Ministry of Higher Education and
Scientific Research
University of Baghdad
College of Science
Department of Biology

Theoretical Pathogenic Bacteria 2020-2021

المرحلة الرابعة / الدراستين الصباحية والمسائية

الكورس الاول

تدريسي المادة:
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Lecture : (1)

Gram positive cocci

Genus: *Staphylococcus*

- **General characteristics**

Staphylococci are non sporulating, non-motile Gram-positive cocci. Microscopically, these organisms are typically found in grape-like clusters and tetrads, as well as in pairs and sometimes in short chains. Usually unencapsulated or have limited capsule formation. When grown on blood agar, staphylococci form small (1 to 2 mm), smooth, round colonies that are often pigmented and may be surrounded by a zone of β-hemolysis.

Staphylococci are very hardy organisms and can resist drying, withstand 10% NaCl broth, and will survive at temperatures between 10° and 45° C. Because staphylococci are facultative anaerobes, they will grow in the presence or absence of oxygen. Staphylococci are catalase positive. They reside on mucous membranes, skin and anterior naris.

Classification of species depends mainly on:

1. Aerobic acid production from different carbohydrates.
2. Coagulase activity.
3. Haemolysis.
4. Nitrate reduction.
5. Genetic methods: ribotyping, DNA - DNA hybridization.

Coagulase production separates the staphylococci into **two** major groups:

A- Coagulase positive: The coagulase-positive species *S. aureus, S. intermedius, S. delphini, S. schleiferi* subsp. *coagulans* and the coagulase-variable species *S. hyicus* are regarded as potentially serious pathogens.

B- Coagulase negative:

S. epidermidis, S. saprophyticus and many other species.

In general, the staphylococci are variably sensitive to antimicrobial drugs. Resistance could be produced by:

1. Production of β- lactamase (penicillinase): It is controlled by a plasmid, thus they are resistant to penicillins and cephalosporins (more than **95** % of staphylococci isolates are resistant to those antibiotics).

2. Methicillin Resistant *Staphylococcus aureus* (MRSA): Methicillin is β- lactamase resistant. The responsible genes may be resides in the plasmid or mostly in the chromosome. This character is important especially in nosocomial infection. Bacteria that are resistant to methicillin are also **resistant to all** other antibiotics in the beta-lactam class, which includes penicillin derivatives and cephalosporins. The only antibiotics available to kill MRSA are powerful and potentially toxic options such as **vancomycin**

3. Plasmids - mediated resistance to tetracycline, erythromycin, and aminoglycosides.

I- *Staphylococcus aureus*

❖ Antigenic structure

1- **Tcichoic acids** (PG linked); **lipoteichoic** acids (membrane associated). Regulates cationic environment.

2- **Protein A:** It is responsible for agglutination test known as **COAGGLUTINATION** it combined with Fc portion of IgG molecule. The Fab portion of IgG bound to protein **A** is free to combine with a specific antigen. Thus, it reduces the false positive reactions.

3- **Capsular polysaccharides.** Eleven serotypes have been reported. Types 1 and 2 are highly encapsulated, mucoid strains that are virulent for experimental animals, but rarely encountered among clinical isolates of

S. aureus. These strains produce a **"microcapsule"** which may be antiphagocytic.

4- **Peptidoglycan:** can lead to fever and alternative complement activation that leads to inflammation followed by leukopenia, thrombocytopenia, and shock.

5- **Adhesins:** Specific cell wall associated surface proteins of *S. aureus* that bind to matrix proteins such as fibronectin, fibrinogen (clumping factor), collagen, bone sialoprotein, etc. These binding activities are thought to be involved in binding and colonization of various body sites and specific cell types.

❖ Toxins and enzymes

1- **Staphylolysins:** exotoxins and they are α, β, γ, δ haemolysins:

- *Alpha- haemolysin;* It is a dermonecrotic protein, dissolves rabbit erythrocytes, damages platelets and has powerful action on vascular smooth muscles.
- *Beta- haemolysin:* Dissolves sheep erythrocytes upon incubation for 1 hr at 37°C, and 18 hr at 10°C (this is called hot and cold reaction). Antigenicity is distinct from other haemolysins. Toxic for many kinds of cells including human erythrocytes.
- *Gamma- haemolysin:* is lytic for erythrocytes from different mammalian species and also cytotoxic for leukocytes. Gamma-toxin has also been proposed to play a role in the pathogenesis of toxic shock syndrome (TSS) together with toxic shock syndrome toxin 1 (TSST-1).
- *Delta- haemolysin:* Dissolves erythrocytes of sheep, mice, guinea pigs, rabbits, horses, rats, as well as human. Leukotoxic as well as dermonecrotic.

2- Leukocidin (Panton-Valentine Leukocidin):

Found in 5% of all *S. aureus* strains and in 50% of ones from abscesses. Lethal to PMNs, disrupts their membranes through pore formation which leads to increased permeability.

3- Enterotoxins:

It is a neurotoxin affects the vomiting center in the CNS, which causes vomiting and diarrhea. The infection needs the presence of the bacteria, while the toxication does not.

4- Toxic shock syndrome toxin-1 **(TSST**-1):

Found in almost all *S. aureus* strains, isolated from individuals suffering from TSST. About 90% of healthy individual have antibodies against TSST-1. TSST patients either do not have antibodies or have them at very low levels.

5- Exfoliatine toxin (ET):

It is involved in Staphylococcal scalded skin syndrome **(SSSS).** Mostly phage group II strains of *S. aureus* controlled by a plasmid or chromosomal gene or both.

6- Coagulase:

Plasma clotting protein deposits fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase is considered as an **invasive** factor rather than spreading factor.

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Lecture : (2)

7- Clumping Factor (fibrinogen binding protein):

Clumping factor allows *S. aureus* to adhere to fibrinogen. Coagulase and clumping factor are **distinct** entities.

8- Other enzymes: Such as hyaluronidase, staphylokinase, proteases, lipases, β-lactamase and thermostable nucleases (DNases, RNases).

❖ **Pathogenicity**

a. <u>Infections of the skin and associated structures</u>:

Pyoderma (purulent skin infections): abscesses, furuncles, carbuncles, and impetigo.

b. <u>Staphylococcal Scalded Skin Syndrome (SSSS):</u>

Most often observed in infants and young children. The syndrome begins as erythema around the mouth and nose and spreads rapidly to affect the skin of the neck, the trunk, and sometimes the extremities. The epidermal necrolysis mediated by the toxin exfoliation results in extensive areas of denuded skin. The intraepithelial split in **SSSS** is in the granulosa layer.

c. <u>Gastrointestinal tract Staphylococcal diseases:</u>

i. **Staphylococcal enteritis:** patients receiving chemotherapeutic agents especially via the oral route (reduce the normal flora).

ii. **Staphylococcal food poisoning.**

d. <u>Deeper infections:</u>

They are either primary infections or may stem from an infection that has metastasized form a cutaneous infection or from carrier site. They are not likely to occur in healthy individuals but rather in those who have a precondition such as extensive surgery, burns, diabetes, cystic fibrosis, lower respiratory

tract viral infections, ulcers and immunodefective (or immunosupressed) individuals, *S. aureus* can cause infection in many tissues and systems and organs: e.g. endocarditis, cystitis, meningitis, pneumonia, septicaemia, infection of post-operative wounds and others like osteomyelitis

e. Toxic shock syndrome: a multisystem, febrile illness with hypotension, vomiting, diarrhea, rash with subsequent palmer and plantar desquamation. Hyperaemia of mucous membranes can occur regularly.

❖ **Diagnostic lab tests**

a) Specimens: it depends on the localization of infection: surface swab, pus, blood, tracheal aspirate, spinal fluid. Antibody determination in serum is rarely of value in the beginning of infection but when there is chronic infection antibodies are very important to be detected.

b) Stained smears: "after taking the specimens" typical staphylococci appear; gram positive bacteria in grape shaped could be seen in stained smear of pus and sputum.

c) Culture: on blood agar, typical colonies appear in 18 hrs at 37 °C after several days we could see the haemolysis and pigment production. In case of mixed flora, we could use media containing 7.5% NaCl (Staph 110 and mannitol salt agar. On the latter they develop yellow color). On Baired Parker agar colonies appear black surrounded by clear halo.

d) Coagulase test: it separates staphylococci into two groups; coagulase positive and coagulase negative.

e) Catalase test: it differentiates the staphylococci, which are positive, from the streptococci, which are negative.

f) **Serological tests:** Antibodies to teichoic acid specially can be detected in prolonged, deep infections (e.g. endocarditis). These serologic tests have low practical value.

g) **PCR.**

❖ **Epidemiological (nosocomial) infections**

In case of hospital acquired infection or an epidemic spread out in the hospital, due to *S. aureus* it is so important to diagnose the type or strain of *S. aureus* that cause the infection. This goal can be achieved by following a kind of tests known as "EPIDEMIOLOGICAL MARKERS":

1) **Biotyping:** not all isolates that had been isolated from different sites, are 100% identical in all characters.
2) **Phage typing:** there are about 100 phages (can infect more than 22 specified types of *S. aureus)* classified into four groups called lytic groups.
3) **Antibiogram**: similar or identical patterns of resistance to antibiotics can also be used as presumptive evidence of the relatedness of strains in epidemiological tracing. Methicillin resistance is very important.
4) **Serotyping**: there are more than 30 specific antigens (agglutinogens)
5) **Geneting methods**: DNA hybridization or plasmid profile.
6) **Polymerase chain reaction (PCR)**

❖ **Treatment**

Gram positive bacteria usually treated by penicillin but that were until 1970 then resistant strains appeared and penicillin becomes to cause allergy. So we can use: Cephalosporin, Methicillin, Oxacillin or an aminoglycoside.

Refampin (in case of endocarditis, since it can reach inside the valve). In case of methicillin resistance vancomycin is appropriate alternative.

❖ **Sources of infections**

1. **Patients** with lesions are discharging staphylococci.
2. **Healthy carriers:** they are healthy, but carrying the microorganisms, hence they considered as source of infection, especially food handler.
3. **Animals:** some of them have strains that affect humans and could be transmitted to human by direct contact with animals raised at homes and cause disease.

❖ **Prophylaxis**

No vaccine had been developed against *S. aureus* because antigens are very wide, toxins have variable mode of action. However, cleaning is very important to prevent infection.

II- COAGULASE NEGATIVE STAPHYLOCOCCI (CONS)

Interest had increased in CONS when we started using clinical prosthetic devices. They may cause wound infection, UTI, bacteraemia, catheters infections, vascular grafts, and infections of prosthetic devices.

☒ ***S. epidermidis*:** It a major skin inhabitant it causes endocarditis, colonization of prosthetics, bacteraemia, wound infections, and UTI especially in elderly hospitalized patients, sensitive to novobiocin.

☒ ***S. saprophytics*:** Causes 10-20% of primary Urinary Tract Infections (UTI) in young women (16-35 yrs). They are resistant to novobiocin.

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Lecture : (3)

THE GRAM NEGATIVE COCCI and related organisms

I- *Neisseria*

General characteristics:

1. All are fastidious Gram negative diplococci with a bean shaped configuration, the flat or concave sides are adjacent.
2. Flagella and swimming motility are absent. The cells are nonmotile in liquid media but surface-bound motility ("twitching motility") is frequently observed.
3. All species are aerobic or facultatively anaerobic. The pathogens grow better in 5 to 10% CO_2.
4. All species produce cytochrome oxidase (oxidase positive).

The medically important members are ***N. meningitidis*** **(the meningococcus)** and ***N. gonorrhoeae*** **(the gonococcus),** both are pathogenic for humans (obligate parasite) and typically are found associated **with or inside polymorphonuclear cells**. The organisms are sensitive to low temperature and drying.

Neisseria meningitidis **(the meningococcus)**

Isolated by Weichselbaum in 1887

❖ **Antigenic composition of meningococci**

Antigens are organized in three ways:

1. **Serogroups:** meningococci are divided into at least 12 serogroups based on **chemical and structural characteristics of capsular polysaccharides.** Serogroups: A, B, C, X, Y, Z, 29E, and W-135 are of medical important. A, B, and C produce epidemics. Serogroups X, Y, and Z produce meningitis, single cases; and found in carriers of the disease.
2. **Serotypes:** serotyping based on **outer membrane proteins of the cell wall (e.g. porin proteins).** These serotypes have become important in studies of the epidemiology of infection and in the development of new vaccines.

3. **Immunotypes:** immunotyping based on **lipopolysaccharides.** Neisserial LPS is distinguished from enteric LPS by its highly-branched basal oligosaccharide structure and the absence of repeating O-antigen subunits. For these reasons, neisserial LPS is referred to as **Lipooligosaccharides (LOS)**.

❖ **Pathogenicity of meningococci**

The most important virulence factors contributing to disseminated disease are pili, IgA1 protease, LOS, outer membrane proteins, and capsule polysaccharides.

Infection with *N. meningitidis* has two presentations, **meningococcemia,** characterized by skin lesions, and **acute bacterial meningitis.** The fulminant form of disease (with or without meningitis) is characterized by multisystem involvement and high mortality.

