Ministry of higher education and scientific research
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PATHOGENIC BACTERIA LECTURES

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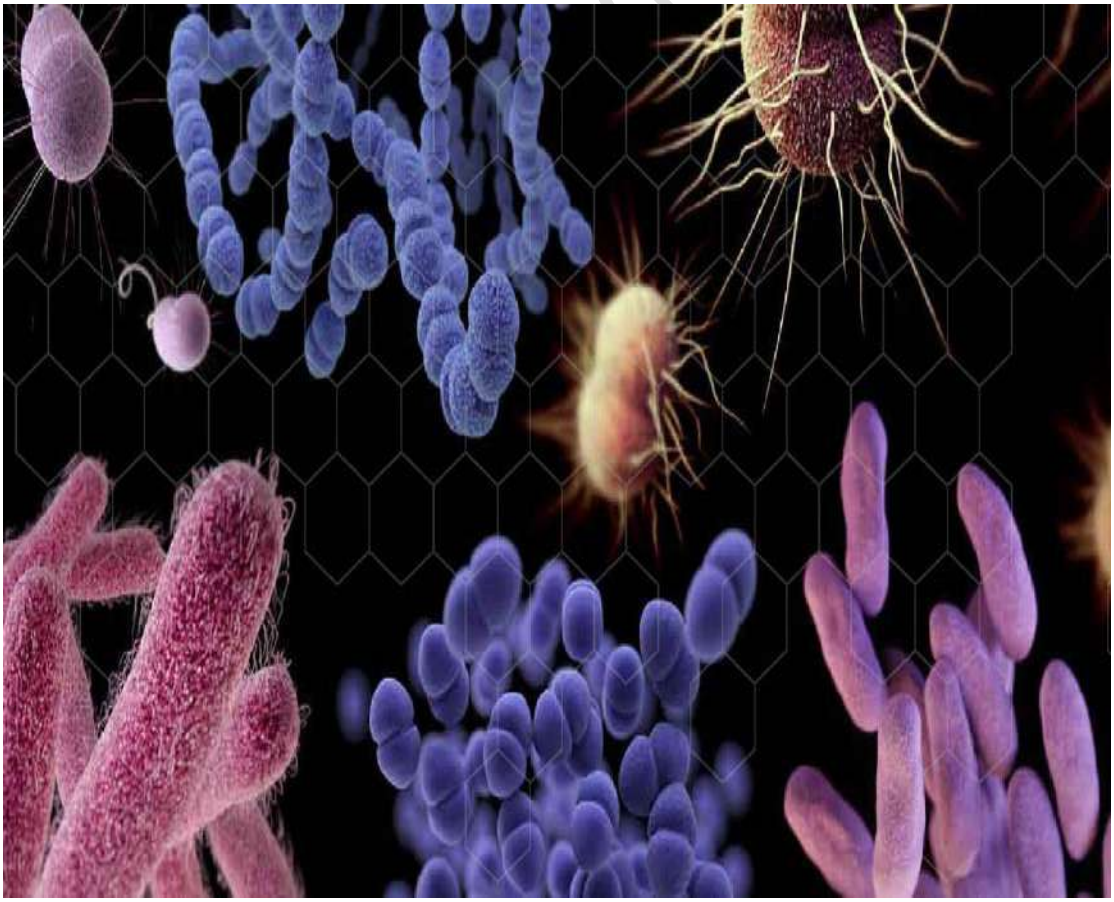
THIRD LEVEL /FIRST COURSE



First lecture *Dr. Mouruj Al aubydi and Dr. Jenan Al Saffar*

Pathogenic bacteria: are bacteria that cause bacterial infection. This subject deals with human pathogenic bacteria .Although most bacteria are harmless or often beneficial, several are pathogenic. One of the bacterial diseases is tuberculosis, caused by the bacterium *Mycobacterium tuberculosis* discovered by Robert Kock (1843-1910).

Koch's postulates are four criteria designed to establish a causative relationship between a microbe and a disease (**scientific basis which provides the evidence for microorganism to be the causative agent of an infectious disease)**. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884**.**

Koch's postulates are the following:

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be re isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

In (1865-1869) Louis Pasteur (French Scientist) discovered the parasite infects the silk worm. Joseph Lister (British scientist) in 1867 used phenol as disinfectant .

