium.



(b) **Endotoxins** are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

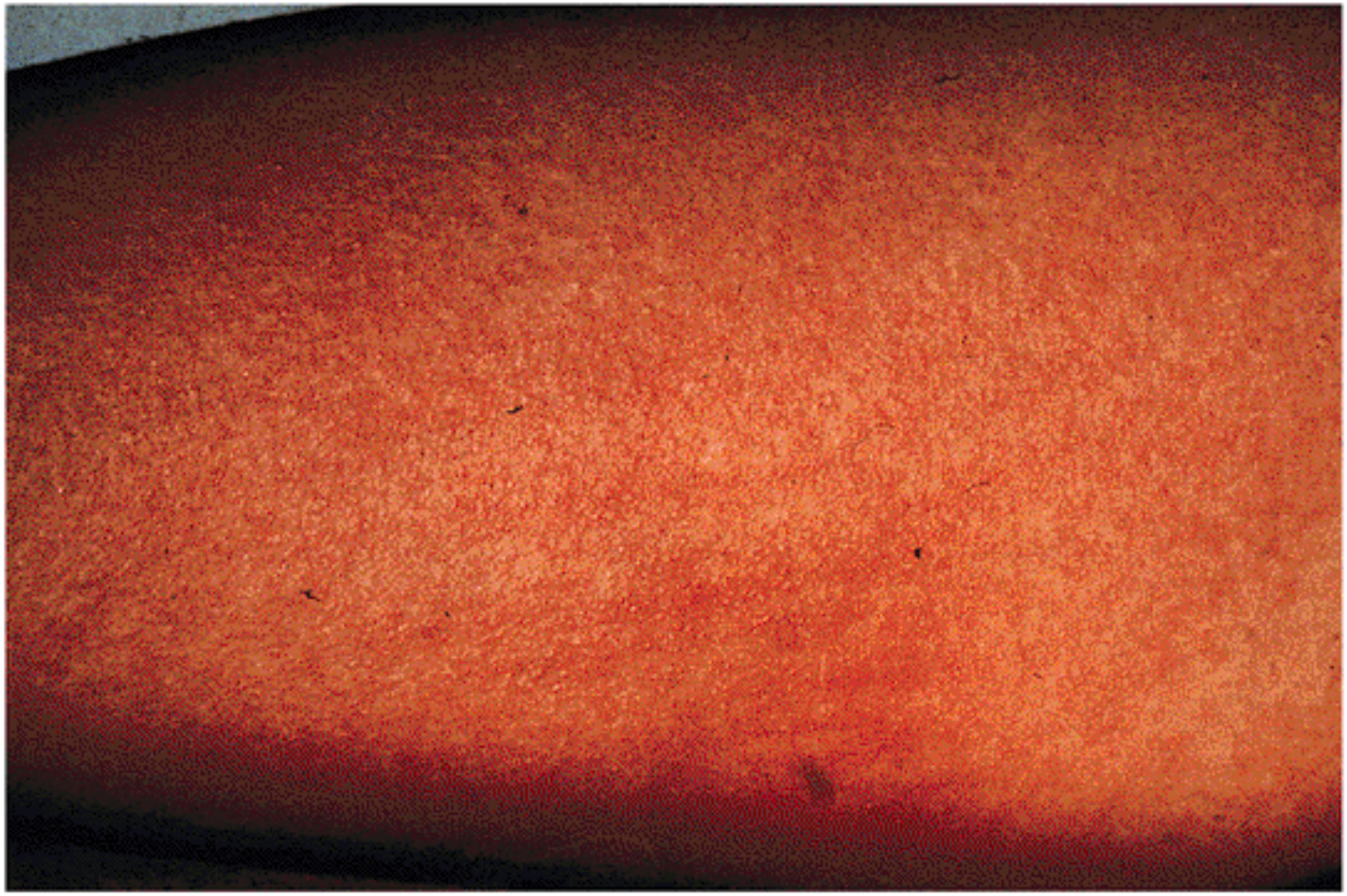
A. Exotoxins

- **Proteins**: Enzymes that carry out specific reactions.
- **Soluble** in body fluids, **rapidly transported** throughout body in blood or lymph.
- Produced mainly by **gram-positive bacteria**.
- Most genes for toxins are carried on **plasmids** or **phages**.
- Produced inside bacteria and released into host tissue.
- Responsible for disease symptoms and/or death.
  - **Cytotoxins**: Kill or damage host cells.
  - **Neurotoxins**: Interfere with nerve impulses.
  - **Enterotoxins**: Affect lining of gastrointestinal tract.
- Antibodies called **antitoxins** provide immunity.
- **Toxoids**: Toxins that have been altered by heat or chemicals. Used as vaccines for diphtheria and tetanus.