Infection is by aspiration of infective bacteria, which attach to epithelial cells of the nasopharyngeal and oropharyngeal mucosa by the aid of pili, cross the mucosal barrier, and enter the bloodstream. It is not clear whether blood-borne bacteria may enter the central nervous system and cause meningitis.

The mildest form of disease is a transient bacteremic illness characterized by a fever and malaise; symptoms resolve spontaneously in 1 to 2 days. The most serious form is the fulminant form of disease complicated by meningitis.

Chills, fever, malaise, and headache are the usual manifestations of infection. Signs of meningeal inflammation are also present.

The majority of people who contract bacterial meningitis and meningococcal septicemia survives and make a full recovery; however, some are left with major residual effects such as deafness, mental retardation, and behavioral defects.

❖ **Diagnostic laboratory tests of meningococci**

- ☒ **Specimens:** specimens of blood are taken for culture, and specimens of spinal fluid (lumbar puncture) are taken for smear, culture, and chemical determination.
- ☒ **Smears:** gram stained smears of the sediment of centrifuged spinal fluid often show typical *Neisseriae* **within** polymorphonuclear leukocytes or extracellularly.
- ☒ **Culture:** specimens are cultured on media such as chocolate agar, Mueller-Hinton agar or modified Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin). Yielded cultures can be further identified by carbohydrate fermentation reactions.
- ☒ **Oxidase test.**
- ☒ **Sugar fermentation test.**
- ☒ **Serology:** it usually performed on CSF, such as capsular swelling, fluorescent antibody staining, and counter Immunoelectrophoresis (CIE). Negative capsular swelling tests and negative CIE test results ***do not necessarily rule out*** a meningococcal infection because serogroups other than A and C frequently possess insufficient capsular antigens to be detected by these methods.

❖ **Prevention and control of meningococci**

A) Chemotherapy

Antimicrobial drugs; Sulpha drugs were drug of choice until bacteria developed resistance against these drugs. Therefore penicillin is the drug of choice. In cases of penicillin allergic patients, chloramphenicol or ampicillin with moxalactam could be used.

Ciprofloxacin, ceftriaxone or rifampicin may be used prophylactically as a means of preventing the disease state in carriers.

B) Active immunization

Immunizing materials have been developed against serogroups A, C, Y, and W-135 meningococci. However, Group C vaccine does not

induce protective levels of antibody in children younger than 2 years. Group B are poorly immunogenic.

❖ **Epidemiology**

Meningococcal are endemic worldwide. They inhabit mainly the nasopharynx and transmitted via droplets or close kissing contact. Of the young adult population, 10-30% carry meningococci. Carriage is highest among enclosed populations. The most vulnerable age groups are children aged < 5 years, particularly those < 2 years, followed by teenagers and young adults. This disease is more common during autumn and winter seasons.

Neisseria gonorrhoeae **(gonococcus GC)**

Isolated in 1879 by Neisser. They considered as one of the most common sexually transmitted pathogen.

❖ **Antigenic structure of gonococci**

Neisseria gonorrhoeae is antigenically heterogeneous and capable of changing its surface structures in vitro, and presumably in vivo, to avoid host defenses. Surface structures include: pili, porins proteins, opacity associated proteins (opa), LOS and others. Gonococci do not have capsular polysaccharides.

❖ **Virulence factors of gonococci**

The following factors are currently regarded as possible agents of gonococcal pathogenicity:

1) **Pili:** mediate initial attachment of gonococci to epithelial cells. Gonococci produce four colony forms: T1, T2, T3, and T4. Only the bacteria in T1 and T2 colonies are piliated, and are regarded as virulent because they produce infection in volunteers.

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Lecture : (4)

2) Por proteins (protein I): when the gonococcal membrane is in intimate contact with the host cell membrane, the Por protein is transferred to the host cell, resulting in alterations in ionic permeability of the host cell plasma membrane. May contribute to the intracellular survival of gonococci inside of neutrophils.

3) Opa (protein II): afimbrial adhesins that mediate firm attachment of gonococci to epithelial cells.

1) **IgA protease:** cleaves and inactivates the SIgA subclass IgA1. Nonpathogenic *Neisseria* species do not produce IgA protease.
2) **Epithelial endocytosis:** the gonococci attach to the epithelial cells of the cervix, are subjected to endocytosis, multiply within the cell, and are protected from the phagocytic activities of leukocytes.
3) **Endotoxin (LOS):** mediates most of the toxic damage in the epithelial cells, and regulates complement activation on the surface of the organism. It also is implicated in the attachment of gonococci to host cells by piliated and nonpiliated organisms. The sialylation of the LPS results in the conversion of a serum-sensitive organism to serum-resistant. Gonococcal endotoxin is probably responsible for some disseminated gonococcal infections (DGI).

❖ **Gonococcal diseases**

A. Gonorrhea

Gonorrhea is one of the most common bacterial venereal diseases. Complicating the high incidence of disease is the increasing appearance of multiple antibiotic resistance. The disease is generally spread via sexual activity.

Disease in men occurs after an incubation period of from 2-14 days. Onset of disease is usually marked by mild discomfort in the urethra, followed a few hours later by dysuria and a purulent yellowish green urethral discharge. In women, symptoms usually begin within 7-21 days after infection. A significant portion of infected women is

asymptomatic (70 - 80%). However, onset is sometimes severe, with dysuria and vaginal discharge. The cervix and deeper reproductive organs are the sites most frequently infected.

Complications, in men, postgonococcal urethritis, a common sequela, actually results from infection with other organisms (e.g., *Chlamydia trachomatis*). Epididymitis is uncommon and usually unilateral. Prostatitis and urethral stricture are less common. Ascending infection may result in infertility. In women, salpingitis (pelvic inflammatory disease) is the most important clinical problem. This syndrome has two important consequences: 1) sterility and ectopic pregnancy; and 2) susceptibility to chronic infections. DGI with bacteremia is more common among women than men.

B. Extragenital infections

i. **Pharyngitis and conjunctivitis**: Infants born to a mother with cervicovaginal gonorrhea may develop gonococcal conjunctivitis.

ii. **Gonococcal arthritis** may be preceded by symptomatic bacteremia. The onset typically is acute, with fever, severe pain and limitation of movement in one or a few joints.

❖ **Pathogenesis of gonococci**

Gonococci initially attach to host epithelial cells via **pili**. Closer attachment is then quickly mediated by the **Opa** protein. Secretory IgA protease protects the organisms from antibodies present at the mucosal surface. Some epithelial cells are damaged by gonococcal LOS, but **others** are probably **invaded** by the organism. After attachment and initial colonization, the sequence of events probably includes: **1)** Entry of gonococci into the host cell by endocytosis. **2)** Intracellular replication inside the endocytic vesicle. (Host cell killing of bacteria within the vesicles is inhibited by the membrane perturbing activities of **Por**). **3)** Transport of the vesicle to the basal of the cell, fusion with the cell membrane, and release of the

gonococci into the subepithelial tissue, the lamina propria. **4)** Multiplication in the lamina propria aided by iron acquisition systems. **5)** The gonococci have opportunities to spread because of the proximity of the lamina propria to regional lymphatics and blood vessels; DGI happens in approx. 1% of cases.

❖ **Laboratory diagnosis of gonococci**

Laboratory identification procedures include the following:

1) **Specimens:** The specimens selected for diagnosing gonorrhea depend on the gender, age and sexual preference of the patient. Urethral and cervical specimens routinely are collected from men and women, respectively. However, the organism may be isolated from blood, the nasopharynx, skin lesions, CSF and the anal canal of homosexual.
2) **Direct examination:** Gram stain of purulent materials may reveal Gram-negative diplococcic in polymorphonuclear leukocytes.
3) **Culture:** Specimens are cultured on media such as supplemented chocolate agar or Thayer-Martin medium in an elevated CO_2 environment". Blood cultures and cultures of synovial aspirates or skin lesions should be attempted for patients with suspected disseminated gonococcal infection (DGI).
4) **Oxidase test.**
5) **Superoxol test:** catalase test using 30% H_2O_2 was used to differentiate *N. gonorrhoeae* from other *Neisseria* species.
6) **Carbohydrate fermentation**.
7) **Serological tests:**
   a. Direct fluorescent antibody technique.
   b. Staphylococcal coagglutination technique.
8) **Molecular methods:** The microbiological diagnosis of gonorrhea based on culture on selective medium produces about 80 - 95% sensitivity, with false-negative results attributed to poor specimen storage, transport problems, and inhibition of growth by the

components of selective media. As an alternative diagnostic test, molecular techniques have been developed and offer the promise of eliminating transport and specimen collection issues, which are believed to affect test sensitivity in the field setting.

❖ **Treatment of gonococci**

The recommended treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolone plus an antibiotic (e.g. doxycycline or erythromycin) effective against possible coinfection with *Chlamydia trachomatis*. Sex partners should be referred and treated. The current **CDC Treatment Guidelines** recommend treatment of all gonococcal infections with antibiotic regimens effective against resistant strains. The recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin, or oflaxacin.

Prophylaxis of "ophthalmia neonatorum" can be achieved by local application of 0.5 % erythromycin ophthalmic ointment or 1% tetracycline ointment.

No vaccine was prepared until now. The patients failed to develop a solid immunity.

I- *Acinetobacter*

Includes many species of gram negative diplococci or coccobacilli. Widely distributed in nature and are part of the normal human flora (skin).

Acinetobacter calcoaceticus an opportunist causes variety of infections especially hospital associated infections; Wound infections, Meningitis, Pneumonia, Bacteraemia, and Urethritis.

II- *Moraxella*

The moraxella group includes six species. They are nonmotile, nonfermentative, and oxidase positive. On staining, they appear as

small gram negative bacilli, coccobacilli, or cocci. They are members of the normal flora of the upper respiratory tract and occasionally cause bacteraemia, endocarditis, conjunctivitis, meningitis, or other infections. Most of them are susceptible to penicillin.

A) ***Moraxella catarrhalis***

It was previously named ***Branhamclla catarrhalis*** and before that ***Neisseria catarrhalis.*** It is a member of the normal flora in nasopharynx. It causes bronchitis, pneumonia, sinusitis, otitis media, and conjunctivitis.

B) ***Moraxella lacunata*****:** Causes conjunctivitis.

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Lecture : (5)

I- *Bacillus*

Saprophytes found in soil, water, air and vegetation. The mostly important genera are *B. anthracis, B. cereus, B. thuringiensis, B. subtilis* and *B. megaterium*.

B. anthracis

General characteristics

Members of this species are non-motile, facultative anaerobes, large bacilli measuring 4-8 µm in length and 0.5-1.0 µm in width. They have square ends and arranged in long chains, usually encapsulated with centric spore. Colonies on agar plate are large, opaque, white, medusa head, and have "cut glass" appearance in transmitted light.

Antigenic structure

1. **Capsular polypeptide:**

This capsule composed of Polyglutamic acid and it is antiphagocytic.

2. **Somatic polysaccharides:**

Found in cell wall, it does not considered as a virulence factor and it is not immunogenic.

3. **Protein exotoxin complex:**

The toxin components include the edema factor, lethal factor and protective antigen. The latter protects the former two from body proteases before entering host cells, and it also induces protective antibodies when used as a vaccine.

Pathogenicity

B. anthracis is a pathogen of animals (herbivorous). Spores found in soil and on vegetation ingested, inhaled or gain entrance through abraded skin or mucosa. The spores germinate in the tissue and transformed into vegetative cells, which

produce the exotoxin. The capsule inhibits phagocytosis. In human, the disease considered as an occupational hazard.

Diseases in human

1- Skin infection (cutaneous anthrax):

The organism enters through abraded skin, after 12-36 hrs a papule is formed which changed into a vesicle, then to a pustule, eventually into a dark necrotic area surrounded by a rim of edema.

2- Wool sorters disease

Inhaled spores settle in the respiratory tract, Producing local hemorrhage and edema.

3- Septicemia

It is rare; however it leads to meningitis.

4- Gastrointestinal anthrax

Occurs in countries where contaminated meats are sold. Toxin produced in the intestinal tract forms a necrotic lesion in the iluem or cecum. High fatality rate is occurring.

Treatment

Penicillin is the drug of choice. Streptomycin, tetracycline and erythromycin are alternative drugs. Treatment before the appearance of bacteremia is important because the antibiotics are no effective against the toxin.

Prevention

Anthrax is an endemic in many areas of the world, and can produce epizootic outbreaks. The control of this disease can be achieved via:

A) Vaccination of cattle.

B) Cremation or burial with quick lime of infected animals.

C) Restricted movement of livestock.

Control in human is provided by immunization with toxoid. Recovery from the disease produces permanent immunity for human and animals.

II- ***Clostridium***

Saprophytes found in soil, water, air and vegetation. Gram positive spore forming bacilli. Measuring 3-8 µm in length and 0.4-1.2 µm in width. Obligate anaerobes. Some of them are non-motile. Most of them are unencapsulated. Few species parasitize intestinal tract of human and animals. Produce highly toxic exotoxins.