Host parasite relationship:

Symbiosis - long-term interactions between different biological species, which can be mutualistic, commensal or parasitic

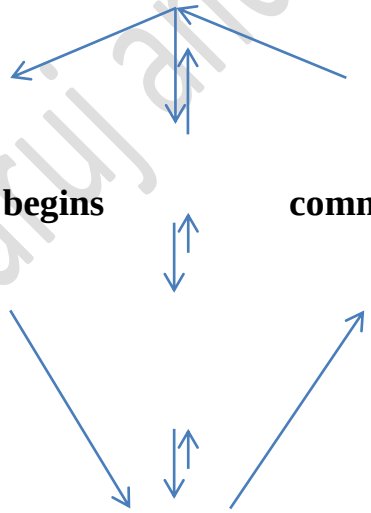
Parasitism the two (both) organisms of different species exist in a relationship in which the **parasite where benefits while the another is harmed**.

Mutualism is individual benefits, in which **both organisms benefit** (such as individual) and bacteria within their intestines (normal flora) .

Commensalism is a class of relationship between two organisms **where one organism benefits without affecting the other**.

Amensalism, where **one is harmed while the another is unaffected.**

Antagonism refers to **the action of any organism that suppresses or interfere the normal growth and activity of a pathogen,** such as the main parts of bacteria or fungi.



All these relationship are dynamic , and can be quickly shifts among them.

NORMAL FLORA

The microorganisms are found normally in the tissues such as intestine, skin , and other mucous membranes . But the **blood, brain, muscle, urine etc., are normally free of microorganisms**. However, the surface tissues,

i.e., **skin and mucous membranes**, are constantly in contact with environmental organisms and become readily colonized by various microbial species. **The mixture of organisms regularly found at any anatomical site is referred to as the normal flora**. The normal flora of humans consists of a few **eukaryotic fungi and protists**, but bacteria are the most numerous and obvious microbial components of the normal flora. Thus we can classify normal flora in to

1-TRANSIENT FLORA

Some of These organisms may be **Pathogens** (more frequently among the transient flora group).Some among the normal flora may be **opportunists** (may be found for hours. Days , weeks) .

2- Resident flora : Regularly found inside the body (endosymbionts) or on its surfaces (ectosymbionts) usually not pathogenic but may be opportunistic.

Table 1. Bacteria commonly found on the surfaces of the human body.

BACTERIUM	S	C	N	Ph	MG	Ur	V
Staphylococcus epidermidis	++	+	++	++	++	+	++ ++
Staphylococcus aureus*	+	+/-	+	+	+	++	+/- +
Streptococcus mitis				+	++	+/-	+ +
Streptococcus salivarius				++	++		
Streptococcus mutans*				+	++		
Enterococcus faecalis*				+/-	+	++	+ +
Streptococcus pneumoniae*		+/-	+/-	+	+		+/-
Streptococcus pyogenes*	+/-	+/-		+	+	+/-	+/-
Neisseria sp.		+	+	++	+		+ +
Neisseria meningitidis*			+	++	+		+
Enterobacteriaceae*(Escherichia coli)		+/-	+/-	+/-	+	++	+ +
Proteus sp.		+/-	+	+	+	+	+ +

Pseudomonas aeruginosa*				+/-	+/-	+	+/-	
Haemophilus influenzae*		+/-	+	+	+			
Bacteroides sp.*						++	+	+/-
Bifidobacterium bifidum						++		
Lactobacillus sp.				+	++	++		++
Clostridium sp.*					+/-	++		
Clostridium tetani						+/-		
Corynebacteria	++	+	++	+	+	+	+	+
Mycobacteria	+		+/-	+/-		+	+	
Actinomycetes				+	+			
Spirochetes				+	++	++		
Mycoplasmas				+	+	+	+/-	+