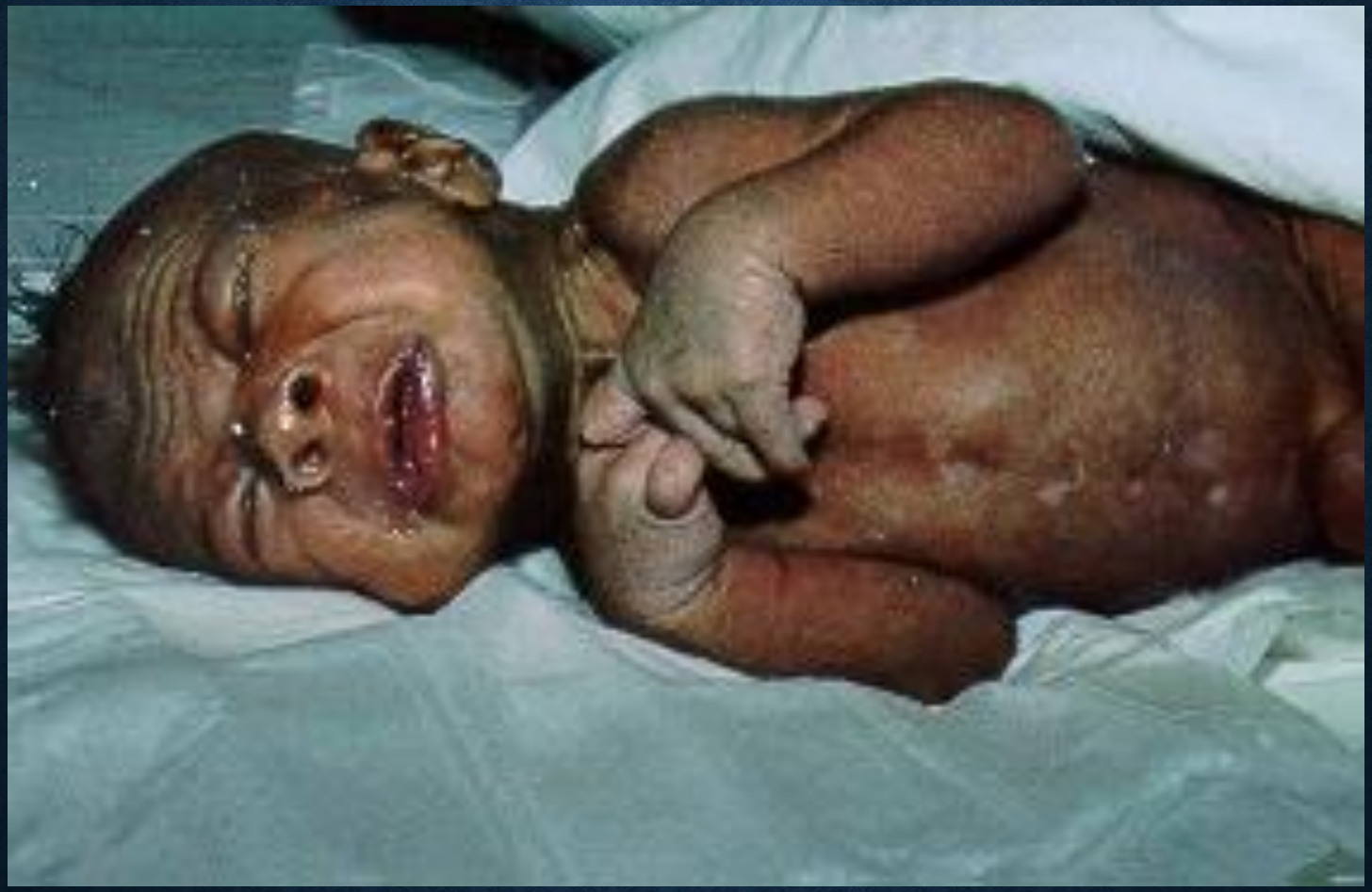
Important Exotoxins

- **Diphtheria Toxin**: *Corynebacterium diphtheriae* when infected by a phage carrying tox gene. **Cytotoxin** inhibits protein synthesis in eucaryotic cells. Two polypeptides: A (active) and B (binding).
- **Erythrogenic Toxins**: *Streptococcus pyogenes* produces three **cytotoxins** which damage blood capillaries, causing a red rash.
- **Botulinum Toxins**: Produced by *Clostridium botulinum*. **Neurotoxin** that inhibits release of neurotransmitter acetylcholine and **prevents transmission of nerve impulses to muscles**, causing flaccid paralysis. Extremely potent toxins.
- **Tetanus Toxin**: Produced by *Clostridium tetani*. A **neurotoxin** that **blocks relaxation of skeletal muscles**, causing uncontrollable muscle spasms (lockjaw) and convulsions.
- **Vibrio Enterotoxin**: Produced by *Vibrio cholerae*. Two polypeptides: A (active) and B (binding). The A subunit of **enterotoxin** causes epithelial cells to discharge large amounts of fluids and electrolytes.
- **Staphylococcal Enterotoxin**: *Staphylococcus aureus* produces an **enterotoxin** similar to cholera toxin. Other enterotoxins cause toxic shock syndrome.

RASH OF SCARLET FEVER CAUSED BY ERYTHROGENIC TOXINS OF *STREPTOCOCCUS PYOGENES*



Muscle Spasms of Tetanus are Caused by Neurotoxin of *Clostridium tetani*

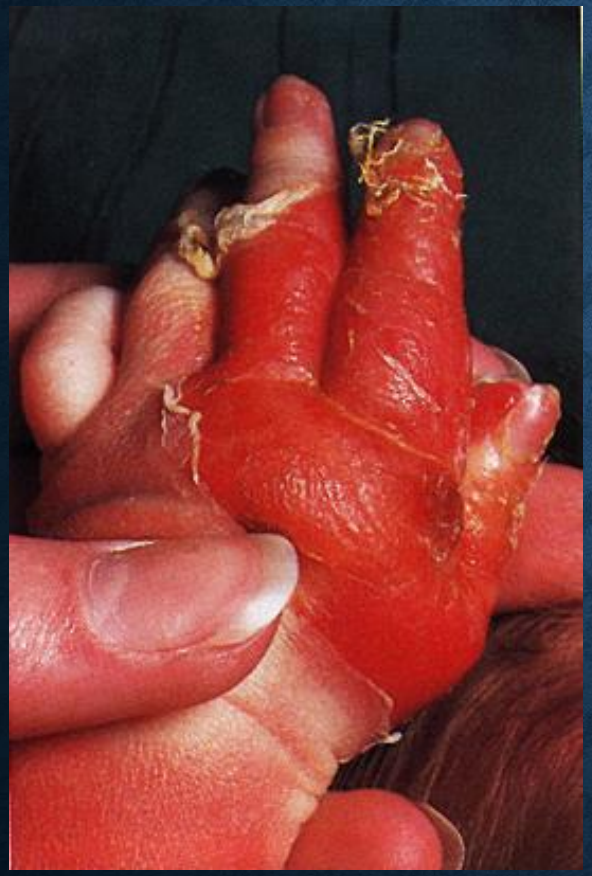


Neonatal Tetanus (Wrinkled brow and risus sardonicus)
Source: Color Guide to Infectious Diseases, 1992

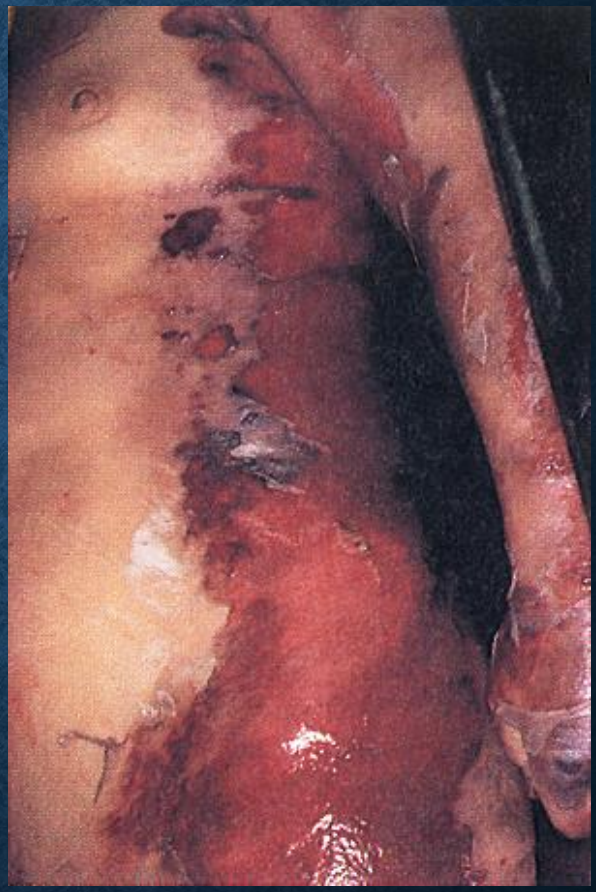
Vibrio Enterotoxin Causes Profuse Watery Diarrhea

Rice-water stool of cholera. The A subunit of enterotoxin causes epithelial cells to discharge large amounts of fluids and electrolytes.
Source: Tropical Medicine and Parasitology, 1995

DISEASES CAUSED BY STAPHYLOCOCCAL TOXINS



Scalded Skin Syndrome



Toxic Shock Syndrome

Endotoxins

- Part of outer membrane surrounding gram-negative bacteria.
- Endotoxin is lipid portion of lipopolysaccharides (LPS), called **lipid A**.
- Effect exerted when gram-negative cells die and cell walls undergo lysis, liberating endotoxin.
- **All produce the same signs and symptoms:**
  - Chills, fever, weakness, general aches, blood clotting and tissue death, shock, and even death. Can also induce miscarriage.
  - **Fever**: Pyrogenic response is caused by endotoxins.

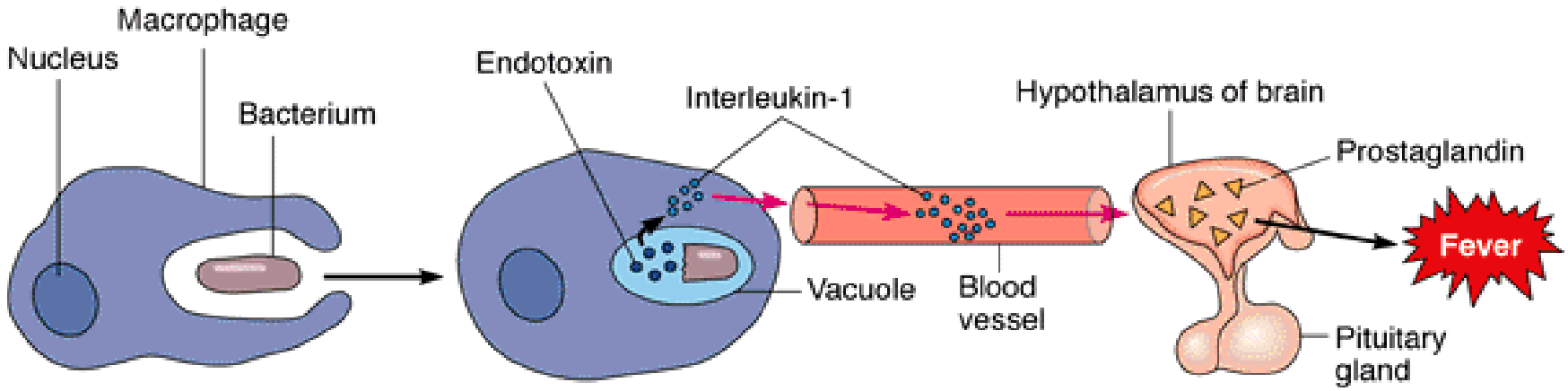
Endotoxins (Continued)

- **Endotoxins do not promote the formation of effective antibodies.**
- **Organisms that produce endotoxins include:**
  - ***Salmonella typhi***
  - ***Proteus spp.***
  - ***Pseudomonas spp.***
  - ***Neisseria spp.***
- **Medical equipment that has been sterilized may still contain endotoxins.**
- ***Limulus* amoebocyte assay (LAL) is a test used to detect tiny amounts of endotoxin.**

Events leading to fever:

- Gram-negative bacteria are digested by phagocytes.
- **LPS** is released by digestion in vacuoles, causing macrophages to release **interleukin-1** (IL-1).
- IL-1 is carried via blood to **hypothalamus**, which controls body temperature.
- IL-1 induces hypothalamus to release **prostaglandins**, which reset the body's thermostat to higher temperature.