C. botulinum

Found in soil and occasionally in animal feces. Spore are highly resistant to heat, withstanding 100°C for 3-5 hours. Diminish at acid pH or high salt concentration. During growth and autolysis, the bacterium liberates toxins. Seven distinct antigenic varieties (A-G). Type A toxin is a complex consisting of neurotoxin and heamagglutinin which protect the neurotoxin from stomach acid and enzymes. Probably 1-2 µg is lethal to human.

Pathogenicity

C. botulinum causes botulism "an intoxication resulting from the ingestion of food in which *C. botulinum* has grown and produced toxin". Spiced, smoked vacuum packed or canned alkaline foods are eaten without cooking. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular joints

producing respiratory paralysis. Symptoms begin after 18-36 hrs. after ingestion of toxic food; visual disturbance, inability to swallow, speech difficulty and death occurs from respiratory paralysis or cardiac arrest.

Botulism is a disease of high mortality rate. There is no fever, patient remains fully conscious until short time before death. Those who recover does not develop antitoxin in the blood.

Laboratory diagnosis

Toxin can often be demonstrated in serum from the patient, and toxin may be found in leftover food. Mice injected intraperitoneally die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. *C botulinum* may be grown from food remains and tested for toxin production, but this is rarely done and is of questionable significance. Toxin may be demonstrated by ELISA, passive hemagglutination or radioimmunoassay.

Treatment

Antitoxins to three types of *C. botulinum* toxins have been prepared, reduced mortality rate from 65-25%.

Prevention

Strict regulation of commercial canning. Chief danger lies in home canned foods. Toxoid used for active immunization of cattle.

C. perfringens

It is implicated in gas gangrene. *C. perfringens* is indigenous members of the intestinal tract of humans and animals. Saprophytes found in soil. Large encapsulated, non-motile, spore former bacilli, produces eleven toxins. Alpha toxin is the most important (acts as lecithinase). Also they produce collagenase, hemolysin, proteinase, DNase, and some of them produce enterotoxin.

Disease produced

1. **Gas gangrene.**

An infection requires sites for germination of spores and multiplication of vegetative cells. The traumatized tissue offers an anaerobic environment. The organism does not invade healthy tissue. Most cases appear during war, surgeries or car accidents. Symptoms are included; Local pain in the area of the wound, swelling of the wound, skin rupture, revealing a necrotic, foul smelling wound. The most symptoms; delirium, apathy, disorientation, toxemia and death.

2. Food poisoning

Mostly caused by *C. perfringens* type A. When more than 10^{18} are ingested, sporulated and produced enterotoxin will causes secretion of fluid and electrolytes. Symptoms are; abdominal pain, diarrhea, nausea and vomiting.

3. **Enteritis necroticans**

Food born disease caused by *C. perfringens* type C. producing hemorrhage and gangrene in the intestine due to beta toxin.

4. Uterine infections

Follow instrumental abortion and it occurs in 5% of women.

Diagnostic lab tests

1. Specimens: Wound materials, Pus, Tissue, Blood, Urine and Feces.
2. Smears: Presence of large Gram positive bacilli but the Spores not regularly found.
3. Culture: Blood agar, chopped meat-glucose medium, and thioglycolate medium, anaerobically. The growth transferred into milk agar will produce clot and gas.

Colonies on blood ager are surrounded by an inner zone of clear hemolysis and an outer zone of incomplete hemolysis. Colonies on egg yolk agar will form a precipitate around the colonies.

Treatment and prevention

- Immediate debridement of the wound to remove all dead tissue.
- Application of anitserum.
- Antibiotic therapy (penicillin and tetracycline).
- Treating patients with high concentration of oxygen at elevated pressure.

In case of food poisoning, treatment does not require antibiotics since the disease is mild and self-limiting. It can be prevented by proper cooking.

C. tetani

General characteristics

Found in soil, human intestine (25%) and animal feces. Motile bacilli forming tennis racket shaped terminal spores. Obligate anaerobe but can stand certain concentration of oxygen. Can be distinguished by specific flagellar antigen. All share common somatic antigen. All produce the same antigenic type of toxin.

Toxin produced

1- Tetanospasmine

Responsible for tetanus. Heat labile protein. Neurotoxin released upon autolysis. It acts on CNS blocking the transmission of nerve impulses producing spastic.

2- Tetanolysin

A hemolysin can be reversibly inactivated by oxygen.

Pathogenesis

Tetanus results from small wounds which contaminated by *C. tetani* spore that germinate and produce toxin. The infection remains localized with minimal inflammatory damage. The toxin produced upon growth, sporulation and lysis of cells. The toxin migrates along neural paths from a local wound to sites of action in the CNS. Severe painful spasms and rigidity of voluntary muscles. The characteristic symptom of lock jaw involves spasms of masseter muscle, followed by progressive rigidity and violent spasms of trunk and limb muscles. Spasms of the pharyngeal muscles cause difficulty in swallowing. Death usually results from interference with mechanisms of respiration.

Symptoms appear after 4-6 days to 6 weeks incubation time depending on two factors:

1. The time of anaerobic condition to develop at the site of infection.
2. The time required for any toxin to reach CNS.

Laboratory diagnosis

- Clinical signs:

Patients usually have a wound, exhibit trismus. Have a history of no immunization.

- Culture:

Material from wound on blood agar or cooked meat medium heated to 80° C for 10-15 min to eliminate non spore former. 24 hrs later on blood agar swarming motility is examined.

- Spore stain.

Treatment

Tetanus antitoxin will neutralize any toxin in blood but already nerve fixed toxin. Penicillin used to destroy any cells thus preventing toxin production. Surgically removal of necrotic tissue which create anaerobiosis. Convulsions and spasms treated by barbiturate.

Ministry of Higher Education and
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University of Baghdad
College of Science
Department of Biology

Theoretical Pathogenic Bacteria 2020-2021

المرحلة الرابعة / الدراستين الصباحية والمسائية

الكورس الاول

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Lecture : (6)

Gram positive cocci
Genus: *Streptococcus*

- **General characteristics**

Gram positive cocci that appear in chains or clusters, most species are facultative anaerobes except one group "strict aerobes" which inhabits the intestinal tract and female genital tract. All streptococci are catalase negative. Some streptococci elaborate capsular polysaccharide. Hair-like pili project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid.

They are fastidious requiring many amino acids, vitamins, purine and pyrimidine bases. Primary habitat of upper respiratory tract of human.

- **Classification**

1 - Depending on haemolytic behaviour on blood agar:

a. Alpha haemolytic / incomplete haemolysis / viridans group.

b- Beta haemolytic / complete haemolysis / major human pathogens.

c- Gamma haemolytic / no haemolysis / not primary pathgens.

2- Rebecca Lancefield and co-workers / classification depending on antigenic characteristics of cell wall; carbohydrate C- substance in **beta haemolytic** streptococci / serological groups **A-V** (except I and J)**.**

3- Biochemical reactions and resistant to physical and chemical factors.

4- Molecular genetics and ecologic features.

- **Group A streptococci (*Streptococcus pyogenes*)**
  - **Antigenic structure**

1- **Capsule:** most group A, B, and C strains produce capsules composed of hyaluronic acid. Those who has capsule can impede the phagocytosis. This capsule is antigenic but not immunogenic.

2- **Cell wall proteins**

- **M protein:** this substance is a major virulence factor of group A ***S. pyogenes.*** When M protein is present, the streptococci are virulent, and they are able to resist phagocytosis. *The **M** protein (found in fimbriae) binds fibrinogen from **serum** and **blocks** the binding of complement to the underlying peptidoglycan*. It is immunogenic. There are more than 80 different types of M protein. Strains of ***S. pyogenes*** that produce certain M protein types are rheumatogenic, whereas strains of ***S. pyogenes*** that produce other M protein types are nephritogenic.
- **T protein:** this antigen has no relationship with virulence of Streptococci. It permits differentiation of certain types of streptococci, especially those whom isolated from impetigo patients, by agglutination with specific antisera.
- **Cell surface proteins: R protein** is used as an epidemiologic marker and has no known role in virulence. **F protein** (fibronectin binding), **G** protein binds the Fc portion of antibodies, and **P substance,** which probably make up most of the streptococcal cell body.

3- **Cell wall carbohydrates:** according to them, Streptococci are classified into serological groups (Lancefield groups). The serologic specificity is determined by an amino sugar. For group A Streptococci, this is rhamnose-*N*-acetylglucosamine; for group B, rhamnose-glucosamine polysaccharide; for group C, rhamnose-*N*-acetylgalactosamine; for group D, glycerol teichoic acid containing D-alanine and glucose; for group **F,** glucopyranosyl-N-acetylgalactosamine. **Group A sugar (also called**

C-substance) can cross-react with heart valves glycoprotein. C-substance found to cause arthritis to some animals.

4- **Cell wall peptidoglycan:** causes fever, lysis of red blood corpuscles in rabbits and other animals, and dermal necrosis.

5- **Cytoplasmic membrane antigens:** cross- reacts with tissues of heart, kidneys, and connective tissues. Group D specific antigen belongs to the cytoplasmic membrane antigens.

- **Extracellular streptococcal products**

The Streptococci elaborate extacrellular products in vitro. Many of these products are believed to be important in the pathogenesis of streptococcal infections. They are used in diagnosing such infections.

1- **Pyrogenic exotoxin** (formerly known as Erythrogenic toxin)**:** Three **streptococcal pyrogenic exotoxins** (SPE), are recognized; A, B and C. In the non-immune individual, it causes rash that is characteristic of scarlet fever.

2- **Cardiohepatic toxin and nephrotoxin:** a low molecular weight compound excreted by virulent strains of Streptococci is capable of producing lesions in the heart and liver tissue when injected into susceptible animals.

3- **Haemolysins:** the haemolytic activity of many streptococcal strains is due to the production of two distinct extracellular haemolysins called streptolysin O (SLO) and streptolysin S (SLS).

☒ **SLO:** is oxygen labile and immunogenic. Detection of antistreptolysin O antibodies is extensively used in the diagnosis of streptococcal infections. SLO has been implicated as a factor in the pathogenesis of rheumatic fever, a post streptococcal complication.

☒ **SLS:** is oxygen stable, responsible for the zone of beta haemolysis seen around surface colonies of streptococci on blood agar plates. It is capable of lysing mammalian red blood corpuscles and leukocytes, it is non-immunogenic. Intravenous injections of SLS in rabbits cause intravascular haemolysis and acute liver necrosis.

3- Spreading factors:

a. Hyaluronidase: can digest host connective tissue hyaluronic acid, as well as the organism's own capsule. Depolymerization could therefore enhance the spread of streptococci in the tissue. However, it could be used as treatment in certain cases.

b. Protease: a proteolytic activity has been shown in strains causing soft tissue necrosis or toxic shock syndrome.

c. Streptokinase:

produced by most of group A streptococci. It can interact with the proenzyme plasminogen of human serum, converting it to plasmin. Plasmin can digest fibrin and other serum factors important in the formation of blood clots. This activity is now used in the treatment of acute myocardial infarction.

d. Nucleases (streptodornases A-D): At the site of infection, the host's inflammatory response results in the accumulation of nuclear exudates from dead or injured white blood cell. Virulent streptococci can produce nucleases such as ribonuclease and deoxyribonuclease that will digest DNA and RNA of the exudates thus facilitating the spreading of streptococci.

e. Other extracellular products: such as C5a peptidase: is an extracellular enzyme that degrades complement component C5a, the main factor that attracts phagocytes to sites of complement deposition.

This large repertoire of products is important in the pathogenesis of *S. pyogenes* infections. Even so, antibodies to these products are relatively insignificant in protection of the host.

- **Pathogenesis**

Ninety percent of streptococcal diseases caused by group A beta haemolytic Streptococci. Streptococcal diseases are divided into two categories:

I- Suppurative diseases:

a. Impetigo: a highly contagious skin disease found primarily in children, involves the infection of ***epidermal layers of skin.*** The infection begins as small blisters that can spread to adjacent areas.

b. Cellulites: inflammatory condition associated with streptococcal invasion of ***subcutaneous tissue.*** This type of disease results in gangrene and invasion of blood stream.

c. Erysipelas: involves the infection of ***dermis*** characterized by a spreading inflammation with massive brawny oedema and a rapidly advancing margin of infection.

d. Necrotizing fasciitis (streptococcal gangrene): this is an infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues. Group A streptococci that cause necrotizing fasciitis have sometimes been termed **"flesh-eating bacteria".**

e. Puerperal fever: uterine infection that frequently accompanies delivery when aseptic techniques are not followed.

f. Streptococcal sore throat: in children and adults, the disease is acute and is characterized by intense nasopharyngitis, tonsillitis, and intense redness and oedema of the mucous membranes, with purulent exudates; enlarged, tender cervical lymph nodes; and (usually) high fever.

g. Sepsis: infection of traumatic or surgical wounds with streptococci results in sepsis or surgical scarlet fever.

h- Toxic shock: is caused by a few strains that produce a toxic shock-like toxin.