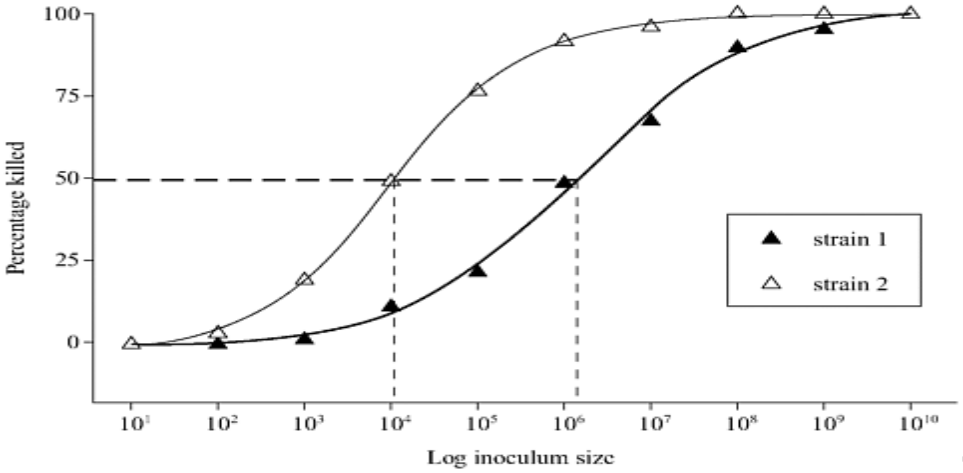
S:skin C:conjunctiva N: Nose ph: pharynx M: mouth G: GIT Ur: urethra V: vagina

++ = nearly 100 % + = common (about 25 %) +/- = rare (less than 5%)

What are the bacterial virulence factors?

Virulence factors can most simply be defined as the character(s) that are directly involved in the development of disease. The term **virulence** is used to grade the ability of an organism to cause disease. The measurement of virulence is made by comparing the numbers of organisms necessary to cause disease in a suitable model.

1- Adherence to host cells
2- Invasiveness
3- Iron sequestering
4- Virulence factors that inhibits phagocytosis
5- Bacterial toxins include (Exotoxin , Endotoxin)
6- Antibiotic resistance
7- Super antigen



The slope is greatest at the mid- point, reflecting the biggest increases in the proportion of animals
dying per dose. This means that the difference between the LD30 and LD70 will be reasonably close
to that of the LD50, but values of the LD90 may be indistinguishable between 10^7 and 10^{10}
organisms.

- **Infectivity:** Describes the ability of an organism to establish itself in a new host and this is defined by the ID50. The LD50 is used in the measurement of virulence between two strains of the same organism.

1. Colonization

The first stage of microbial infection is **colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. **Sites of entry in human hosts include skin and mucous membrane, such as (the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva).** In its simplest form, bacterial adherence or attachment to a eukaryotic cell or tissue surface requires the participation of two factors: a **receptor** and a **ligand**. **The receptors so far defined are usually specific carbohydrate or peptide residues on the eukaryotic cell surface**. The bacterial ligand, called an **adhesin**, is **typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor**.
There are several terms used to describe adherence factors in microbiology such as (Adhesin, Receptor, Fimbriae, Biofilm, Capsule..etc)

second lecture *Dr. Mouruj Al aubydi and Dr. Jenan Al Saffa*

Specific Adherence of Bacteria to Cell and Tissue Surfaces

Several types of observations have provided indirect evidence for **specificity of adherence** of bacteria to host cells or tissues:

1. **Tissue tropism**. Particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue.
2. Species specificity. Certain pathogenic bacteria infect only certain species ,e.g. Group A streptococcal infections occur only in humans.

3. Genetic specificity within a species: certain strains or races within a species may be genetically immune to a pathogen, e.g. certain pigs are not susceptible to *E. coli* K-88 infections; males are not susceptible to mastitis; females are not susceptible to orchitis.

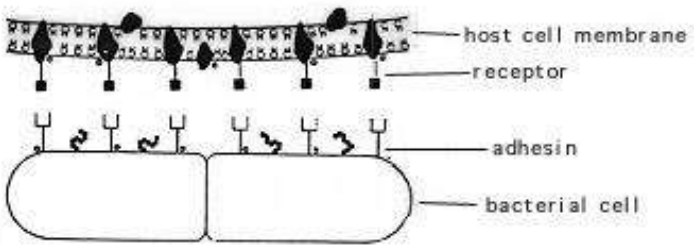
Mechanisms of Adherence to Cell or Tissue Surfaces

The mechanisms for adherence may involve two steps:

1. **Nonspecific adherence: reversible attachment** of the bacterium to the eukaryotic surface (sometimes called "docking") involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surface. Possible interactions and forces involved are:

1. Hydrophobic interactions
2. Electrostatic attractions
3. Atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies
4. Brownian movement
5. Recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

2. **Specific adherence: irreversible permanent attachment** of the microorganism to the surface (sometimes called "anchoring").The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur .Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface.