ENDOTOXINS AND THE PYROGENIC (FEVER) RESPONSE



1. A macrophage ingests a gram-negative bacterium
2. The bacterium is degraded in a vacuole, releasing endotoxins that induce the macrophage to produce interleukin-1 (IL-1)
3. IL-1 is released by the macrophage into the bloodstream, through which it travels to the hypothalamus of the brain
4. IL-1 induces the hypothalamus to produce prostaglandins, which reset the body's "thermostat" to a higher temperature, producing fever

Shock: **Any life-threatening loss of blood pressure.**

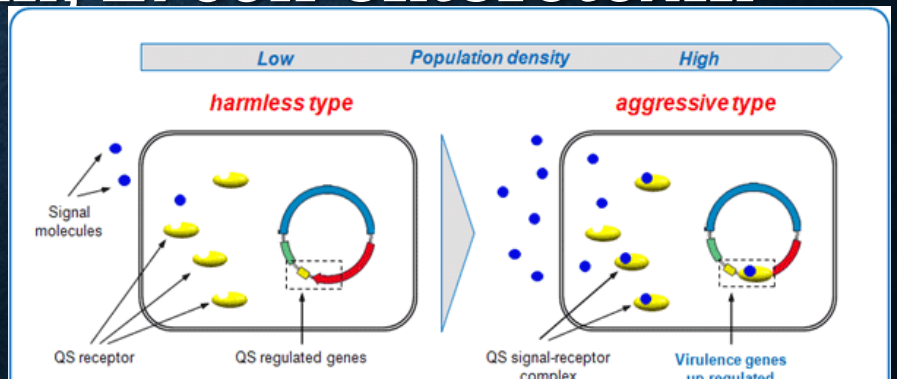
Septic shock: **Shock caused by endotoxins of gram-negative bacteria.**

- ***Phagocytosis of bacteria leads to secretion of tumor necrosis factor (TNF), which alters the permeability of blood capillaries and causes them to lose large amounts of fluids.***
- ***Low blood pressure affects kidneys, lungs, and gastrointestinal tract.***

Plasmids, Lysogeny, and Pathogenicity

Plasmids: Small, circular pieces of DNA that are not connected to chromosome and are capable of independent replication.

- **R (resistance) factors contain antibiotic resistance genes.**
- **Other plasmids contain genes for toxins and pathogenic factors: tetanus toxin, staphylococcal enterotoxin, *E. coli* enterotoxin (heat-labile), adhesins, and coagulase.**



Bacteriophages:

Can incorporate genetic material into chromosomal DNA and remain latent (lysogeny). Bacterial cell can change characteristics (lysogenic conversion) and produce certain toxins or pathogenic factors:

- **Diphtheria toxin**
- **Capsules in *S. pneumoniae***
- **Botulinum neurotoxin**
- **Staphylococcal enterotoxin**
- **Cholera toxin.**

Barrier systems

Host cell membrane	**Taken up by phagocyte and resist killing**	**Inhibitory molecule**	***Mycobacterium***
Production Of antibody	**Degrade antibody**	**IgA protease**	***Streptococcus***
Antimicrobial cell-mediated response	**Activate T cells non-specifically and Productively**	**Superantigen**	***Staphylococcus***
Antimicrobial immune response	**Vary presenting microbial antigen**	**Switch on production of different antigens**	***Borrelia***
		Genetic recombination	***Streptococcus***

MICROBIOLOGY

Proteus is a member of the *Enterobacteriaceae* family. The genus of *Proteus* consists of motile, aerobic and facultatively anaerobic, Gram-negative rods. *Proteus* is a member of the tribe *Proteeae*, which also includes *Morganella* and *Providencia*. The genus *Proteus* currently consists of five named species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. myxofaciens* and *P. hauseri* and three unnamed genomospecies: *Proteus* genomospecies 4, 5, 6 (104). However, a recent study indicated that *P. myxofaciens* may represent a separate genus with low similarity to tribe *Proteeae*, and it has been suggested that this organism be renamed *Cosenzaea myxofaciens* (47).

A striking microbiologic characteristic of *Proteus* species is their swarming activity. Swarming appears macroscopically as concentric rings of growth emanating from a single colony or inoculum. On a cellular level, swarming results from bacterial transformation from "swimmer cells" in broth to "swarmer cells" on a surface such as agar, in a process involving cellular elongation and increased flagellin synthesis (62). The genus name *Proteus* originates from the mythological Greek sea god *Proteus*, who was an attendant to Poseidon (62). *Proteus* could change his shape at will. This attribute reminded early microbiologists of the morphologic variability of the Protei on subculture, including their ability to swarm.

EPIDEMIOLOGY

Members of the genus *Proteus* are widespread in the environment and are found in the human gastrointestinal tract (9). The most common infections caused by *Proteus* spp. are urinary tract infections (UTIs). *Proteus* spp. can be found to colonize the vaginal introitus prior to onset of bacteruria.

Therefore, like *Escherichia coli*, *Proteus* spp. causes urinary tract infections by ascending from the rectum to the periurethra and bladder.

P. mirabilis is by far the most common species identified in clinical specimens. *P. mirabilis* is a common cause of both community-acquired and catheter-associated UTI, cystitis, pyelonephritis, prostatitis, wound infections, and burn infections, and occasionally causes respiratory tract infections, chronic suppurative otitis media, eye infections (endophthalmitis), meningitis, and meningoencephalitis (3, 4, 51, 81, 137). It is a common cause of bacteremia following catheter-associated UTI (90), and in rare cases has been reported to cause cellulitis, endocarditits, mastoiditis, empyema, and osteomyelitis (24, 61, 86, 137). It has also been suggested that *P. mirabilis* could have a role in the etiology of rheumatoid arthritis (145).

P. vulgaris, previously considered biogroup 2, has been reported to cause UTIs, wound infections, burn infections, bloodstream infections, and respiratory tract infections (71, 137). There has also been one case study of *P. vulgaris* causing bacteremia and brain abscesses, with the suspected point of entry being the digestive tract(16).>

P. penneri, previously biogroup 1, generally causes UTIs, wound infections, burn infections, bloodstream infections, and respiratory tract infections (71, 137).There has been one case study of*P. penneri* Fournier's gangrene in a child with congenital genitourinary anomalies (33). There has also been one recent report of *P. penneri* causing "red body disease" of the Pacific white shrimp *Penaeus vannamei* (25). Notably, *P. penneri* may be incorrectly identified as *P. mirabilis* due to being indole-negative (72), and it cannot be clearly resolved from *P. vulgaris* by 16S sequencing unless using the 16S-23S internal transcribed spacer (26). Thus, the burden of human infections caused by this organism may be underestimated.>

P. myxofaciens was originally isolated from a gypsy moth and has been isolated from UTIs in India (129).

P. hauseri, previously considered biogroup 3, has not been associated with infections in humans.

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CLINICAL MANIFESTATIONS

The clinical manifestations of infections with *Proteus* spp. are, in the main, non-specific. However, urinary tract infections involving struvite stones are characteristic. By producing urease, *Proteus* spp. can hydrolyze urea into ammonia and carbon dioxide, and therefore raise urinary pH. Alkalinization of urine promotes precipitation of magnesium-ammonium phosphate salts leading to the formation of struvite stones, which may serve as a nidus for the persistence of infection or may directly obstruct the urinary tract, thereby promoting infection.

LABORATORY DIAGNOSIS

The members of the genus *Proteus* are Gram negative, motile facultative anaerobic rods. On culture plates, *Proteus* species are distinguished by their ability to swarm. *Proteus* spp. have 2-3mm colorless, flat, colonies on MacConkey agar, whereas they swarm in waves to cover blood agar plates and LB agar plates.