II- Non suppurative diseases

a. Scarlet fever: associated with the formation of erythrogenic toxin by group A streptococci.

b. Rheumatic fever: M protein cross reacts with sarcolemma. Antibodies cross-react with heart tissue, fixes complement, and cause damage accompanied by inflammation of the joints. Highest incidence found in age group 5-19 years.

c. Acute glomerulonephritis (AGN): Antigen-antibody complexes may be deposited in kidney, fix complement, and damage glomeruli. Only a few M-types are nephritogenic.

d. Erythema nodosum: skin condition, small red nodules appear under the surface of the skin. It could be the result of hypersensitivity to the peptidoglycan of the streptococcal cell wall.

- **Identification**

Preliminary identification:

1 - Haemolytic behaviour. Cultures on sheep blood agar plates are the gold standard.

2- Bacitracin sensitivity: group A streptococci are sensitive to Bacitracin, while group B streptococci are resistant.
3- Fluorescent antibody.
4- Phadebact coagglutination test: Major beta haemolytic groups A, B, C, and G detected by this kit.
5- Streptex: latex particles are conjugated to group specific streptococcal antibodies mixed with unknown growth of streptococci result in agglutination.
6- Directly from throat swab (large numbers of streptococci required) is mixed with nitrous acid (extraction) in combination with coagglutination. The test required 30 minutes.
7- PCR.

- **Diagnostic test for post streptococcal diseases:**

1- Determination of anti streptolysin O titer (ASOT). An elevated ASO is a useful diagnostic marker for rheumatic fever. In contrast to rheumatic fever, antistreptolysin O (ASO) titers are low in acute glomerulonephritis.

2- Streptozyme test (slide agglutination test): Sheep red blood corpuscles are sensitized with extracellular products from group A streptococci mixed with diluted patient serum results in agglutination. The extracellular products are Hyaluronidase (HA), deoxyribonuclease (DNase), nicotinamide adenine dinucleotidase (NADase), and streptokinase (SK).

- **Treatment and prevention**

Penicillin is the drug of choice for treatment of group A beta haemolytic streptococci. In case of individuals sensitive to **penicillin, erythromycin** is a suitable alternative. **Vaccines** are prepared from streptococcal type specific M protein against group A infections.

- **Other groups**

1- Group B Streptococci: they found in oral cavity, intestinal tract and vagina ***S. agalactiae,*** which surrounded by a polysaccharide capsule. They cause neonatal meningitis by transporting to the baby during delivery, UTI, and puerperal fever.

2- Group C Streptococci: cause infections in many animals' species but rarely in human. ***S. dysgalactiae*** **subsp.** ***equisimilis*** cause human illnesses such as endocarditis, pneumonia, meningitis, and wound infections. Outbreaks of disease associated with unpasteuresid milk or cheese made from unpasteurized milk. ***S. equi*** causes strangles in horses. ***S. equi*** **subsp.** ***zooepidemicus*** infections are infrequent in human e.g. meningitis and pneumonia.

3- Group D Streptococci: alpha or gamma haemolytic on sheep blood agar, of intestinal origin (Enterococci): ***E. faecium, S. bovis, S. equinus, Enterococcus durans, Enterococcus faecalis,*** the latter is of medically important since is causes UTI and abdominal lesions.

- **Non-Lancefield Group Streptococci**

Includes **the viridans Streptococci** (*S. mutans*, *S. sanguis*; *S. salivarius; S. mitis*) and ***S. pneumonia*****.**

Streptococcus pneumoniae

Formerly, ***Diplococcus.*** Inhabitants of the upper respiratory tract, gram positive cocci that occur singly, in pairs, or in chains. The clinical specimens are Lancet shaped and surrounded by a capsule, only encapsulated (smooth form) strains are virulent. On repeated subculture in the laboratory, the capsule is lost (rough form). The pneumococci are facultative anaerobes that

are very fastidious in their cultural requirements. Some strains need and elevated level of CO_2 (5-10 %) for initial isolation.

The addition of blood to culture media supplies the enzyme catalase. Under aerobic conditions pneumococci produce hydrogen peroxide, which can be toxic to the pneumococci. The enzyme catalase acts to remove the accumulated hydrogen peroxide.

On blood agar pneumococcal colonies **exhibit alpha haemolysis** and closely resemble colonies of alpha haemolytic Streptococci.

☒ Virulence factors

1- Capsular polysaccharide: the primary virulence factor of the pneumococci. The capsule prevents binding of antibody to the cell wall of the pneumococcus and thus inhibits phagocytosis.

2- Pneumolysin: all pneumococci produce pneumolysin. This protein is related to ***S. pyogenes*** streptolysin O. Pneumolysin is toxic to pulmonary endothelial cells. Pneumolysin deficient pneumococci are less virulent than the toxin producing isolates. Most patients with pneumococcal disease exhibit an antibody response to pneumolysin and pneumolysin immunization is protective in animals.

3- Pneumococcal surface protein A (PspA): found on all pneumococci, and highly variable both immunologically and in molecular mass. Passive immunization with anti PspA is protective.

4- Neuraminidase: like pneumolysin, is released upon autolysis. Despite the involvement of this enzyme in the colonization by other bacteria, there is no proven role in pneumococcal virulence.

5- SlgA protease: all pneumococci produce SIgA protease, which is a property shared with other species causing pneumonia and meninigitis. This enzyme could play a part in establishment of the microorganisms in the nasopharynx.

☒ Infections produced by Pneumococci

1- Primary infections: caused by certain serotypes and responsible of approximately 75 % of pneumonia cases and responsible of more than half of lethal cases caused by pneumococcal bacteraemia. Lobar pneumonia, Septicaemia, Peritonitis, Purulent meningitis and purulent otitis are examples of primary infections.

2- Secondary infections

Produced by any serotype, 40-70% of the population is carriers of pathogenic ***S. pneumoniae.*** Many authorities believe that pneumonia and related pneumococcal infections are acquired **endogenously** through **lowered host resistance** rather than exogenously by direct contact.

☒ Laboratory diagnosis of pneumococci

Specimens: sputum, laryngeal swabs, transtracheal aspirates, blood, and CSF. A definitive diagnosis of pneumococcal pneumonia can be made only if the microorganisms are isolated directly from the blood or from other clinical specimens by plating onto blood agar. Alternatively, by identification of specific pneumococcal antibodies by counter **Immunoelectrophoresis** of body fluids: pleural fluid, blood, and urine.

The pneumococci can be differentiated from other alpha haemolytic streptococci by the following procedures:

1- Quellung reaction: it is the most accurate and specific test of identification of the pneumococci. Sputum or exudative material is spread on a slide and mixed with antiserum against type specific polysaccharide or the polyvalent antiserum results capsular swelling. A capsular halo can be observed around the diplcocci.

2- Optochin susceptibility test: optochin is an antimicrobial drug derived from quinine. When disks containing the drug are placed on blood agar

previously seeded with pneumococci, zones of inhibition can be observed around the optochin. Alpha haemolytic Streptococci are resistant to optochin.

3- Bile solubility test: the pneumococci pruduce an enzyme (amidase) that cleaves specific covalent bonds in the peptidoglycan layer. This enzyme is activated by bile or bile salts such as sodium deoxycholate solution are added to a broth culture of pneumococci, the cells are rapidly lysed. While, Alpha haemolytic streptococci are not lysed by bile.

4- Mouse virulence test: most pneumococcal strains, when injected intraperitoneally into mice, can induce death of animals within 24-48 hours. Alpha haemolytic streptococci, used under the same procedures, are not lethal to mice.

☒ Treatment of pneumococci

Penicillin is the drug of choice. In **penicillin** allergic patients, **erythromycin** or one of its derivatives **azithromycin,** can be used. **Vancomycin** is the drug of choice for the **penicillin** resistant pneumococci.

☒ Prevention of pneumococci

In **1983,** a polyvalent vaccine containing polysaccharide antigen from 23 types of *S. pneumoniae* was recommended for children over 2 years. In **1998,** a vaccine containing pneumococcal polysaccharide **coupled to a carrier protein (diphtheria toxoid)** as the immunogen was shown to be effective in young children. The vaccine contained the polysaccharide of the seven most common pneumococcal serotypes. This vaccine was approved by the FDA in **2000.**

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Lecture : (7)

Infectious diseases are complex and involve a series of complex and shifting interactions between the invading organism and the host, these interactions include the following:

1. The organism's ability to breach host barriers and to evade destruction by innate local and tissue host defenses.
2. The organism's biochemical tactics to replicate, to spread, to establish infection, and to cause disease.
3. The microbe's ability to transmit to a new susceptible host.
4. The body's innate and adaptive immunologic ability to control and eliminate the invading parasite.

Infection may imply colonization, multiplication, invasion or persistence of a pathogen on or within a host.

Infectious disease is used to describe an infection that causes significant overt damage to the host. There are two broad qualities of pathogenic bacteria underlie the means by which they cause disease: **invasiveness** and **toxigenesis**.

Invasiveness is the ability to invade tissues. This encompasses mechanisms for colonization (adherence and initial multiplication), ability to bypass or overcome host defense mechanisms, and the production of extracellular substances ("invasins") which facilitate the actual invasive process.

Toxigenesis is the ability to produce toxins. Toxic substances may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth.

Pathogenesis is the process of causing disease. It depends on the:

- immune status of the host
- nature of the species or strain (virulence factors)
- Phase of microbial growth; bacteria in log phase are more likely to overcome host resistance than those in late phases.
- number of organisms in the initial exposure (dosage of pathogen); for example, *Salmonella typhi* infection need small dose, while *Salmonella enteriditis* infection need to large dose.

ID50 (Infectious Dose) is number of microbes required to produce infection in 50% of the population.

LD50 (Lethal Dose) is amount of toxin or pathogen necessary to kill 50% of the population in a particular time frame.

Virulence is a term which refers to the degree of pathogenicity of the microbe.

Virulence might be lost by the following:

1- Attenuation; successive transfer of pathogen.
2- Loss of capsule; for example loss of capsule in pneumococcus
3- Colony variation; conversion from smooth to rough, for example *Salmonella* & *Shigella*
4- Loss of temperate phage: for example *Corynebacterium diphtheria*.
5- Heat & desiccation

Enhancement of virulence by:

1- Successive passage in lab animals
2- Presence with other bacteria; for example *Corynebacterium diphtheria* & *Streptococcus pyogenes*

Conserving of virulence:

1- Lyophilization
2- Using enrichment culture media containing blood and store in dark at low temperature
3- Spore forming bacteria resist adverse conditions, therefore they have permanent virulence.

Bacterial virulence differs according to the host factors which includs:

1- Age
2- Malnutrition
3- Drugs addiction
4- Metabolic diseases such as diabetes
5- Haemological disease
6- Immune deficiency disease
7- Sex
8- Race

Types of diseases:

1. **Local**: A disease that is restricted to a certain area in the body.
2. **Focal**: Localized site of disease from which bacteria and their products can spread to other body parts.
3. **Primary**: A disease caused by one microbial species.
4. **Secondary**: A primary disease complicated with second pathogen. For example, pneumonia following primary influenza especially in aged people.
5. **Mixed**: A disease caused by two or more microbial species.
6. **In apparent:** A disease that doesn't give rise to any detectable manifestation.
7. **Latent:** A disease that persist in the tissues in dormant state and later becomes manifested usually when the host resistance is lowered.
8. **Bacteremia:** A condition in which blood serves as site of presence of bacteria, but the bacteria usually cleared from the vascular system with no harmful effect.
9. **Septicemia:** A condition in which blood serves as a site of bacterial multiplication as well as a mean of transfers of infectious agents from one site to another.
10. **Pyemia:** The presence of pyogenic bacteria like *Staphylococcus* in the blood as they spread from one site to another.
11. **Toxemia:** The presence of bacterial toxins in the blood.

The Mechanisms of Bacterial Pathogenicity

To cause disease a pathogen must:

1. Gain access to the host (Entry into host)
2. Adhere to host tissues (Adhesion)
3. Penetrate or evade host defenses (Invasion)
4. Survival in the host
5. Damage the host, either:

- directly
- accumulation of microbial wastes

Entry into host

A. Mucous membranes (moist mucosa): most common route for most pathogens

1. Respiratory tract (most common)
2. Gastrointestinal tract
3. Urinary/genital tracts
4. Conjunctiva

B. Skin (keratinized cutaneous membrane);
-some pathogens infect hair follicles and sweat glands
-few can colonize surface
-unless broken, skin is usually an impermeable barrier to microbes

C. Parenteral route
-penetrate skin: punctures, injections, bites, cuts, surgery, etc.
-deposit organisms directly into deeper tissues

D. Many opportunistic pathogens are carried as part of the normal human flora. and this acts as a ready source of infection in the compromised host.

Adhesion:
Is an essential preliminary to colonization and then penetration through tissues. At the molecular level, adhesion involves surface interactions between specific **receptors** on the mammalian cell membrane (usually carbohydrates) and **ligands (adhesin)** (usually proteins) on the bacterial surface. The presence or absence of specific receptors on mammalian cells contributes significantly to tissue specificity of infection.