2. Multiply: Multiplication of bacterium at the site of entry **.**

3. Invasion

Invasiveness is the **ability of a pathogen to invade tissues.** The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substances which act against the host by breaking down primary or secondary defenses of the body. Medical microbiologists refer to these substances as **invasins**. Most invasins are proteins (enzymes) that act locally to damage host cells and/or have the immediate effect of facilitating the growth and spread of the pathogen. The damage to the host as a result of this invasive activity may become part of the pathology of an infectious disease.

Invasiveness encompasses (1) mechanisms for colonization (adherence and initial multiplication), (2) production of extracellular substances ("invasins"), that promote the immediate invasion of tissues and (3) ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process.

4. Spread within the host: using

"Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and

intercellular spaces, thereby promoting the spread of the pathogen such as Hyaluronidase, Collagenase , Neuraminidase , Hemolysins.

5. Ability to persist within the host (evade the host immune response)

The consisting of polysaccharides, capsules**,** Outer membrane proteins, IgAses, Antigenic variation. All these factors are considered as virulence factors for pathogen that can be overcome the human or animals immune system.

6. SHEDDING

For many pathogenic bacteria multiplication within the human body provides a means of generating large numbers of progeny, thus increasing the numbers that can be shed into the environment.

How we can differentiate between exotoxin and endotoxin for G+ and some G-

Exotoxin	Endotoxin
Excreted by living cells, found in high conc. In fluid medium	Part of the cell wall and G- bacteria liberated up on their disintegration
Polypeptides , molecular weight 10000-900000 Daltons	Lipopolysaccharide complex. lipid apportion responsible for toxicity
Relatively unstable to temperature above 60 C	Relatively stable to temp. above 60C for several hours with no loss activity

Highly antigenic, stimulates the formation of high – titer antitoxin (neutralized toxin)	Do not stimulate formation of antitoxin ,stimulate formation of antibodies to polysaccharide moiety
Can be converted to a toxoid	Cannot be converted to a toxoid
Do not produce fever in the host	produce fever in the host "pyrogenic effect"
Highly toxic in microgram quantities to laboratory susceptible animals	Weekly toxic, hundreds of microgram quantities required to be lethal for animals

Microbial epidemiology

- **Epidemiology** is the science that studies the patterns, causes, and effects of health and disease conditions in defined populations. The distinction between
- **Epidemic diseases** that are visited upon a population , from
- **Endemic** disease that reside within a population (endemic).

Epidemiologists also study the interaction of diseases in a population, a condition known as **a Syndemic**, is the aggregation of two or more diseases in a population.

Reservoir of infection: From the French reservoir, from reserve. Mean the primary habitat of the organism. **Any person, animal, plant ,water, soil or substance (part of a device) in which an infectious agent normally lives and multiplies.** The reservoir typically harbors the infectious agent without injury to itself and serves as a source from which other individuals can be infected. The infectious agent primarily depends on the reservoir for its survival. It is from the reservoir that the infectious substance is transmitted to a human or another susceptible host .

Epidemiological markers

Biological markers which are used to characterize microorganisms or discriminate between genomes based on genetic variation among microbial isolates. Which are include:

1- ***Genotype*** *: Genetic constitution of an organism as assessed by a molecular method such as* ***plasmid typing . SNP typing***
2- ***Phenotype*** *:Observable characteristic of an isolate such as* ***serotyping ,bacteriocin typing ,antibiotic typing***

Stability epidemiological marker should remain stable for each isolate after **its primary isolation & during laboratory storage & subculture across generations.**

How Do Infectious Diseases Spread?

Microbes invade the host body and begin multiplying using the host body resources. Thus, this causes problems in the normal functioning of the infected part, organ or tissue. Microbes can enter the body by the following mode of entry:

Respiratory Tract Illnesses: through inhalation of airborne droplets containing microbes by sneezes or coughs.

Food Borne Illnesses: Infections can spread through contaminated food and drinks.

Vector Borne Illness: Infections that spread by a vector who serves as an intermediate host to a healthy person is called as vector borne illness. Diseases such as malaria

Person-To-Person Contact: Many illnesses spread by direct contact with the infected person. Body fluids that contain microbes can enter the body through saliva, blood, semen, pus or an open wound.