Proteus spp. are identified by the following biochemical characteristics: positive methyl-red reaction, negative Voges-Proskauer reaction, phenylalanine deaminase production, growth on KCN and urease production. *P. mirabilis* and *P. penneri* are indole-negative, while other *Proteus* species are indole-positive. The *Proteus* genomospecies (4, 5, and 6) can be distinguished from other *Proteus* species based on five

biochemical characteristics: esculin hydrolysis, salicin fermentation, L-rhamnose fermentation, and elaboration of DNase and lipase.

PATHOGENESIS

Proteus spp. possess several virulence factors that explain their uropathogenic potential, many of which have been investigated in a murine model of UTI (>9). They have pili or fimbriae for adherence to uroepithelium. Additionally, they elaborate cytotoxic hemolysins that lyse red cells and release iron, a bacterial growth factor. *Proteus* isolates possess flagella for motility. As noted above they produce urease, leading to the formation of struvite stones.

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SUSCEPTIBILITY IN VITRO AND IN VIVO

Proteus spp. can be naturally resistant to antibiotics, such as benzylepenicillin, oxacillin, tetracycline, and macrolides (137). *Proteus* spp. can acquire resistance to ampicillin through plasmid mediated beta-lactamases, and chromosomal beta-lactamase expression has now been reported (136). In the last decade there have also been numerous reports of production of extended-spectrum beta-lactamases (ESBLs) by *Proteus* spp.. The ESBLs can confer resistance to third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime, as well as the monobactam, aztreonam (115). The cephamycins (cefoxitin, cefotetan and cefmetazole) and the carbapenems (imipenem and meropenem) are generally not hydrolyzed by ESBLs (115). However, resistance to carbapenems is starting to be observed in*Proteus* spp. (63, 109, 128).

It should be noted that the MICs for third generation cephalosporins or aztreonam may not reach widely used breakpoints for resistance with some

ESBL producing *Proteus* isolates. In 2010, there was a change in the CLSI recommendations for susceptibility breakpoints, resulting in many ESBL-producing isolates previously considered to be resistant to these antibiotics now being regarded as susceptible (39, 93, 142).For instance, 78-97% of ESBL-producing strains tested were considered susceptible to ceftazidime, cefepime, and aztreonam using the new breakpoints (93, 142). Another change in CLSI recommendations occurred in 2012, and SENTRY data from North America indicates that this change decreased the level of imipenem susceptibility compared to the 2010 criteria (64.5% of 1244 isolates were susceptible by 2012 criteria vs 99.8% by 2010 criteria) (117). Due to these changes in breakpoints for susceptibility, data concerning resistance to celphalosporins, aztreonam, and carbapenems may be underestimated.

Single Drug

Proteus mirabilis: Overall, the majority of *P. mirabilis* isolates from the past two decade have been susceptible to commonly used antibiotics (58). SENTRY data from the US and EU of isolates collected in 2009-2011 reported <10% of isolates resistant to amikacin, aztreonam, cefepime, ceftazidime, ceftriaxone, meropenem, and piperacillin/taxobactam (120), and a study of *P. mirabilis* catheter-associated UTI isolates from Poland similarly reported only 14% of isolates being resistant to amikacin. However, vancomycin, teicoplanin, linezolid, quinupristin/ dalfopristin, daptomycin, clindamycin, metronidazole, macrolides and ketolides do not have clinically useful activity against >*P. mirabilis*, and a high level of resistance (>60% of isolates) has been observed for cefuroxime, tetracycline, polymyxin B, colistin sulfate, and nitrofurantoin (2). The new glycylcycline, tigecycline, also has surprisingly poor *in vitro* activity, compared to its activity against other Gram negative bacilli (45). High

levels of ciprofloxacin resistance have been reported in Poland (94), though norfloxacin remained effective against these isolates (94), and *qnr* quinolone resistance genes have been identified in *P. mirabilis* isolates (52, 92).A compendium of antibiotic resistance of *P. mirabilis* is given in Table 1.

A wide variety of ESBLs have been detected in *P. mirabilis,* and recent reports indicate a rise in ESBL-producing *P. mirabilis,* for instance in Japan (29). CTX-M-type ESBLs have been detected in > *P. mirabilis* isolates from Korea and Taiwan (136, 141). CTX-M2 is the most common ESBL in Japan (64, 65, 97), as well as Israel (5) and it appears to be spreading rapidly (64, 98).CTX-M type β-lactamases also appear to be evolving in *P. mirabilis* via recombination (44). CTX-M has been found on the *P. mirabilis* chromosome as part of an integrative and conjugative element (ICE) in addition to being plasmid-encoded (87). TEM is another common ESBL in *P. mirabilis* (69), and the most common type of ESBL in *P. mirabilis* isolates from Croatia and Italy (5, 125, 138).A new TEM (TEM-187) has been reported in *P. mirabilis,* which has broad activity against penicillins but lower activity than TEM-1 (31, 32).It has been suggested that TEM-187 may represent an evolution of TEM enzymes from penicillinases to ESBLs, leading to underestimation of ESBLs in *P. mirabilis* (31). Other ESBL types include: VEB-1, an integron borne ESBL that was detected in a *P. mirabilis* isolate from a Vietnamese patient hospitalized in France (95), a multidrug-resistant isolate from Greece (109), and in Taiwan (59); PER-1, which was detected in a *P. mirabilis* isolate from Italy (106); VIM-1, detected in three ESBL *P. mirabilis* isolates from Bulgaria (128); and SHV-type β-lactamases, detected in *P. mirabilis* isolates from Bulgaria (128) and Taiwan (59).>

Metallo-beta-lactamases (MBLs) are also being reported in recent *P. mirabilis* isolates. For instance, one study from France identified a *P. mirabilis* isolate with a metallo-beta-lactamases (11), and a New Dehli metallo-beta-lactamase (NDM-1) has been identified in *P. mirabilis* isolates from New Zealand and India (15, 49, 144). Interestingly, NDM-1 was present in a genomic island in one isolate of *P. mirabilis* and co-occurred with a VEB-6 ESBL and SGI-1 (described below) (49), and it has been proposed that the presence of NDM-1 in a genomic island structure may enhance the spread of carbapenemases.

Multidrug resistance in *P. mirabilis* is also becoming more common (92). SGI-1 (>*Salmonella* genomic island 1), an integrative mobilizable element of multidrug-resistant *Salmonella* Typhimurim, has recently been detected in a surprisingly high percentage of *P. mirabilis* clinical isolates from France and indicates that *P. mirabilis* is a bacterial species of concern involved in dissemination of this multidrug-resistant element (41, 132, 133). SGI-1 confers resistance to a wide variety of older drugs that are no longer commonly used to treat human infection, but the multidrug-resistant regions of SGI-1 from *P. mirabilis* isolates had complex mosaic structures and rearrangements capable of facilitating acquisition and/or movement of antibiotic resistance genes that jeopardizes use of third-generation cephalosporins and quinolones (>132, 133).An ESBL-producing *P. mirabilis* isolate has also been identified with both TEM and CTX-M (110). Interestingly, ESBL production was found to be a risk factor for ciprofloxacin-resistant bacteremia due to *P. mirabilis* (135), and recent treatment with quinolone antibiotics was a risk factor for carriage of ESBL-producing *P. mirabilis* (5). A recent study from Tunisia also identified a high prevalence of plasmid-mediated quinolone resistance determinants among ESBL-producing *P. mirabilis* isolates (83).

Importantly, ESBL and non-ESBL producing isolates of *P. mirabilis* are frequently susceptible to beta-lactam/beta-lactamase inhibitor combinations. However, there have been some reports of inhibitor resistant TEM mutants (IRT) occurring in *P. mirabilis* (18, 84, 102). These beta-lactamases are not inhibited by clavulanic acid, sulbactam and tazobactam. It should be noted that these beta-lactamases do not have extended-spectrum activity (that is, they do not hydrolyze third generation cephalosporins).