Invasion or invasiveness
It's the ability of microorganisms to enter host tissues, multiply and spread. it is mediated by a complex array of molecules, often described as **'invasins'** which act against the host by breaking down defenses of the body.

Types of Bacterial Invasins

- **Spreading factors;** bacterial enzymes such as hyaluronidase, collagenase, etc.

- **Enzymes that cause cell lysis**; such as lecithinases, phospholipases, hemolysin, etc.

- **Staphylococcal coagulase**
- **Extracellular digestive enzymes;** such as proteases, lipases, glycohydrolases, nucleases, etc.

- **Toxins with short-range effects related to invasion;** such as anthrax toxin.

Some bacteria, e.g. *Corynebacterium diphtheriae* or *Clostridium tetani*, (toxin producers) are non-invasive. Others such as Staphylococci & Streptococci are moderately invasive, while anthrax & plague bacilli are highly invasive.

Survival in the host

Most successful pathogens possess structural or biochemical features which allow them to resist the main lines of host internal defense against them, i.e., the phagocytic and immune responses of the host. For example;

- the poly-D-glutamate capsule of *Bacillus anthracis* protects the organisms against cell lysis by cationic proteins in sera or in phagocytes.
- The outer membrane of Gram-negative bacteria is a formidable permeability barrier that is not easily penetrated by hydrophobic compounds such as bile salts which are harmful to the bacteria.
- Pathogenic mycobacteria have a waxy cell wall that resists attack or digestion by most tissue bactericides.
- Intact lipopolysaccharides (LPS) of Gram-negative pathogens may protect the cells from complement-mediated lysis or the action of lysozyme.
- Almost all principal pathogens that cause pneumonia and meningitis have antiphagocytic polysaccharide capsules.

Damage the host

Tissue damage and the manifestations of disease may also result from interaction between the host's immune mechanisms and the invading organism or its products. Reactions between high concentrations of antibody, soluble microbial antigens, and complement can deposit immune complexes in tissues and cause acute inflammatory reactions and immune complex disease.

Epidemiology, it's derived from the Greek word "Epidemios" which means among people. It is the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems.

Lab technique in Epidemiology

For epidemiological investigations the strains of bacteria can be divided into types, which are taxonomic categories below the species level. Various typing methods can be used: biotyping, antibiograms, serotyping, bacteriocin typing, and phage typing.

1- **Biotyping**: biochemical identification of isolates.
2- **Serotyping**: the use of antigenic differences among bacterial strains. For example; flagellar Ag, cell wall Ag, capsular Ag.

- Commonly used to identify *Salmonella*, *Shegilla* and Enteropathogenic *E.coli*

- M-protein serotyping is useful in epidomiology of glomeruli nephritis caused by *Streptococcus pyogenes*
- Capsular swelling test (Quelling test) is used in serotyping of *Klebsiella* spp.

3- **Antibiogram**: A test used to determine susceptibility of microorganisms to antibiotics or other antimicrobial substances.

4- **Phage typing**: Using the phages in identification of bacterial strains. For example; *S. aureus* has 22 phages for identification.

5- **Bacteriocin typing**: Used in identification of *Serratia marscence*, *E.coli*, *Proteus mirabilis* & *Shigella* spp.

6- **Genetic methods** which including:
   - Direct method ,hybridization of DNA
   - Indirect method, restricted endonucloase analysis

Pattern of infections or diseases:

- **Endemic**, a disease found constantly in low percentage with a certain area. For example, Cholera in South –east Asia
- **Epidemic**, the outbreak that happens suddenly in high percentage of disease in susceptible persons within certain population. For example, Meningitis in Iraq at 1976
- **Pandemic**, communicable infections that are widespread in a region, sometimes worldwide, and have high attack rates. For example, Asian influenza

Patterns of transmission of diseases

1. **Horizontal**: Disease is spread through a population from one infected person to another
   (Kissing, sneezing)
2. **Vertical**: The disease is transmitted from parent to offspring (Ovum, sperm, placenta, milk)
3. **Contact Transmission**:
   - Direct - Kissing, sex
   - Droplets - Talking
   - Vertical – Mother to fetus
   - Vector
4. **Indirect Contact** :
   - Food, water, and biological products (blood, serum, tissue)
   - Fomite (door knobs, toilet seats, etc.)
   - Air
   - Droplet nuclei (dried microscopic residue)
   - Aerosols (dust or moisture particles)

Sporadic a disease occurs in an irregular pattern.

Prevalence is the total numbers of cases in the population at any given time.

Incidence is the number of new cases over a defined period of time.

Mortality rate is the number of individuals that die as a result of the outbreak of disease within certain period of time.

Nosocomial infections: hospital acquired infections.

Clinical stages of a disease in the host:

A. **Acute diseases;** are characterized by symptoms that usually appears quickly and become very intense, and then subsides when the host immune system has overwhelmed the pathogen or its toxic products. For example, childhood diseases like measles, mumps, various types of influenza and chicken box.

Stages of acute disease:

1. **Incubation** period, the time between exposure to the organism and appearance of the first symptoms of the disease.
2. **Acute period,** symptoms of the disease are at their peak. For example, fever and cough in respiratory, diarrhea and vomiting in intestinal tract, etc.
3. **Convalescent period**, a period characterized by a sharp decline in symptoms.

B. **Chronic diseases and persistence;** symptoms are expressed over a long period of time; infectious agent here is an intracellular parasite like brucellosis and tuberculosis.

Some microbes that persist in host give rise to mild symptoms or no symptoms, and its actively shed from the host, the individual shedding these microorganisms are called carriers and the microorganisms may be persist in the host for days, months or even year like bacteria of cholera, typhoid and diphtheria.

Reservoirs & sources of diseases:

1. **Animate, which include;**
   - **Patients**, they represent the most important reservoirs for many viral and bacterial diseases like measles, mumps, poliomyelitis, whooping cough and sexually transmitted diseases.
   - **Carriers**, infected healthy individuals, no symptoms (asymptomatic), or very mild form of disease, yet they both can spread disease to others – many bacterial pathogens.

     Carriers can be:
     1. **Convalescent carrier**, a person who has recovered from the symptoms of an infectious disease but is still capable of transmitting pathogens to others.
     2. **Healthy carrier**, a person who or animal that harbors a specific infectious agent in the absence of discernible clinical disease and serves as a potential source of infection.
     3. **Chronic carriers**, they carry infectious agents for long periods of time.

   - **Animals,**

Zoonosis; it is any infection or disease that is transmitted to man from animals (a disease occurring primary in animal and transmitted secondary to human).

- ✓ For example; Salmonella transmitted from cattle and poltry through contaminated food causing salmonellosis.
- ✓ Two classes of arthropods serve as reservoirs and sources of infection:
  1. **Insecta;** flies, mosquitoes, fleas and lice
  2. **Arachnida;** ticks and mites

The relationship between infectious agents and arthropods:

1. **Mechanical**; arthropods carry infectious agents on their appendages and not involved in their life cycle like flies and *Salmonella*.
2. **Biological**; arthropods serve like hosts and reservoirs of the microbial agents like mosquitoes and malaria parasites.

2.Inanimate, which includes:

- **Soil**, example; *Clostridium tetani* and *Clostridium perfrengens*
- **Water**, example: *Vibrio cholerae*
- **Food**, example; *Salmonella typhi, Clostridium botulinum* & *Campylobacter*

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Pathogenic Bacteria
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Lecture : (8)

The science of medical microbiology dates back to the pioneering studies of Pasteur and Koch. The methods they developed lead to the first golden age of microbiology (1875–1910), when many bacterial diseases and the organisms responsible for them were defined. In the first half of the 20th century, scientists studied the structure, physiology, and genetics of microbes in detail and began to answer questions relating to the links between specific microbial properties and disease.

The contributors:

- **Louis Pasteur (1822 - 1895)**

A French microbiologist, He is considered as "**Father of Microbiology**".

1862 – Proposed germ theory of disease
1867 – Pasteur devised the process of destroying bacteria known as **pasteurization**.
1881 – Development of anthrax vaccine. Resolved Pebrine problem of silkworms.
1885 – Development of a special vaccine for rabies (the **Pasteur treatment**)

- **Robert Koch (1843 - 1910)**

A German scientist, He perfected many bacteriological techniques and known as "**Father of Practical Bacteriology**".

1876 – Koch demonstrated that anthrax is caused by *Bacillus anthracis*.
1882 – Isolated the bacterium—*Mycobacterium tuberculosis*—that causes tuberculosis.
1883 – Isolation of *Vibrio cholerae*, the cause of cholera.
1883 – Verification of the germ theory of disease by relating a specific organism to the specific disease.
1884 – Koch put forth his postulates—known as Koch's postulates.

The four Original postulates are:

1- The suspected microorganism must always be found in diseased but never in healthy individuals.
2- The microorganism must be isolated in pure culture (one free of all other types of microbes) on a nutrient medium.
3- The same disease must result when the isolated microorganism is inoculated into a healthy host.
4- The same organism must be reisolated from the experimentally infected host. (Fig. 1).

Proposed fifth postulate

5- Elimination of the disease-causing microbe from the infected host or prevention of exposure of the host to the microbe should eliminate or prevent disease.

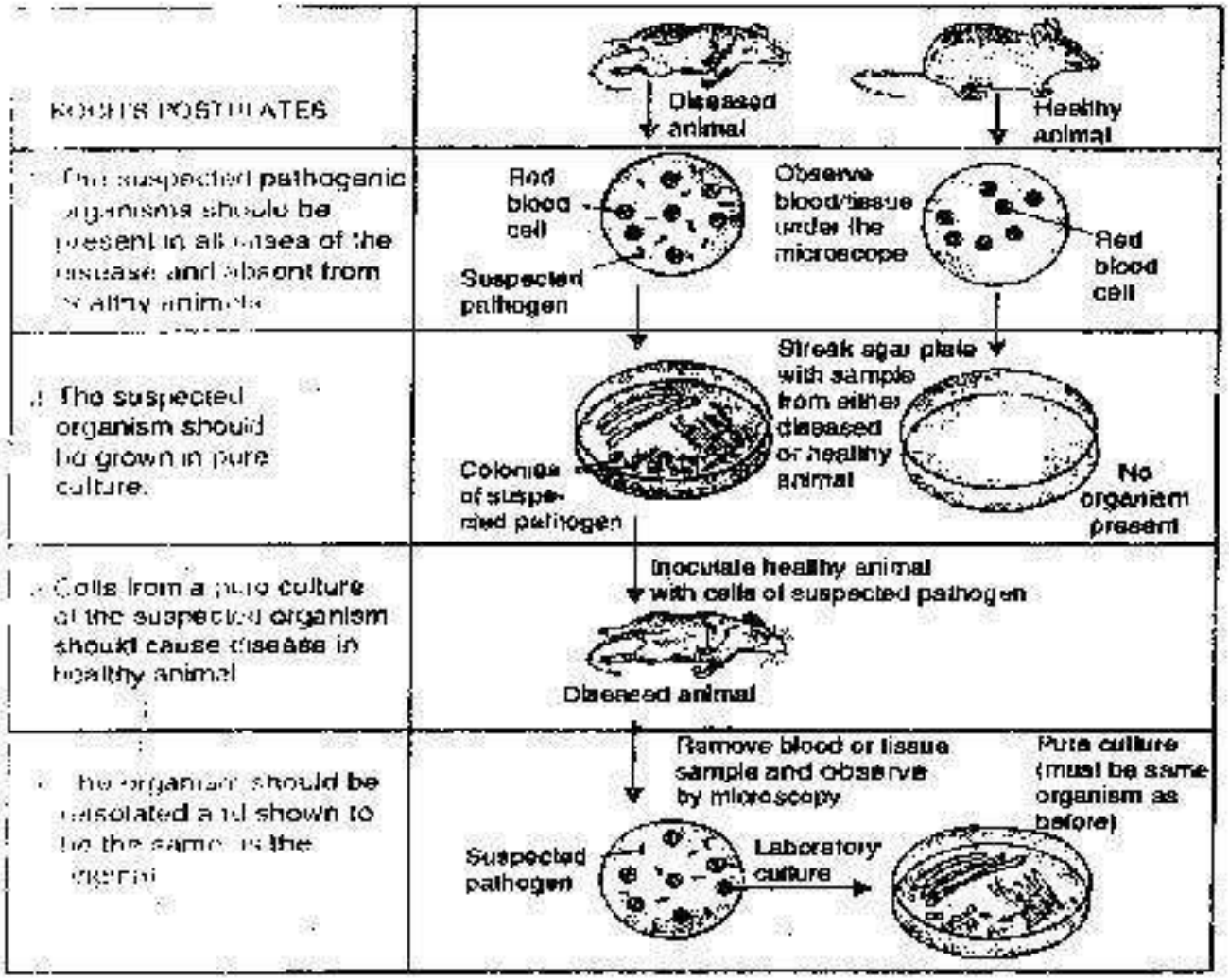


Fig. 1: Koch's postulates

- **Joseph Lister (1827-1912)**

British surgeon, he first introduced the technique to reduce microbes in a medical setting and prevent wound infections in 1867. Lister will always be known as the **Father of antiseptic surgery or father of modern surgery.**

Specific relationships between human and germs:

From the moment of birth human been are continuously exposed to microorganisms including bacteria. Some of the interactions between the human and microorganisms are essential for the wellbeing of individual but other result in disease.