Venereal/Sexual Transmission : like gonorrhea, syphilis, HIV/AIDS, etc. spread through unsafe intercourse with an infected person.

Vertical Transmission: When an infected woman gets pregnant or acquires an infection during pregnancy, it results in vertical transmission. This means, the infection can spread from the mother to her embryo, fetus or child during pregnancy or childbirth.

Iatrogenic transmission: Infection that spreads due to medical error or lapse, that is injection or transplantation of an infected material into a healthy individual.

Animal to person: infections that can be transmitted between vertebrate animals and humans. The natural host is the animal is called as **zoonotic diseases**. Rabies is an example of such an infectious disease.

Bacterial Infections characterized by following signs:

*Redness, swelling, and heat on the infected part *Pain at the site of infection * Pus

Some of the common diagnostic methods to detect infection include:

*Blood tests * X-rays *Microbial culture *Stool samples *Urinalysis *Microscopically tests *Biochemical tests *Molecular diagnostic tests *Biopsy

Bacterial antigenic structures :

There are three different antigenic structures found in bacteria which are

1- O antigen which is represent to a somatic bacterial antigen (Ag) such as lipopolysaccharide (LPS) .
2- K antigen which is represent to capsular part of bacteria
3- H antigen which is represent to bacterial flagella .

Several terms are predominantly related with infection

Infectious disease is an infection that can be transmitted between humans (or organisms/animals, etc.).

Acute infections will be those that arise quickly (e.g. tonsillitis) and progress rapidly

Chronic infections which have a longer course (e.g. tuberculosis), lasting for weeks up to years without resolution.

Latent infections are those in which the microbe is able to persist for years within a site in the host and cause minimal clinical disease for most of the time the organism is present.
Generalized infections are often more severe. The involvement of numerous organs throughout the body will lead to more complications. Another term for systemic infection is 'multi-organ infection'.

Localized infections: simply infecting a site such as on the skin**.**

Nosocomial infections : People often acquire an infection during their stay in hospital.

Fourth lecture Dr.Mouruj alaubydi and Dr. Jenan Al Saffar

Staphylococcus genus

Staphylococcus (from the Greek: staphylē, "grape" and coccus, "granule") is a genus of dark Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. produces catalase which is one feature that distinguishes them from catalase negative Streptococcus , has an appropriate cell wall structure (including peptidoglycan type and teichoic acid presence) and G + C content of DNA in a range of 30–40 mol%.

The Staphylococcus genus includes at least 40 species. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora. are facultative anaerobes (capable of growth both aerobically and anaerobically). All species grow in the presence of bile salts.

The most virulence species is S. aureus , almost all isolates of which secrete coagulase , an enzyme that causes citrated plasma to clot.

Staphylococci are hardly ,being resistant to heat and drying ,and thus can present for longtime on inurement objects , thus serve as source of infection .

Taxonomy The taxonomy is based on 16s rRNA sequences, and most of the staphylococcal species fall into 11 clusters, a twelfth group has now been moved to a new genus Macrococcus, the species of which are currently the closest known relatives of the Staphylococci. Staphylococcus aureus A- Epidemiology : IS frequently carried by healthy individuals on the skin and mucous membrane carriers serve as a source of infection to them . B- Pathogenesis : Virulence factors are the genetic , biochemical , or structural features that enable an organism to produce disease .Staphylococcus aureus expresses many potential virulence factors

1- Coagulase : Activity results in localized clotting , which restricts access by polymorphonuclear neutrophils (PMNS) and other immune defenses. 2- Cell wall virulence factors : a- Capsule : Most clinical isolates express a polysaccharide "microcapsule" of type 5 or 8 , its very thin , but has been associated with increased resistance to phagocytosis . b- Protein A: Is a major component of the S. aureus cell wall . It act as anti-phagocytic factor . c- Fibronectin – binding protein : Its one of surface proteins promote binding to mucosal cells and tissues matrices . 3- Cytolytic exotoxins: α ,β ,γ , and δ toxins attack mammalian cell (including RBC) membranes . , and often referred to as hemolysis . 4- Panton – Valentine leukocidin : Its pore forming toxin lyses PMNs . 5- Super antigens exotoxins : Which are responsible for causing toxic shock syndrome . a- Enterotoxins : there are six types (A,B,C,D,E,and G), these enterotoxins cause food poisoning . Enterotoxins are super antigens that are even more heat – stable than S . aureus . b- Toxic shock syndrome toxin (TSST-1): This is a classic cause of toxic shock syndrome (TSS). Because of similarities in molecular structures , its sometime reffered to as staphylococcal enterotoxin F although it does not cause food poisoning when ingested. c- Exfoliatin (Exofoliative toxin, (ET)): Is also a super antigen , it causes Scalded skin syndrome in children . Clinical significance : 1- Localized skin infections are small, superficial abscesses involving hair follicles (folliculitis) or sweat or sebaceous glands. 2- Deep, localized infections or skin carriage or may result from trauma. 3- Acute endocarditis: Generally associated with intravenous drug abuse , acute endocarditis is caused by