Another mechanism of beta-lactamase inhibitor resistance in *P. mirabilis* isolates is presence of plasmid-mediated AmpC beta-lactamases. AmpC type beta-lactamases (also termed group 1 or class C beta-lactamases) can either be chromosomally encoded or plasmid encoded in *P. mirabilis* (99, 116). AmpC has also been found on the chromosome as part of integrative and conjugative elements (ICE) (87). Strains with plasmid-mediated AmpC beta-lactamases are consistently resistant to aminopenicillins (ampicillin or amoxicillin), carboxypenicillins (carbenicillin or ticarcillin) and ureidopenicillins (piperacillin). These enzymes are also resistant to third generation cephalosporins and the 7-α-methoxy group (cefoxitin, cefotetan, cefmetazole, moxalactam). MICs for aztreonam are usually in the resistant range but may occasionally be in the susceptible range. AmpC beta-lactamases generally do not effectively hydrolyze cefepime or the carbapenems.>One type of AmpC beta-lactamase is CMY, and clonal spread of CMY-producing *P. mirabilis* has been reported in Europe (36). CMY is also the predominant AmpC in Taiwan (141), and AmpC has been reported in *P. mirabilis* isolates from Korea (136) and Spain (87).

Carbapenems are generally active against *P. mirabilis*. However, imipenem MICs are frequently higher for *P. mirabilis* compared to other

members of the *Enterobacteriaceae,* and a recent study from Taiwan found that only 11.4% of *P. mirabilis* isolates were susceptible to imipenem (139). Meropenem is more potent than imipenem against *P. mirabilis* (46, 139). Carbapenemases have been found in >*P. mirabilis* (130), albeit rarely. A recent report has documented the presence of the class D carbapenemase, OXA-23, in *P. mirabilis* (19).

Proteus vulgaris: *Proteus vulgaris* produces a chromosomally encoded beta-lactamase (23), referred to as the cefuroxime-hydrolyzing beta-lactamase (cefuroximase or CumA) (34), which hydrolyzes cephalosporins. The enzyme can be induced by ampicillin, amoxicillin and first generation cephalosporins, weakly induced by carboxypenicillins, ureidopenicillins, cefotaxime and ceftriaxone, and inhibited by clavulanate. Strains of *P. vulgaris* that have a mutation in the regulatory genes of this beta-lactamase produce high levels of the enzyme and are resistant to penicillins, cefuroxime, ceftriaxone and cefotaxime. However, these isolates will generally be susceptible to ceftazidime, aztreonam, cephamycins, carbapenems and beta-lactam/beta-lactamase inhibitor combinations. Ertapenem and meropenem are substantially more active than imipenem (80). A compendium of antibiotic resistance of *P. vulgaris* is included in Table 1.

Recent reports have indicated the presence of ESBLs in *P. vulgaris* isolates (69, 78, 130), similar to *P. mirabilis,* including TEM and PER (60, 69). It has been noted that the MICs of several oxyimino type expanded-spectrum cephalosporins, such as cefotaxime and cefpodoxime, are much higher when broth microdilution methods are used than when agar dilution methods are used *in vitro* susceptibility testing of *P. vulgaris*. Proposed mechanisms for this MIC gap phenomenon are unclear (105).

Quinolones and aminoglycosides are usually active against *P. vulgaris* strains (45), though *qnr* genes for quinolone resistance have been detected in recent isolates (52, 92). Tigecycline has lesser activity against >*P. vulgaris* than against other *Enterobacteriaceae* (for example, MIC_{50} 4 µg/mL against *P. vulgaris* but 0.25 µg/mL against *E. coli*) (45).

P. penneri: Like *P. vulgaris*, *P. penneri* is naturally resistant to ampicillin, narrow-spectrum cephalosporins and cefuroxime, by virtue of production of a similar beta-lactamase (77).*P. penneri* is considered to be a nosocomial pathogen with an underestimated potential to cause disease, and a recent case report identified a multidrug-resistant ESBL-producing *P. penneri* isolate (107).

P. myxofaciens: One report of *P. myxofaciens* from UTIs in India discussed antibiotic susceptibility, and found this species to be susceptible to imipenem, ciprofloxacin, amikacin, gentamicin, trimethoprim-sulfamethoxazole, aztreonam, ofloxacin and piperacillin and resistant to methicillin and nalidixic acid (129).

In Vivo Experiments

Very few *in vivo* (animal) models of *Proteus* infections have been established in which antimicrobial activities were assessed. Treatment of *Proteus* sepsis in rats with ceftazidime or carbapenems was associated with an increase in the plasma endotoxin concentration (57). However, the antibiotic concentrations in those animals treated with carbapenems were significantly lower than for animals treated with ceftazidime. The significance of this finding is uncertain.

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ANTIMICROBIAL THERAPY

General

Urinary tract infection is the most common clinical manifestation of *Proteus* infections. Empiric treatment for community-acquired urinary tract infection will depend more on susceptibilities of *E. coli* than of *P. mirabilis*, since *E. coli* is by far the more common pathogen. For hospitalized patients or those with urinary catheters, the first decision is whether the isolate is clinically significant. Isolates which are not accompanied by pyuria or symptoms do not warrant treatment. Based on the compiled antibiotic resistance data provided in Table 1, trimethoprim or cotrimoxazole may no longer be viable treatment options for *P. mirabilis* infections. Quinolone resistance is also increasing, and *P. mirabilis* is almost uniformly resistant to nitrofurantoin, tetracycline, and polymyxins. The most appropriate treatment for *P. mirabilis* may be aminoglycosides, carbapenems (except imipenem), and 3rd generation cephalosporins. Recent *P. mirabilis* isolates were also mostly susceptible to augmentin, ampicillin-sulbactam, and piperacillin/tazobactam. In general, treatment should be with intravenous agents (or oral therapy for quinolones) until fever has resolved. Correction of the underlying anatomical abnormality or removal of a urinary catheter is also frequently necessary.

The treatment of choice of *P. mirabilis* bacteremia depends on whether or not the organism is an ESBL producer. Carbapenems are the treatment of choice for ESBL producing isolates causing bacteremia (112). The basis for this statement is not just the almost uniform *in vitro* susceptibility but also increasingly extensive clinical experience. However it must be pointed out that this experience is in organisms such as *K. pneumoniae* rather than *P. mirabilis*. Meropenem is preferred over imipenem for ESBL producing *P. mirabilis* in view of the superior *in vitro* susceptibility of

meropenem against *P. mirabilis* (46). Piperacillin/tazobactam has been successfully used to treat ESBL producing *P. mirabilis* infections in Italy (82). Quinolones are probably a reasonable option if the isolate is susceptible. Cephalosporins are not recommended for the treatment of ESBL producing *P. mirabilis* isolates; failures have been observed (82).

In view of the presence of an inducible beta-lactamase in *P. vulgaris*, we would not recommend penicillins, cefuroxime, ceftriaxone or cefotaxime as first line therapy for serious infections due to this organism. However, the MICs of ceftazidime and aztreonam are almost always less than 1 µg/mL, these antibiotics do not induce production of the beta-lactamase of *P. vulgaris* and the enzyme does not hydrolyze these antibiotics. Therefore, aztreonam, beta-lactam/ beta-lactamase inhibitor combinations, or carbapenems would be reasonable, since these drugs are resistant to the hydrolytic activity of class A beta-lactamase.

The development of resistance to ceftriaxone, occurring during treatment, has been seen with *P. penneri* (82). Treatment recommendations are the same for this organism as for *P. vulgaris*.

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Special Infections

Meningitis:

Proteus meningitis usually follows neurosurgical procedures (28). Third generation cephalosporins are indicated in the treatment of *P. mirabilis* meningitis only if the organism is proven not to be an ESBL producer. Aztreonam has also been successfully used in the treatment of *Proteus* meningitis, and may be an option in penicillin allergic patients (70). Meropenem (2 grams every 8 hours intravenously, in adult patients

with normal renal function) should be regarded as the therapy of choice for meningitis due to >*P. vulgaris*, *P. penneri* and ESBL producing *P. mirabilis*. Although only a single case report of failure exists, ceftriaxone or > cefotaxime should probably be avoided for >*P. vulgaris* or *P. penneri* meningitis (77). Removal of neurosurgical hardware should be considered wherever possible.

Endocarditis:

Infective endocarditis due to *P. mirabilis* has been rarely reported. The few cases that have been reported appear to have been related to prosthetic valves (8, 50). Therefore early surgical intervention is likely the key to successful outcome. Therapeutic options would appear to be an appropriate beta-lactam (see section on therapy of bacteremia above) plus an aminoglycoside.

Underlying Diseases

The therapeutic recommendations are not different for those patients with immunosuppression.

Alternate Therapy

Serious infections in patients with life-threatening allergies to beta-lactam antibiotics could comprise aminoglycosides or possibly either quinolones or cotrimoxazole. Nitrofurantoin is not an option nor is tetracycline or the glycylcycline class.