- **Parasite** is an organism that resides on or within another living organism in order to find the environment, nutrient that required for growth and reproduction.

Symbiotic relationships:
The surface tissue of the skin, oral cavity, respiratory tract, gastrointestinal tract, genital and urinary tract are populated by bacteria and other microorganisms.

- **Normal micro flora (Microbiota)** are microbial population frequently found in association with particular tissue in normal, healthy individuals.

- **Transients or Transient flora** are acquired from the environment and establish themselves briefly but tend to be excluded by competition from residents or by the host's innate or immune defense mechanisms. They found in association with skin and mucous membrane, they may be present for hours, days, few weeks, they may or may not be pathogenic.

- **Residents** or **Resident flora** are strains that have an established niche at one of the many body sites, which they occupy indefinitely. They found regularly inside the body (endosymbiosis) or on surface (ectosymbiosis) usually not pathogenic but may be opportunistic.

Microbiota of the skin
- *Staphylococcus epidermidis*
- *Staphylococcus aureus* (in small numbers)
- *Micrococcus* species
- α-Hemolytic and nonhemolytic streptococci (eg, *Streptococcus mitis*)
- *Corynebacterium* species
- *Propionibacterium* species
- *Peptostreptococcus* species
- *Acinetobacter* species
- Small numbers of other organisms (*Candida* species, *Pseudomonas aeruginosa*, etc)

Microbiota of the mouth
- *Staphylococcus*
- *Neisseria*
- *Lactobacillus*
- *Bacteriodes*

The mucous membrane of mouth and pharynx are often sterile at birth, after birth some viricans streptococci become established.

Microbiota of the upper respiratory tract
- α-Hemolytic and nonhemolytic streptococci
- *Haemophilus* species
- *Pneumococci*
- *Mycoplasma*
- *Bacteroides*

Microbiota of the eye

- *Staphylococcus* spp.
- *Streptococcus* spp.
- *Corynebacterium xerosis*

Microbiota of the ears

- *Staphylococcus* spp.
- *Streptococcus* spp.
- *Bacillus* spp.

Microbiota of the gastrointestinal tract

✓ **Upper intestine**

- *Lactobacillus*
- *Enterococcus*

✓ **Colon**

96 – 99% of bacteria consist of anaerobic:

- *Lactobacillus spp.*
- *Streptococci*
- *Fusobacterium spp.*
- *Bifidobacterium*
- *Bacteroides*
- *Clostridium perfringens*
- Small numbers of *Proteus* and *Pseudomonas*

The important functions of intestinal microbiota can be divided into three major categories:

1. **Protective functions**: in which the resident bacteria displace and inhibit microbial pathogens indirectly by competing for nutrients and receptors or directly through the production of antimicrobial factors, such as bacteriocins and lactic acid.

2 **The development and function of the mucosal immune system.** Such as induce the secretion of IgA.

3. **Metabolic functions**. Such as synthesize vitamin K, biotin, and folate and enhance ion absorption. Certain bacteria metabolize dietary carcinogens and assist with fermentation of nondigestible dietary residue.

Pathogenicity of microorganisms:

Pathogenicity: The ability or capacity of an infectious agent (microorganism) to cause disease.

Pathogen: A microorganism capable of causing disease. There are two types of pathogens:

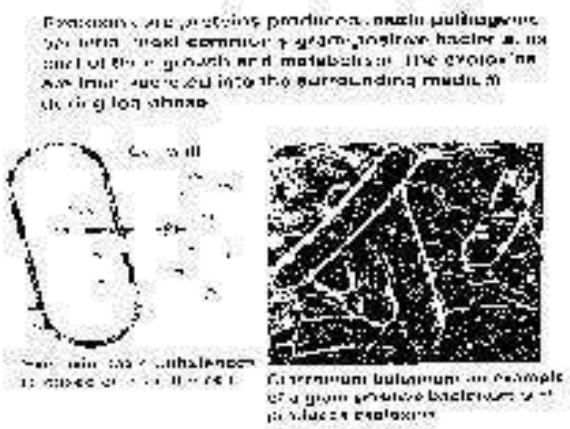
1. **Opportunistic pathogens:** agents capable of causing disease only when the host's resistance is impaired (ie,when the patient is "immunocompromised").Or they are normal flora cause disease when the immune response of the host is suppressed.
   For example: *Clostridium perfringens* is normal flora in colon but cause "gas gangrene" in locally damaged tissue.

2. **True pathogens:** they possess properties that enable them to overcome the body defenses and infect the tissue of normal healthy subject.

The microbial factors contributing infection:
A few substances play role in reproduction of diseases:

1. Toxins

Toxins produced by bacteria are generally classified into two groups: exotoxins and endotoxins. Exotoxins are proteins that are most often excreted from the cell. However some exotoxins accumulate inside the cell and are either injected directly into the host or are released by cell lysis. Endotoxins are lipid molecules that are components of the bacterial cell membrane. The primary features of the two groups are listed in Table 1.



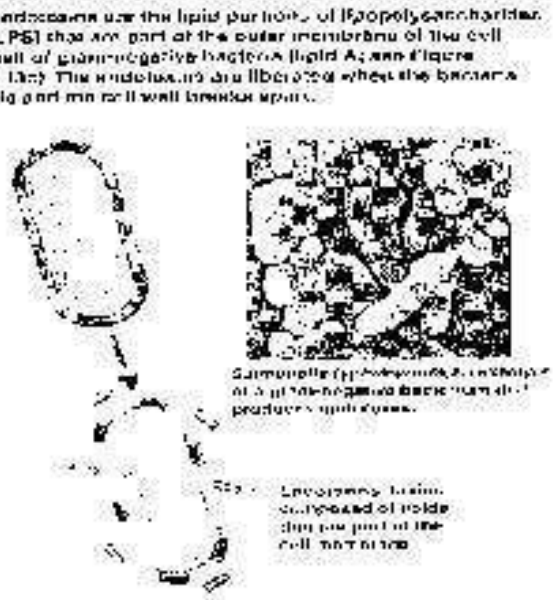


Table 1: Essential features of exotoxin and endotoxin

Exotoxin	Endotoxin
Excreted by living cell; found in high concentrations in liquid medium	Integral part of the cell wall of gram-negative bacteria; released on bacterial death
Produced by both gram-positive and gram-negative bacteria	Found only in gram-negative bacteria
Polypeptides with a molecular weight of 10,000–900,000	Lipopolysaccharide complexes; lipid A portion probably responsible for toxicity
Relatively unstable; toxicity often destroyed rapidly by heating at temperatures above 60°C	Relatively stable; withstand heating at temperatures above 60°C for hours without loss of toxicity
Highly antigenic; stimulate formation of high-titer antitoxin; antitoxin neutralizes toxin	Weakly immunogenic; antibodies are antitoxic and protective
Converted to antigenic, nontoxic toxoids by formalin, acid, heat, and so on	Not converted to toxoids
Highly toxic; fatal to animals in microgram quantities or less	Moderately toxic; fatal for animals in tens to hundreds of micrograms
Usually do not produce fever in the host	Usually produce fever in the host

2. **Extracellular enzymes:**
Many species of bacteria produce enzymes that are not intrinsically toxic but do play important roles in the infectious process.

A. Tissue-Degrading Enzymes

Many bacteria produce tissue-degrading enzymes. The best-characterized are enzymes from *C. perfringens*, and, to a lesser extent, anaerobic bacteria, *S. aureus* , and group A streptococci.

- **collagenase** a proteolytic enzyme degrades collagen. the major protein of fibrous connective tissue, and promotes spread of infection in tissue. Produce from *C. perfringens*

- **coagulase**, many pathogenic *Staphylococcus* produce this enzyme which works in conjunction with blood factors to coagulate plasma. Coagulase contributes to the formation of fibrin walls around staphylococcal lesions, which helps them persist in tissues.

- **Hyaluronidases** are enzymes that hydrolyze hyaluronic acid, a constituent of the ground substance of connective tissue. They are produced by many bacteria (eg, staphylococci, streptococci, and anaerobes) and aid in their spread through tissues.

- **Streptokinase (fibrinolysin)**, this enzyme is able to dissolve coagulated plasma and probably aids in the rapid spread of streptococci through tissues. Many hemolytic streptococci produce it.

- **Cytolysins**—that is, they dissolve red blood cells **(hemolysins)** or kill tissue cells or leukocytes **(leukocidins)**. Many bacteria produce these substances such as Clostridia and Staphylococci.
Most gram-negative rods isolated from sites of disease produce hemolysins. For example, whereas *E. coli* strains that cause urinary tract infections typically produce hemolysins, strains that are part of the normal gastrointestinal flora may or may not produce hemolysins.

B. IgA1 Proteases

Some bacteria that cause disease produce IgA1 proteases. IgA1 protease is an important virulence factor of the pathogens *N.gonorrhoeae*, *N meningitidis*, *H influenzae*, and *S pneumoniae*.
The enzymes are also produced by some streptococci associated with dental disease, and a few strains of other species that occasionally cause disease. Production of IgA1 protease allows pathogens to inactivate the primary antibody found on mucosal surfaces and thereby eliminates protection of the host by the antibody.

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Lecture : (9)

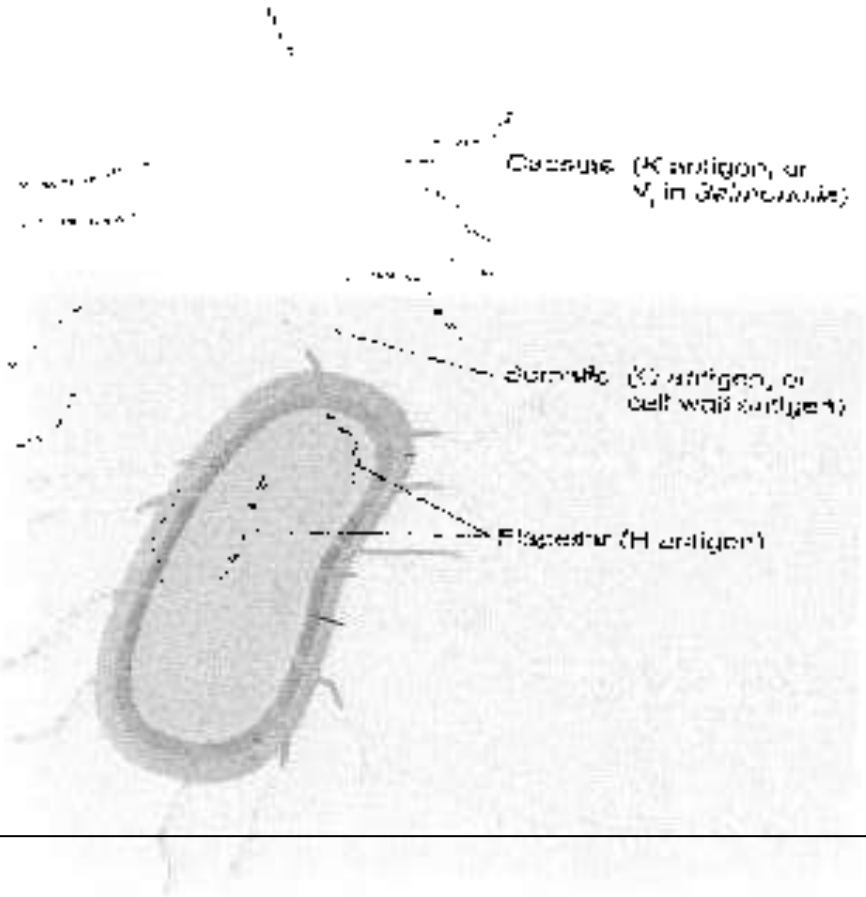
They are a large heterogeneous group of aerobes or facultative anaerobes, gram-negative rods, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of bacteriocins such as colicins, toxins (endotoxin & exotoxins) and other virulence factors. The natural habitat is the intestinal tract of humans and animals.

Bacteriocins: proteins are produced by certain strains of bacteria active against some other strains of the same or closely related species.

The family includes 51 genera (*Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus,* and others). The clinically significant *Enterobacteriaceae* comprise 20–25 species, and other species are encountered infrequently.

Antigenic Structure:

- more than 150 different heat-stable somatic O (lipopolysaccharide) antigens
- more than 100 heat-labile K (capsular) antigens
- more than 50 H (flagellar) antigens



Diseases caused by *Enterobacteriacae* other than *Salmonella* & *Shigella*

Causative Organisms

E.coli, Proteus, Enterobacter, Klebsiella, Morganella, Providencia, Citrobacter and *Serratia* species are found as members of the normal intestinal microbiota. They are sometimes found in small numbers as part of the normal microbiota of the upper respiratory and genital tracts. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal microbiota sites.

The most frequent sites of clinically important infection are the urinary tract, biliary tract, and other sites in the abdominal cavity, but any anatomic site can be the site of disease.