injection of contaminated preparations or by needles contaminated with S. aureus . 4- Septicemia: Is a generalized infection with sepsis or bacteremia that may be associated with a known focus. 5- Pneumonia : S . aureus is a couse of sever necrotizing pneumonia . 6- Nosocomial infections: S . aureus is one of the most common causes of hospital- associated with catheters. 7- Toxinoses : These are diseases caused by the action of a toxin , frequently when the organism that secreted the toxic is undetectable . Toxinoses caused by S . aureus include

a- Toxic shock syndrome . b- Staphylococcal gastroenteritis : caused by ingestion of food contaminated with enterotoxin-producing S . aureus , these food tend to be protein rich. Its heat resistance toxins are able to withstand subsequent reheating . the short incubation period of staphylococcal food poisoning occurs between the toxin in the food has already been formed by staphylococcal before the food is ingested . c- Scalded skin syndrome.

 Laboratory identification: 1- Microscopic identification (G+) in grape like cluster . 2- Colony morphology 3- Catalase positive. 4- Mannitol positive. 5- Coagulase positive.

 Treatment: By using antibiotics . Because S . aureus become resistant to pencillin G , thus its need to replacement of initial agent (pencillin G) by β- lactamase – resistant pencillins , such as methicillin or oxacillin . However , increased use of methicillin and related antibiotics has resulted in S . aureus that is resistant to the number of β- lactam antibiotics , these strains are known as methicillin – resistant S . aureus . 1- Hospital – acquired methicillin resistant S . aureus (MRSA). 2- Community- acquired MRSA. 3- Vancomycin resistance : vancomycin has been the agent of choice for empiric treatment of life –threatening MRSA. S . aureus infections several MRSAs were isolated that had also acquired low- level vancomycin resistance . Coagulase – negative Staphylococcus.

Of 12 coagulase- negative staphylococcal species that have been recovered as normal commensals of human skin and anterior nares , the most abundant and important is S. epidermidis , the second most important coagulase- negative staphylococcus is S. saprophyticus , both bacterium are important agents for hospital-acquired infections A- S. epidermidis : Is present in large numbers as part of the normal flora of the skin , it is frequently recovered from blood cultures, generally as a contamination from skin .Its produces an extracellular polysaccharide material called polysaccharide intercellular adhesion (sometime called slime) that

facilitates adherence to bioprosthatic material surface such as intravenous catheters.

B- S.saprophyticus This organism is a frequently cause of cystitis in women , probably related to its occurrence as part of normal vaginal flora it tend to be sensitive to most antibiotics, even pencillin G . But we can distinguished from S. epidermidis by its natural resistance to novobiocin

fifth lecture *Dr. Mouruj Alaubydi and Dr. Jenan*

Streptococci genus

Are G+ , non-motile , and **catalase negative** . Clinically important genera include Streptococcus and Enterococcus , they are ovoid to spherical in shape , occur as pairs or chains .

Most are aero tolerant anaerobes because they grow fermentatively even in the presence of oxygen . and because of **their complex nutritional requirements** blood enriched medium is generally used for their isolation.

Classification of streptococcus

We can classify Streptococci according to

1- Hemolytic properties on blood agar

- **α –** hemolytic Streptococcus : appearance of **green pigment** that forms a ring around the colony.
- **β-** hemolysis Streptococci: gross lysis of red blood cells resulting in a **clear ring around** the colony.

- γ- hemolytic streptococci: cause **no color change or lysis** of the RBCs.