ADJUNCTIVE THERAPY

As noted above, early surgical consultation is necessary in patients with *Proteus* endocarditis or post-neurosurgical meningitis. Urologic consultation should be sought in patients with recurrent *Proteus* urinary tract infection, especially in the presence of struvite stones (123).

ENDPOINTS FOR MONITORING THERAPY

Generally, standard clinical endpoints are used for determining the adequacy of therapy for *Proteus* infections. After initiation of therapy, a favorable response is signified by resolution of systemic and local symptoms and signs of infection. In patients with primary or secondary bacteremia, blood cultures should become negative. For urinary tract infections, urine cultures should become negative. In patients with *Proteus* meningitis, a repeat spinal tap after 48 to 72 hours may be helpful to document microbiologic clearance. The duration of therapy after an initial favorable clinical response is generally empiric. Pneumonia, bacteremia and urinary tract infections require at least 10 days of therapy. Meningitis should be treated for 21 days, and endocarditis for at least 42 days.

If fever recurs during therapy, then a superinfection or a drug allergy should be considered. Many of the patients infected with *P. vulgaris* will have serious underlying illnesses which predispose them to superinfections.

VACCINES

No vaccines are commercially available at the present time. However, *P. mirabilis* vaccine candidates are being identified and efficacy tested in a murine model of ascending UTI (6, 53, 74, 75, 76, 103, 126, 127).

INFECTION CONTROL MEASURES

Typing methods for *P. mirabilis* have been studied for greater than 30 years (3, 4). The ability of *P. mirabilis* to swarm over the surface of agar media has been utilized in a typing method known as the Dienes mutual inhibition test (114). The Dienes test is based on the mutual inhibition of two different strains as they swarm towards one another on an agar surface. If the two

strains are genetically distinct, a clear line of demarcation will form as the swarming edge of one strain meets the other. In contrast, if the two strains are related or identical, there is no mutual inhibition and the swarming edges merge with no visible line of demarcation (114). Genetic determinants of Dienes line formation have been identified and are an active area of research (7, 22, 48, 143). Discriminatory power of the Dienes test is virtually identical to pulsed field gel electrophoresis or ribotyping (114). Polymerase chain reaction based methods have also been used to characterize the molecular epidemiology of *P. mirabilis* (42).

Outbreaks of ESBL and non-ESBL producing *Proteus mirabilis* infections have occurred. The gastrointestinal tract is the likely reservoir of infection (30). We believe that contact isolation precaution measures should be used as a mode of control of spread of ESBL producing *P. mirabilis*. Such an approach requires the identification of asymptomatic carriers of the organism and then accommodation of such individuals in single rooms or cohorting with other colonized patients. Those who enter the room of a patient colonized with an ESBL producing organism should wear gloves and gowns and practice appropriate hand hygiene on leaving the patient's room and removal of the protective apparel. Restriction of use of third generation cephalosporins should also be considered to reduce selective pressure leading to mutations contributing to ESBL production.

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Back to Top

Quorum sensing

Dr .Azal Alaa

Lec 6

Quorum sensing

- is the main method of communication between the bacterial cells within the biofilm and may also serve as a mechanism to coordinate and regulate the multiple virulence factors in *A. baumannii*. There are an indicating that quorum sensing might possibly be involved in host–pathogen interactions as well. Thus, biofilm formation and quorum sensing are important components in the wide arsenal of virulence determinants produced by *A. baumannii*.

- Quorum sensing (QS) is a bacterial communication process that allows bacteria to share information about cell density and gene expression. Also it involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs). Among the many traits controlled by quorum sensing is the expression of virulence factors by pathogenic bacteria.

Bacteria can communicate by producing and responding to autoinducers (AIs) which are small diffusible molecules that act as signals. They are produced at basal levels and their concentration increases with growth. Because the signals can diffuse through membranes, their concentration inside cells approximates the concentration in the environment.

Upon reaching a critical concentration, the signal molecules can bind to and activate receptors inside bacterial cells. These receptors can then alter gene expression to activate behaviors that are beneficial under the particular condition encountered.

So the AIs features summarize in:

- They are small diffusible signal molecules termed in which bacteria communicate .
- These molecules are produced at basal levels and accumulate during growth. Once a critical concentration has been reached, autoinducers can activate or repress a number of target genes.
- Because the control of gene expression by autoinducers is cell-density-dependent, this phenomenon has been called quorum sensing in which controls virulence gene expression in numerous micro-organisms.

The most studied AIs are the N-acylhomoserine lactone (AHLs) of Gram-negative bacteria. The peptides of Gram positive bacteria and the class of AIs termed **AI-2**, whose structures remain unknown .

AHLs are usually detected through **binding to** and **activation of cytoplasmic receptor** proteins, which dimerize upon signal detection and can bind to promoter regions of target genes to activate or repress their transcription.

- - Peptides are usually detected through binding to membrane sensor proteins of the two-component system family, although some can also be transported to the cytoplasm before interacting with their receptors.
- - **AI-2** binds a periplasmic protein and then interacts with either a two component system or a transporter depending on the organism.
- Binding to a membrane-associated sensor kinase causes the **activation of a phosphorelay cascade**, which results in the activation or repression of a response regulator.

Quorum sensing in *Staphylococcus aureus* virulence

- Many Gram-positive bacteria utilize peptide quorum sensing systems to control gene expression and *S. aureus* has served as a model to study bacterial peptide signaling.
- ***S. aureus* forms biofilms** on many surfaces, including indwelling devices such as urethral stents. These indwelling devices, and subsequent biofilms formed on them, pose a serious risk for *Staphylococcus* infection.

- One of the factors which contribute to *S. aureus* virulence is its peptide-based quorum sensing system, encoded by the accessory gene regulator (agr) locus. The autoinducer in the agr system is an oligopeptide that has been termed the autoinducing peptide (AIP).

- The virulence factors regulated by agr, there are two classes:
- **the first class** contains virulence factors involved in attachment to the host and immune evasion, **while the second class** contains genes involved in the production of exoproteins associated with invasion and toxin production.
- It has been thought that the activation of the agr system essentially switches the bacterium from an adhesive, colonizing commensal to an invasive and aggressive pathogen.

- One of the ways in which agr is thought to impact virulence is through **its role in biofilm formation**.
- The formation of biofilms by bacteria is a multi-step developmental process that starts with adhesion to a surface.
- Attached bacteria divide and give rise to macrocolonies. These macrocolonies later develop into mature biofilms, which can assume multiple topographies. The last step in biofilm development is detachment (dispersal) , which may be important for dissemination during an infectious process.

- Because biofilms are thought to play a critical role in *S. aureus* infection, the role of agr in biofilm formation has been explored. When agr is non-functional, *S. aureus* has enhanced adhesion abilities.
- Therefore, when agr is not active, the bacteria remain in the first stage of biofilm formation, adhering to a surface.
- However, agr is also important for detachment of clusters of cells from the biofilm.
- Agr mutant strain has a detachment defect, and the detachment of bacterial cells from biofilms was found to coincide with agr expression.
- In addition, an agr mutant has increased adherence and more robust formation of static biofilms than its agr-containing counterparts.

- It is thought that this role of agr is brought about by the reduction in adhesin production and an increase in the production of both **δ-haemolysin and proteases**. Thus, an important role of quorum sensing in *S. aureus* is the regulation of biofilm formation, a central factor in *S. aureus* virulence.
- agr may play different roles during the course of infection and this may explain the discrepancy in some of the results regarding its role in bacterial virulence.
- specific agr groups have been associated with some diseases, such as toxic shock syndrome, while other diseases, such as infections of CF patients, are associated with all four agr groups.

Quorum sensing control *Ps. aeruginosa* virulence

- *P. aeruginosa* are difficult to eradicate, due to their high levels of antibiotic resistance and growth in biofilms.
- At least three intertwined quorum sensing systems and one orphan autoinducer receptor affect the ability of *P. aeruginosa* to cause disease. Two of these systems, las and rhl, rely on the production of AHLs as the signalling molecules (AIs).

- Multiple *P. aeruginosa* virulence factors are involved in the development of disease, including secreted factors (such as proteases) and cell-associated factors (such as lipopolysaccharide and flagella), as well as the ability to form biofilms.
- Quorum sensing regulates the production of several extracellular virulence factors, promotes biofilm maturation and regulates the expression of antibiotic efflux pumps, meaning that it has a key role in the pathogenesis of *P. aeruginosa*.