Pathogenesis and Clinical Findings

A. *E. coli*

1. **Urinary tract infection (UTI):** *E. coli* is the most common cause of UTI and accounts for approximately 90% of first UTI in young women.

2. ***E. coli*-associated diarrheal diseases:**

- **Enteropathogenic *E. coli* (EPEC)** are an important cause of diarrhea in infants, especially in developing countries. The duration of the EPEC diarrhea can be shortened and the chronic diarrhea cured by antibiotic treatment.

- **Enterotoxigenic *E. coli* (ETEC)** is a common cause of "traveler's diarrhea" and a very important cause of diarrhea in infants in developing countries. Some strains produce a **heat-labile exotoxin** (LT) and some produce the **heat-stable enterotoxin** STa. Care in the selection and consumption of foods potentially contaminated with ETEC is highly recommended to help prevent traveler's diarrhea. Antibiotic treatment effectively shortens the duration of disease.

- **Shiga toxin-producing *E. coli* (STEC)** they produce two cytotoxic toxins, Shiga-like toxin 1 and Shiga-like toxin 2. Of the *E. coli* serotypes that produce Shiga toxin, O157:H7 is the most common. STEC has been associated with hemorrhagic colitis, a severe form of diarrhea which can be prevented by thoroughly cooking ground beef and avoiding unpasteurized products such as apple cider.

- **Enteroinvasive *E. coli* (EIEC)** produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries.

The Shigellae

Antigenic Structure: There are more than 40 serotypes.

Pathogenesis and Pathology

Shigella infections (Shigellosis) are almost always limited to the gastrointestinal tract; bloodstream invasion is quite rare. Shigellae are highly communicable; the infective dose is on the order of 10^1 organisms. The pathogenic species of *Shigella* are *S dysonteriae, S. flexneri ,S. boydii* and *S. sonnei.*

Toxins

A. Endotoxin

B. *Shigella dysenteriae* Exotoxin

S dysenteriae type 1 (Shiga bacillus) produces a heat-labile exotoxin that affects both the gut (acting as an enterotoxin) and the central nervous system (acting as neurotoxin). The exotoxin is a protein that is antigenic and lethal for experimental animals.

Clinical Findings

After a short incubation period (1–2 days), there is a sudden onset of abdominal pain, fever, and watery diarrhea. In more than half of adult cases, fever and diarrhea subside spontaneously in 2–5 days. However, in children and elderly adults, loss of water and electrolytes may lead to dehydration, acidosis, and even death. The illness caused by *S dysenteriae* may be particularly severe. On recovery, most persons shed dysentery bacilli for only a short period, but a few remain chronic intestinal carriers and may have recurrent bouts of the disease. Upon recovery from the infection, most persons develop circulating antibodies to shigellae, but these do not protect against reinfection.

Immunity: Injection of killed shigellae stimulates production of antibodies in serum but fails to protect humans against infection.

Treatment: Ciprofloxacin ampicillin, doxycycline, and trimethoprim-sulfamethoxazole.

Epidemiology, Prevention, and Control

Shigellae are transmitted by "food fingers, feces, and flies" from person to person. Most cases of *Shigella* infection occur in children younger than 10 years of age. Because humans are the main recognized host of pathogenic shigellae, control efforts must be directed at eliminating the organisms from this reservoir by:

(1) Sanitary control of water, food, and milk: sewage disposal and fly control

(2) Isolation of patients and disinfection of excreta

- **Enteroaggregative *E. coli* (EAEC)** causes acute and chronic diarrhea in persons in developing countries, foodborn illnesses in industrialized countries and have been associated with travcler's diarrhea and persistent diarrnea in patients with HIV.

3. ***Sepsis***—When normal host defenses are inadequate. *E. coli* may reach the bloodstream and cause sepsis.

4. ***Meningitis***— Approximately 75% of *E. coli* from meningitis cases have the K1 antigen.

B. *Klebsiella*—*K pneumoniae* is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion (~1%) of bacterial pneumonias.

C. *Serratia*—*S. marcescens* is a common opportunistic pathogen in hospitalized patients. *Serratia* (usually nonpigmented) causes pneumonia, bacteremia, and endocarditis.

D. *Proteus*—produce infections only when the bacteria leave the intestinal tract. They are found in urinary tract infections and produce bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving contaminated intravenous infusions. *P mirabilis* causes urinary tract infections and occasionally other infections. *P. vulgaris* is important nosocomial pathogen.

Immunity: Specific antibodies develop in systemic infections, but it is uncertain whether significant immunity to the organisms follows.

Treatment

No single specific therapy is available. The sulfonamides, ampicillin, cephalosporins, fluoroquanolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great, and laboratory tests for antibiotic susceptibility are essential. Multiple drug resistance is common and is under the control of transmissible plasmids.

Epidemiology, Prevention, and Control

Some of the enterics constitute a major problem in hospital infection: these bacteria commonly are transmitted by personnel, instruments, or parenteral medications. Their control depends on hand washing, rigorous asepsis, sterilization of equipment, disinfection, restraint in intravenous therapy, and strict precautions in keeping the urinary tract sterile (ie, closed drainage).

(3) Detection of subclinical cases and carriers, particularly food handlers
(4) Antibiotic treatment of infected individuals

The *Salmonella-Arizona* group

Salmonellae are often pathogenic for humans or animals when acquired by the oral route. They are transmitted from animals and animal products to humans, where they cause enteritis, systemic infection, and enteric fever.

Classification

Currently, the genus *Salmonella* is divided into two species; *S. enterica* (contains subspecies; I, II, IIa, IIb, IV & VI) and *S. bongori* . Most human illness is caused by the subspecies I strains, written as *S enterica* subspecies *enterica*. There are more than 2500 serotypes of salmonellae, including more than 1400 in DNA hybridization group I that can infect humans. Four serotypes of salmonellae that cause enteric fever can be identified:
S. Paralyphi A (serogroup A), *S.* Paratyphi B (serogroup B), *S.* Choleraesuis (serogroup C1), and *S.* Typhi (serogroup D)

Pathogenesis and Clinical Findings

S. Typhi, *S.* Choleraesuis, and perhaps *S.* Paralyphi A and *S.* Paralyphi B are primarily infective for humans. The organisms almost always enter via the oral route, usually with contaminated food or drink. The mean infective dose to produce clinical or subclinical infection in humans is 10^5–10^9 salmonellae (but perhaps as few as 10^3 *S* Typhi organisms). Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbiota, and local intestinal immunity. Salmonellae produce three main types of disease in humans:

A. The "Enteric Fevers" (Typhoid Fever)

This syndrome is produced by only a few of the salmonellae, of which *S* Typhi (typhoid fever) is the most important. The organisms multiply in intestinal lymphoid tissue and are excreted in stools. After an incubation period of 10 -14 days, fever, malaise, headache, constipation, bradycardia, and myalgia occur.

B. Bacteremia with Focal Lesions

This is associated commonly with *S choloraesuis* but may be caused by any salmonella serotype.

C. Enterocolitis

This is the most common manifestation of salmonella infection. enterocolitis can be caused by any of the more than 1400 group I serotypes of salmonellae. Eight

to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stools. Low-grade fever is common, but the episode usually resolves in 2–3 days.

Immunity: Infections with *S. Typhi* or *S. Paratyphi* usually confer a certain degree of immunity.

Treatment: ampicillin, trimethoprim–sulfamethoxazole, or a third-generation cephalosporin.

Epidemiology

The feces of persons who have unsuspected subclinical disease or are carriers are a more important source of contamination than frank clinical cases that are promptly isolated, such as when carriers working as food handlers are "shedding" organisms. Many animals, including cattle, rodents, and fowl, are naturally infected with a variety of salmonellae and have the bacteria in their tissues (meat), excreta, or eggs. The high incidence of salmonellae in commercially prepared chickens has been widely publicized.

A. Carriers

After manifest or subclinical infection, some individuals continue to harbor salmonellae in their tissues for variable lengths of time (ie, convalescent carriers or healthy permanent carriers). 3% of survivors of typhoid become permanent carriers, harboring the organisms in the gallbladder, biliary tract, or, rarely, the intestine or urinary tract.

B. Sources of Infection

1. Water, milk and other dairy products (ice cream, cheese, custard)
3. Shellfish, meats and meat products
4. Dried or frozen eggs
5. "Recreational" drugs
7. Animal dyes
8. Household pets

Prevention and Control

1. Sanitary measures must be taken to prevent contamination of food and water by rodents or other animals that excrete salmonellae.
2. Infected poultry, meats, and eggs must be thoroughly cooked.
3. Carriers must not be allowed to work as food handlers and should observe strict hygienic precautions.
4. Vaccination is recommended for travelers to endemic regions.

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Lecture : (10)

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Vibrios, Campylobacters, and Helicobacter

Vibrio, Campylobacter, and Helicobacter species are gram-negative rods that are all widely distributed in nature. The vibrios are found in marine and surface waters. The campylobacters are found in many species of animals, including many domesticated animals. Vibrio cholerae produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death. Campylobacter jejuni is a common cause of enteritis in humans. Helicobacter pylori has been associated with gastritis and duodenal ulcer disease.

The Vibrios

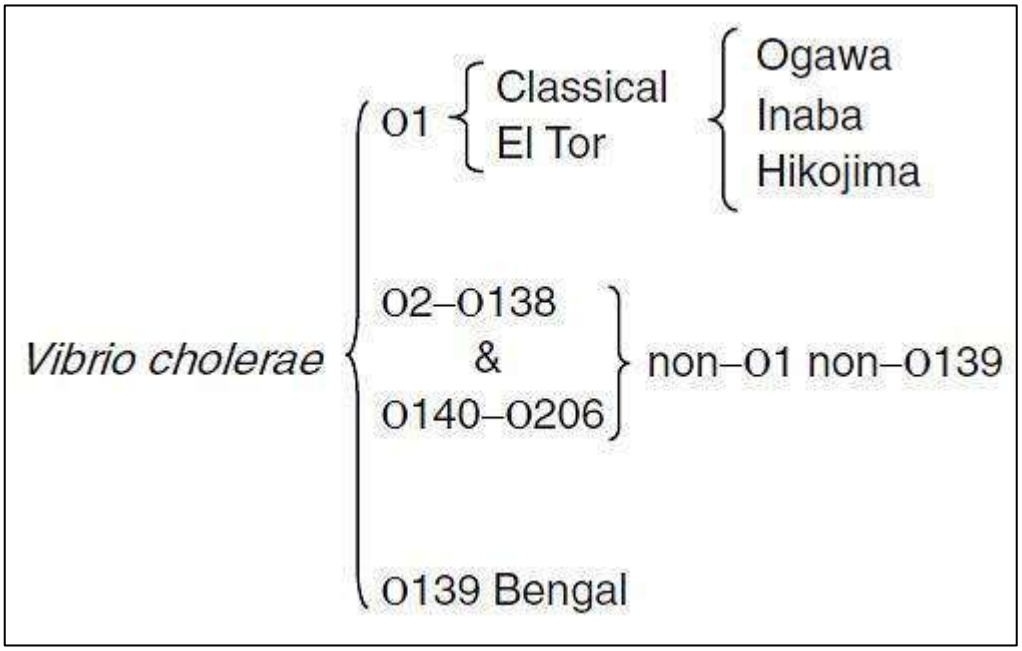
Vibrios are among the most common bacteria in surface waters worldwide. They are curved aerobic rods and are motile, possessing a polar flagellum. V. cholerae serogroups O1 and O139 cause cholera in humans, and other vibrios may cause sepsis or enteritis. The medically important vibrios are listed in Table 1.