2- Serologic (Lancefield) grouping

Many species of Streptococci have a **polysaccharide** in their cell walls known as **C- substance**. Thus on the bases of their C-substance . The **clinically most important groups of β- hemolytic Streptococci are types A and B** .

***Group A β- hemolytic Streptococci**

S. pyogenes : The **most clinically important member** of this group of G+ cocci, it can invade apparently intact skin or mucous membranes, causing some of the **most rapidly progressive infections** known . *S. pyogenes* is usually spread person to person by skin contact and via the respiratory tract .

A- Structure and physiology It is appear as individual cocci, pairs, or clusters of cells in gram stains of samples from infected tissues.

The structural features involved

1- **Capsule: Hyaluronic acid , identical** to that found in **human connective tissue ,** form outer most layer of the cell. This **capsule is not recognized as foreign by the body** , and therefore **, is non-immunogenic** . The capsule also **antiphagocytic** .
2- The cell wall : The cell wall contains a number of clinically important components
   a- M protein: *S. pyogenes* **is not infectious in the absence of M-protein** . This protein is **highly variable** especially the N- terminal regions , resulting in over 80 different antigen types , **M- protein are antiphagocytic** and they **form a coat that interferes with complement binding .**
   b- Group A: Specific C- substance ,this component is composed of rhamnose and N- acetylglucosamine(All group A streptococci contain this antigen).
   c- Protein F (Fibronectin – binding protein):mediates attachment to fibronectin in the pharyngeal epithelium .

3- **Extracellular products** : *S. pyogenes* secretes a wide range of exotoxins that often vary from one strain to another and play roles in the pathogenesis of disease caused by these organisms .

C- Pathogenesis :

S. pyogenes cells , perhaps in an inhaled droplet attach to the pharyngeal via actions of **protein F , lipoteichoic acid , and M protein**. Bacteria may grow and secrete toxins causing damage to surrounding cells , invading the mucosa . there is sufficient spread that the blood stream is significantly invaded , possibly resulting in septicemia and / or seeding of distant sites.

*** Clinical significance :**

1- **Acute pharyngitis or pharyngotonsilitis**

It is the most common type of *S. pyogenes* infection. This type pharyngitis called "Strep throat" the syndrome is designated scarlet fever.
Laboratory confirmation is important for accurate diagnosis and treatment of streptococcal pharyngitis particularly for the prevention of subsequent acute rheumatic fever and rheumatic heart disease .
2-**Impetigo** : Typically affecting children , it can cause severe and extensive lesion on the face and limbs .

3-**Erysipelas** : Affecting all age groups, advancing erythema especially on face

4-**Puerperal sepsis** : Its caused by exogenous transmission (ex. By nasal droplets from an infected carrier) or endogenously from the mothers vaginal flora .

5-**Invasive group A Streptococcal disease (GAS):** Patients may have a deep local invasion either without necrosis (cellulitis) or with it (necrotizing fasciltis / myositis) . Invasive GAS disease often spreads rapidly , leading to bacteremia and sepsis .

6-**Streptococcal toxic shock syndrome**: The syndrome is mediated by the production of streptococcal pyogenic exotoxin that function as super antigen causing massive , nonspecific T-cell activation cytokine release.

7-**Post-streptococcal sequelae** :

a- **Acute rheumatic fever**: this autoimmune disease occurs 2 to 3 weeks after initiation of pharyngitis ,its caused by cross reactions between antigen of the heart and joint tissyes and the streptococcal antigen (especially the M protein epitopes)

b- **Acute glomerulonephritis** : This rare , post infectious squeal occurs as soon as 1 week after impetigo or pharyngitis .

***Laboratory identification:**

Rapid latex antigen kits for direct detection of group A streptococci in patient samples are widely used.

1- Specimens: For lab. analysis can be obtained from throat swabs , pus and lesion samples, sputum , blood or spinal fluid .
2- Sensitivity test: this organism is highly sensitive to bacitracin .
3- *S. pyogenes* is catalase negative .
4- Optochin resistant.
5- Serological tests .

***Treatment:**

1- Penicillin G . For hypersensitive patients to penicillin G , Macrolide such as clarithromycin or azithromycin are preferred drugs.