- The **las and rhl** systems regulate the timing and production of multiple virulence factors, including elastase, alkaline protease, exotoxin A, rhamnolipids, pyocyanin, lectins and superoxidase dismutase.

- The expression of these two quorum sensing systems has also been linked to the regulation of biofilm formation.

- Quorum sensing signalling may start in the early stages of biofilm development, which is characterized by microcolony formation, where lasI mutants are unable to form structurally normal biofilms.

- The expression of rhlI fluctuates during biofilm development and phenotypes of biofilm development with a rhlI mutant vary, according to the media and model used, supposedly due to different iron levels present.

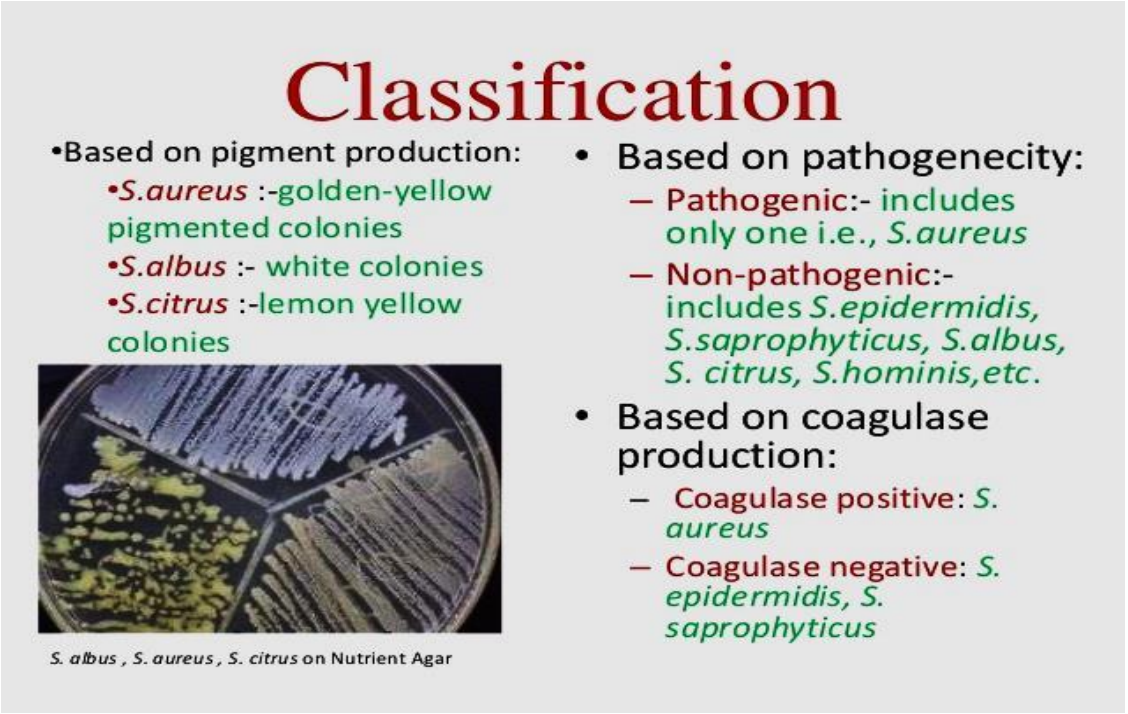
- *Pseudomonas* quinolone signal; PQS has been shown to affect biofilm formation and to regulate several virulence factors in *P. aeruginosa*, including elastase, pyocyanin and LecA lectin, and it is considered essential for full virulence in multiple hosts.
- PQS has been found in sputum, bronchoalveolar fluid and mucopurulent fluid from CF patients, suggesting that it may play an important role during the infection process.
- PQS can also act as an iron chelator, and both the synthesis of PQS and the activity of PqsR–PQS are involved in iron homeostasis, another indication of the global importance of quinolone signalling for *P. aeruginosa.*

Lec.8

Staphylococci

Staphylococci are typical Gram-positive bacteria forming irregular clusters of cocci. Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds, but can cause infection under certain circumstances.

- *Staphylococcus aureus* is more pathogenic than the other common members of the genus, *S. epidermidis* and *S. saprophyticus.*
- *Staphylococcus epidermidis* has been known to cause various hospital-acquired infections (such as prosthetic or indwelling devices), whereas
- *Staphylococcus saprophyticus* is mainly associated with urinary tract infections in young females who are sexually active.



Disease processes with *Staphylococcus aureus* are numerous. The portal of entry is variable, since they gain access to the body via the skin, the respiratory tract or the genitourinary tract. *Staphylococcus aureus* expresses many potential virulence factors:

A. Surface virulence factors:

1. Capsule: Most S aureus strains of clinical importance have polysaccharide capsules, which inhibit phagocytosis**.**

2. Protein A - the bacteria disrupts opsonization and phagocytosis through this surface factor.

B. Secreted virulence factors:

Enzymes produced by staphylococci:

1. **Catalase** converts H_2O_2 to H_2O and O_2.
2. **Coagulase** converts fibrinogen to fibrin and is the primary test used to distinguish S. aureus from the other staphylococcal species
3. **Hyaluronidases** hydrolyze hyaluronic acids and may contribute to tissue breakdown and spread of staphylococci across tissue barriers.
4. **Beta-lactamases** are released by staphylococci and can hydrolyze the beta-lactam ring of penicillins and cephalosporins rendering the antibiotics useless.
5. **Hemolysins** are lipids and proteins that cause lysis of red blood cells by destroying their cell membrane.

Toxins produced by staphylococci:

1. Exfoliated toxins:

These epidermolytic toxins of *S aureus* are two distinct proteins of the same molecular weight. Exfoliative toxin A heat stable (resists boiling for 20 minutes) & Exfoliative toxin B heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis.

2. Toxic Shock Syndrome Toxin

Most *S. aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1). TSST-1 binds to major histocompatibility class (MHC) class II molecules, yielding T-cell stimulation, which promotes the protean manifestations of the toxic shock syndrome. The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash.

3. Enterotoxins

There are multiple (A–E, G–J, K–R and U, V) enterotoxins that, similar to TSST-1, are superantigens. Approximately 50% of *S. aureus* strains can produce one or more of them. The enterotoxins are heat stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25 μg of enterotoxin B results in vomiting and diarrhea.

Clinical Significance:

While *Staphylococcus aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can sometimes cause disease**.**

- **Skin infections** are the most common form of *S. aureus* infection. This can manifest in various ways, including small benign boils, folliculitis, impetigo, cellulitis, and more severe. Invasive soft-tissue infections *S. aureus* is a significant cause of chronic biofilm infections on medical implants.
- *S. aureus* is also responsible for **food poisoning.** It is capable of generating toxins that produce food poisoning in the human body. Its incubation period lasts one to six hours.
- In particular, *S. aureus* is one of the most common causes of **bacteremia** and infective **endocarditis**.

Bacteremia:

Staphylococcus aureus is a leading cause of bloodstream infections.

a) Infection is generally associated with breaks in the skin or mucosal membranes due to surgery, injury, or use of intravascular devices such as catheters, hemodialysis machines, or injected drugs.
b) Once the bacteria have entered the bloodstream, they can infect various organs, causing infective endocarditis, septic arthritis, and osteomyelitis.

This disease is particularly prevalent and severe in the very young and very old. Without antibiotic treatment, *S. aureus* bacteremia has a case fatality rate around 80%.With antibiotic treatment, case fatality rates range from 15% to 50% depending on the age and health of the patient, as well as the antibiotic resistance of the *S. aureus* strain.

Epidemiology

Most people carry Staphylococci on their skin and in their anterior nasal nares. The bacteria also colonize most animals and are transmitted by direct or indirect contact.

Methicillin-resistant *Staph. aureus* (MRSA) strains colonize hospital inpatients and may cause epidemic outbreaks of disease among high-risk patients, such as those with post-operative wounds.