Table 1: The Medically Important Vibrios

Organism	Human Disease
V. cholerae serogroups O1and O139	Epidemic and pandemic cholera
V. cholerae serogroups non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely, extraintestinal infection
V. parahaemolyticus	Gastroenteritis, perhaps extraintestinal infection
Others	
V. mimicus, V. vulnificus, V. hollisae, V. fluvialis, V.damsela, V. anginolyticus, V. metschnikovii, V.cincinnatiensis	Ear, wound, soft tissue, and other extraintestinal infections (all uncommon)

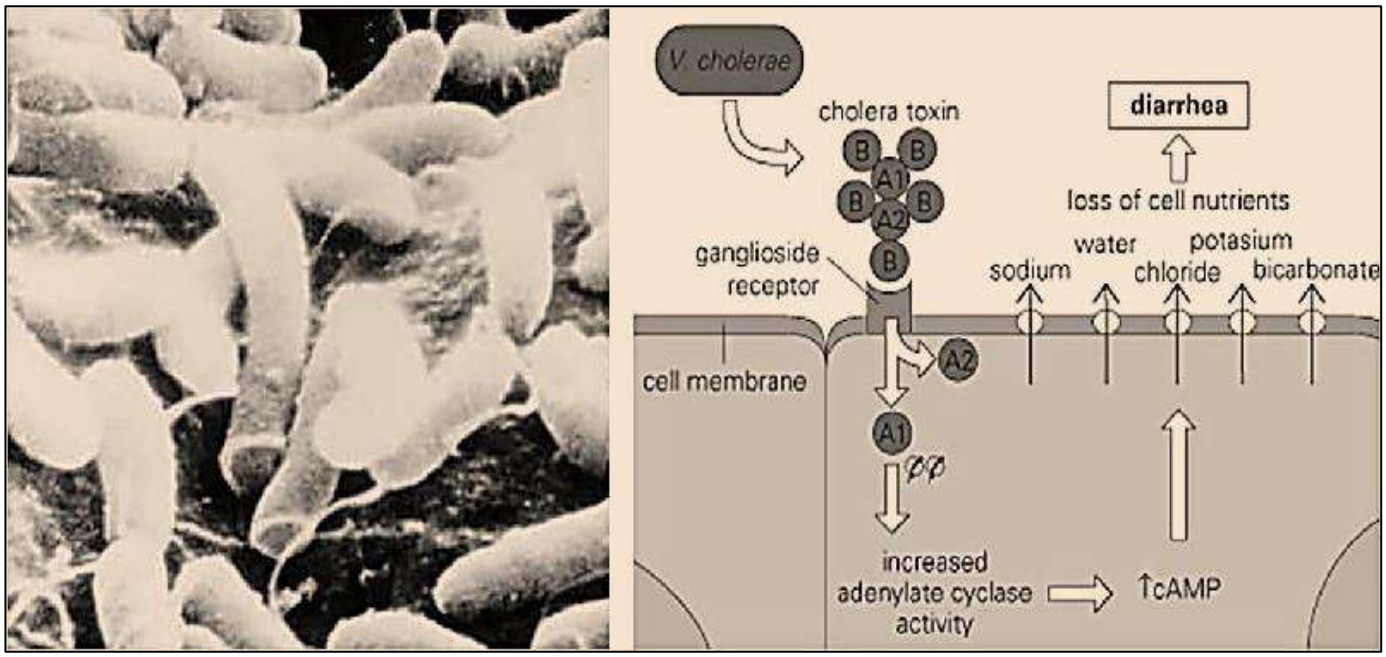
Vibrio cholerae

V. cholerae has at least 206 O antigen groups. The V. cholerae serogroup O1 antigen has determinants that make possible further typing; the serotypes are Ogawa, Inaba, and Hikojima. Two biotypes of epidemic V. cholerae have been defined, classic and El Tor. The El Tor biotype tends to cause milder disease than the classic biotype.



***Vibrio cholerae* Enterotoxin**

V. cholerae produce a heat-labile enterotoxin (MW 84,000), consisting of subunits A and B. Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A1 yields increased levels of intracellular cyclic adenosine monophosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes. There is increased sodium-dependent chloride secretion, and absorption of sodium and chloride by the microvilli is inhibited. Electrolyte-rich diarrhea occurs—as much as 20–30 L/day—with resulting dehydration, shock, acidosis, and death.



Pathogenesis and Pathology

A person with normal gastric acidity to become infected may have to ingest:

- ≥ 10^{10} V. cholerae when the vehicle is water
- 10^{2} –10^{4} organisms when the vehicle is food

Cholera is not an invasive infection. Virulent V. cholerae organisms attach to the microvilli of the brush border of epithelial cells. There they multiply and liberate cholera toxin and perhaps mucinases and endotoxin.

Clinical Findings

About 50% of infections with classic V. cholerae are asymptomatic, as are about 75% of infections with the El Tor biotype. The incubation period is 12 hours–3 days for persons who develop symptoms, depending largely on the size of the inoculum ingested. There is a sudden onset of nausea and vomiting and profuse diarrhea with abdominal cramps. Stools, which resemble —rice water,‖ contain mucus, epithelial cells, and large numbers of vibrios. There is rapid loss of fluid and electrolytes, which leads to profound dehydration, circulatory collapse, and anuria. The mortality rate without treatment is between 25% and 50%.

Immunity

Gastric acid provides some protection against cholera vibrios. An attack of cholera is followed by immunity to reinfection, but the duration and degree of immunity are not known.

Treatment

- The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion.
- Many antimicrobial agents are effective against V. cholerae, oral tetracycline and doxycycline
- In children and pregnant women, erythromycin and furazolidine.

Epidemiology

- 1817 – 1993; 8 pandemics of cholera were occurred by V. cholerae O1of the classic and El Tor biotype and serotype O139 in Asia, Middle East, Africa, South America and Central America
- The disease has been rare in North America.
- Cholera is endemic in India and Southeast Asia.
- The disease is spread:

1. By contact involving individuals with mild or early illness
2. By water, food, and flies.
    - Only 1–5% of exposed susceptible persons develop disease.

Prevention and Control

- Control rests on education and on improvement of sanitation, particularly of food and water.
- Patients should be isolated, their excreta disinfected, and contacts followed up.
- Chemoprophylaxis with antimicrobial drugs may have a place.
- Repeated injection of a vaccine containing either lipopolysaccharides extracted from vibrios or dense Vibrio suspensions can confer limited protection to heavily exposed persons (eg, family contacts) but is not effective as an epidemic control measure.

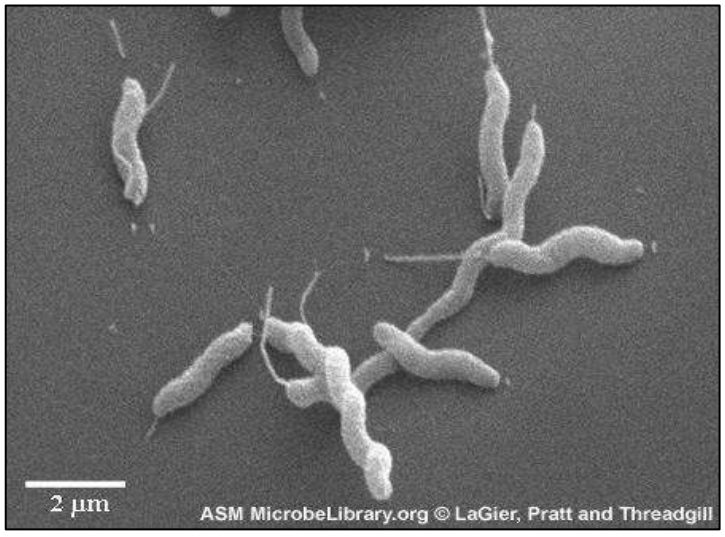
Campylobacter

Campylobacters cause both diarrheal and systemic diseases and are among the most widespread causes of infection in the world. C. jejuni and the other campylobacters are gram-negative rods with comma, S, or ―gull wing‖ shapes. They are motile, with a single polar flagellum, and do not form spores. C. jejuni is a very common cause of diarrhea in humans.

The campylobacters have:

- LPSs with endotoxic activity
- Cytopathic extracellular toxins
- Enterotoxins

The significance of the toxins in human disease is not well defined.



Pathogenesis and Pathology

The source of infection may be:

1. Food, drink (eg, milk, undercooked fowl)
2. Contact with infected animals (especially poultry) or humans and their excreta.

C. jejuni is susceptible to gastric acid, and ingestion of about 10 organisms is usually necessary to produce infection. The organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools. Occasionally, the bloodstream is invaded, and a clinical picture of enteric fever develops.

Clinical Findings

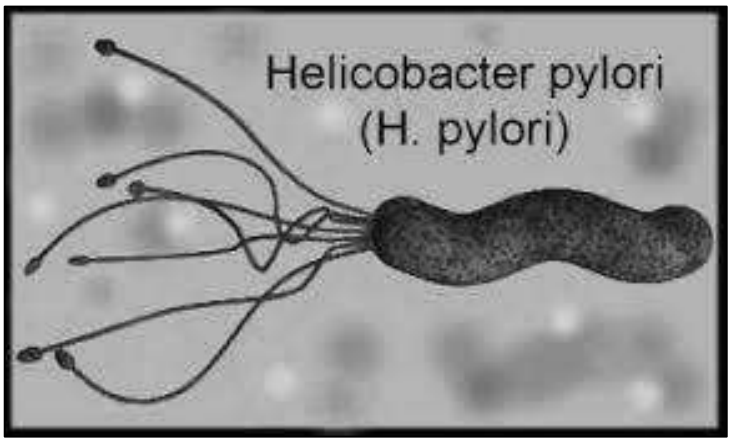
Clinical manifestations are acute onset of cramp abdominal pain, profuse diarrhea that may be grossly bloody, headache, malaise, and fever. Usually the illness is self-limited to a period of 5–8 days, but occasionally it continues longer.

Treatment

C. jejuni isolates are usually susceptible to erythromycin, most cases resolve without antimicrobial therapy; however, in about 5–10% of patients, symptoms may recur.

Helicobacter pylori

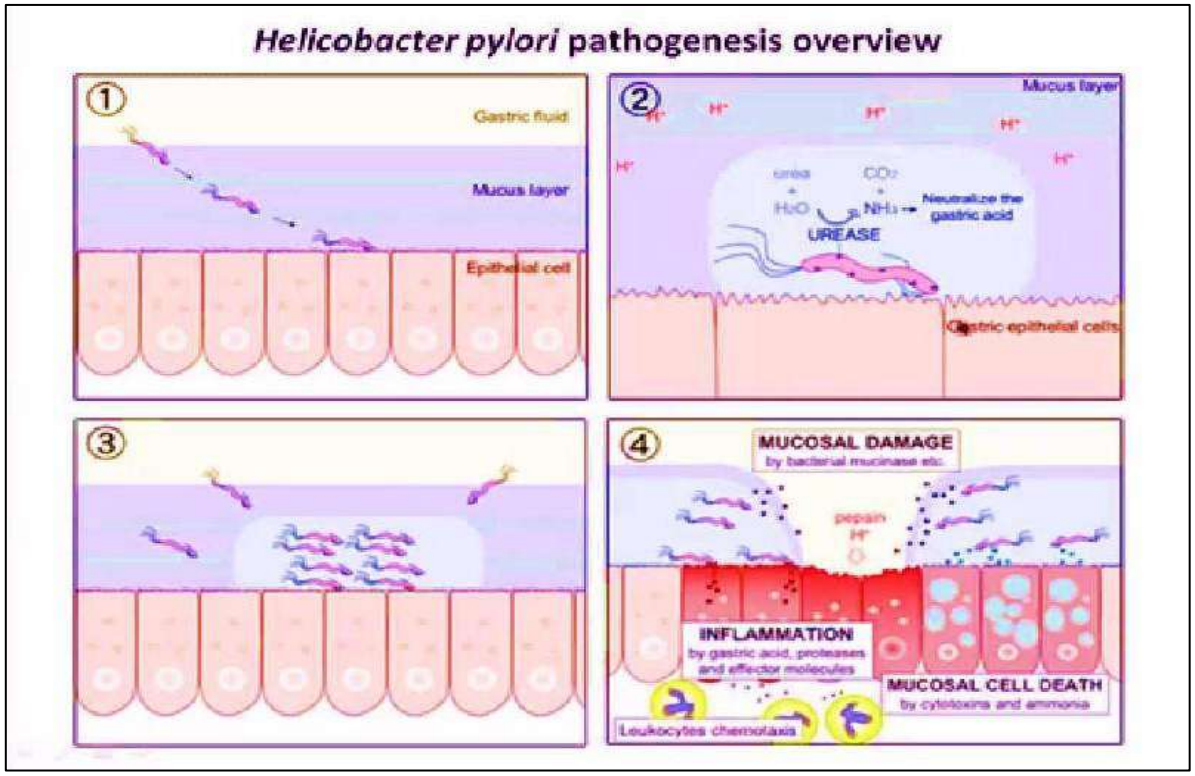
H. pylori is a spiral-shaped gram-negative rod. H. pylori is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphomas. H. pylori is a major risk factor for gastric cancer.



Pathogenesis and Pathology

H. pylori grows optimally at a pH of 6.0–7.0 and would be killed or not grow at the pH within the gastric lumen. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–2.0); on the epithelial side, the pH is about 7.4. H. pylori is found deep in the mucous layer near the epithelial surface where physiologic pH is present. H. pylori also produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus. H. pylori produces potent urease

activity, which yields production of ammonia and further buffering of acid. H pylori is quite motile, even in mucus, and is able to find its way to the epithelial surface. The mechanisms by which H. pylori causes mucosal inflammation and damage are not well defined but probably involve both bacterial and host factors.



Clinical Findings

Acute infection can yield an upper gastrointestinal illness with nausea and pain; vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the H pylori infection persists for years and perhaps decades or evens a lifetime. About 90% of patients with duodenal ulcers and 50–80% of those with gastric ulcers have H. pylori infection.

Treatment

Triple therapy with metronidazole and either bismuth subsalicylate or bismuth subcitrate plus either amoxicillin or tetracycline for 14 days eradicates H. pylori infection in 70–95% of patients. An acid-suppressing agent given for 4 to 6 weeks enhances ulcer healing.

Epidemiology

H. pylori is present on the gastric mucosa of:

- Fewer than 20% of persons younger than years 30
- 40–60% of persons age 60 years, including persons who are asymptomatic.
- In developing countries, the prevalence of infection may be 80% or higher in adults.