Treatment:

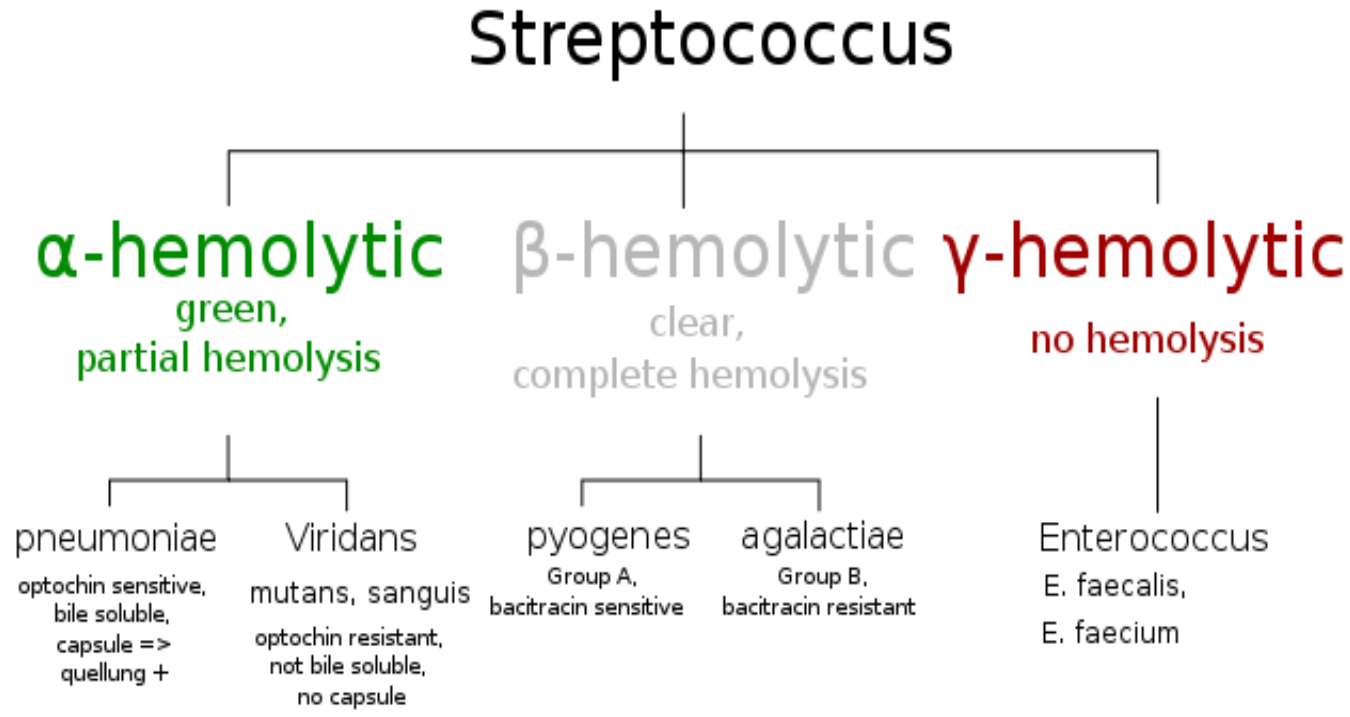
The majority of Staphylococci are resistant to penicillin, but sensitive to some of its derivatives, including methicillin and flucloxacillin. Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin.

Streptococci

Streptococci are oxidase and catalase-negative gram-positive cocci, which are seen in pairs or chains and many are facultative anaerobic. Species of Streptococcus are classified based on their hemolytic properties.

- **Alpha-hemolytic** species cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar.
- **Beta-hemolytic** species cause complete rupture of red blood cells. On blood agar, this appears as wide areas clear of blood cells surrounding bacterial colonies.
- **Gamma-hemolytic** species cause no hemolysis.

Beta-hemolytic streptococci are further classified by **Lancefield grouping**, a serotype classification (that is, describing specific carbohydrates present on the bacterial cell wall).The 20 described serotypes are named Lancefield groups A to V.



In the medical setting, the most important groups are the alpha-hemolytic *Streptococci pneumoniae* and *Streptococcus viridans* group, and the beta-hemolytic streptococci of Lancefield groups A and B (also known as "group A strep" and "group B strep").

A. Alpha-hemolytic

When alpha hemolysis (α-hemolysis) is present, the agar under the colony is dark and greenish. ***Streptococcus pneumoniae*** and a group of oral streptococci (***Streptococcus viridans*** or *viridans streptococci*) display alpha hemolysis. Alpha hemolysis is caused by hydrogen peroxide H_2O_2 produced by the bacterium, oxidizing hemoglobin to green methemoglobin.

1. Streptococcus pneumoniae

S. pneumoniae (pneumococcus) is a leading cause of bacterial pneumonia and occasional etiology of otitis media, sinusitis, meningitis, and peritonitis.

The most important virulence determinant of pneumococci is the **polysaccharide capsule**. This capsule interferes with phagocytosis. The cell wall (peptidoglycan) is believed to contribute to initiating the inflammatory response following infection.

Pneumonia: is a disease of the lung that is caused by a variety of bacteria including Streptococcus, Staphylococcus, Pseudomonas, Haemophilus, Chlamydia, Mycoplasma, several viruses, certain fungi and protozoans. The classical presentation of pneumococcal pneumonia is the sudden onset of fever, chills, pleuritic chest pain and cough associated with rusty colored sputum.

2. *Streptococcus viridans*

The viridans streptococci are a large group of commensal bacteria, that are either α-hemolytic, producing a green coloration on blood agar plates or non-hemolytic.

They are part of the oropharyngeal flora and some species can also be found in the gastrointestinal tract.

They are relatively avirulent organisms however they do cause **dental caries** (like *S. mutans*) and are the most common cause of **subacute infective endocarditis**.

B. Beta-hemolysis

Beta hemolysis (β-hemolysis) is a complete lysis of red cells in the media around and under the colonies, the area appears lightened (yellow) and transparent.

Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells.

1. *Streptococcus pyogenes* (Group A Streptococcus) (GAS)

They cause infections that vary in severity ranging from minor soft tissue to life threatening sepsis.

The outer surface of GAS consists of a unique **hyaluronic acid capsule** that interferes with phagocytosis. The cell wall consists of a large number of different antigenic molecules that include group specific **carbohydrate antigens**, **lipoteichoic acid** and **proteins** that extend to the cellular surface and are involved in adherence and invasion.

These surface proteins facilitate attachment to molecules found in the extracellular matrix and on host cells. **M protein** is the major virulence factor of GAS. The protein interferes with phagocytosis and strains defective in M protein are avirulent. The M proteins appear to promote colonization of tissue surfaces.

Summary of diseases caused by *Strep. pyogenes*

A. **Suppurative infections** (associated with pus occur in the throat, skin & systemically).

1. Respiratory tract infections

Throat: *S. pyogenes* is the leading cause of **Acute follicular tonsillitis**. It is acquired by inhaling aerosols by infected individuals. The symptoms start quickly, pain, red and swollen tonsils, sometimes with white patches or streaks of pus

2. Skin

Impetigo (superficial) infection of epidermal layers of skin.
Cellulitis (deep) occurs when the infection spreads subcutaneous tissues.

3. Systemic

Scarlet fever (rash): is an infection that can develop in people with strep throat. It is characterized by a bright red rash on the body accompanied by high fever and sore throat. It affects children between 5-15 years.

B. Non-suppurative complication:

Two post streptococcal sequelae: **rheumatic fever** & **glomerulonephritis**, may follow streptococcal disease, and occur in 1-3% of untreated infections.

Rheumatic fever, a disease that affects the joints, kidneys, and heart valves, is a consequence of untreated strep A infection caused not by the bacterium itself. Rheumatic fever is caused by the antibodies created by the immune system to fight off the infection cross-reacting with other proteins in the body. This "cross-reaction" causes the body to essentially attack itself and leads to the damage above.

2. *Streptococcus agalactiae* (Group B streptococcus)

Group B streptococci cause a narrow band of beta-hemolysis on blood agar plates. Although they can cause disease in other groups, they are most commonly associated with infections of the **newborn**.

Newborns are at particular risk of Group B sepsis or meningitis if their mother is vaginally colonized with GBS.These infections are life-threatening and can also result in permanent disability to the infant.

C. Gamma hemolysis

If an organism does not induce hemolysis, the agar under and around the colony is unchanged, and the organism is called non-hemolytic.

Enterococcus faecalis displays gamma hemolysis. It is normal flora in the gut but could be pathogenic in other places.

It can cause **bacteremia**, **urinary tract infection**, **endocarditis** and **Periodontitis** which is serious gum infection damages the bones that hold the teeth in place. It's often found in people who've had a root canal.