***Prevention:**

Rheumatic fever is prevented by rapid eradication of infecting organism.

sixth lecture ***Dr. Mouruj Alaubydi and Dr. Jenan***

Group B β-hemolytic streptococci

Represented by the pathogen *S. aglalactiae* are G+ **catalase – negative** organism , is found in the vaginocervical tract of female carriers , and the urethral mucous membrane of male carriers as well as in the GIT. Can be transmitted sexually among adults and from an infected mother to her infant at birth.Group B streptococci are a leading cause of **meningitis and septicemia** in neonates , with **high mortality rate** and may cause infection in post-partum women (endometrities) , also **septicemia and pneumonia** in person with impaired immune system .

- ***Streptococcus pneumonia*** **(Pneumococcus)**

Are G+ , non-motile , encapsulated cocci , and their tendency to occur in pairs as ***Diplococcus pneumoniae*** this bacteria commonly cause

1- Acquired pneumonia
2- Adult bacterial meningitis
3- Important cause of otitis media , sinusitis and mastoiditis .

Like other streptococcus , *Strept. Pneumoniae* is **fastidious** (has complex nutritional requirements)and **routinely cultured in blood agar** . It release **α – hemolysin** that damage red cell membrane , causing colonies to be α – hemolytic .

- **Epidemiology**

Strept. Pneumoniae is an **obligate parasite of human** and **can be found in the nasopharynx of many healthy individuals** . This organism is **extremely sensitive** to environment agents . Pneumococcal infections can be either **endogenous** (residing in the nasopharynx of a carrier who develops impaired resistance to the organism . and also can be **exogenous** , for example , by droplets from the nose of carrier.

- **Pathogenesis**

The bacterial **capsule** of *Strept. Pneumoniae* is the **most important virulence factor** , and is the **basis for classification of serotypes** of this organism . The cell- associated enzymes pneumolysin and autolycin contribute to its pathogenicity.

1- Capsule : its polysaccharide and antiphagocytic and antigenic
2- Pili: Not all pneumococci are pilited , but those clinical isolates that express pili are more virulent.
3- Chronic –binding protein A:Is a major adhesion attach to carbohydrates .
4- Autolysins : Are enzymes that hydrolyze the components of a biological cell in which its produced and responsible for the release of intracellular virulence factors .
5- Pneumolysin : Its retained within the cytosol of intact pneumococci , important virulence factor, this toxin bind to cholesterol therefore interact with all cell types .

- **Clinical significance**

1- Acute bacterial pneumonia
2- Otitis media
3- Bacteremia /sepsis
4- Meningitis

- **Laboratory identification**
  - Specimens for Lab. evaluation can be obtained from nasopharyngeal swab , blood , pus , sputum or spinal fluid.
  - A- hemolytic colonies on blood agar
  - G+ diplococcus
  - Can inhibited by low concentration of surfactant optochin , and the cells are lysed by bile acids .
  - Capsular swelling .when treated with type- specific antiserum (Quellung reaction).
- Prevention :By
  1- Pneumococcal polysaccharide vaccine .
  2- Pneumococcal conjugate vaccine.
  - **Enterococcus :**

Contain **C-substance** that **reacts with group D antisera** . Its clinically most important species are *E . faecalis* and *E. faecium* . Enterococcus can be **α , β or nonhemolytic** . Its **one of nosocomial infection** as a result of their multiple antibiotic resistance .

A- Epidemiology :

It's part of **normal fecal flora** , can colonize oral mucosa membrane and skin , highly resistance to environmental and chemical agents .

B- Diseases:

Enterococcus can spread to normally sterile sites , causing urinary tract infection (UTI), bacteremia /sepsis , endocarditis , biliary tract infection, or intra-abdominal abscesses .

C- Lab. identification :

1- Can survive in the presence of bile salt.
2- Hydrolyze the polysaccharide esculin (producing black colonies on esculin- containing plates.
3- Enterococcus grow in 6.5% NaCl.
4- Yield positive pyrazinamide test.

Non-enterococcal group D streptococci.

Streptococcus bovis is the most clinically of the non-enterococcus group D streptococci part of **normal fecal flora** , they are either **α or non-hemolytic** cause **UTI and endocarditis** , the latter especially in

association with **colon cancer** . It tends to be **sensitive to penicillin and other antibiotics** .

- **Viridans streptococci:**

Include group of streptococci, many G+ , **catalase negative** , **α or γ-hemolytic** ,main facultative oral flora , **relatively virulent**.

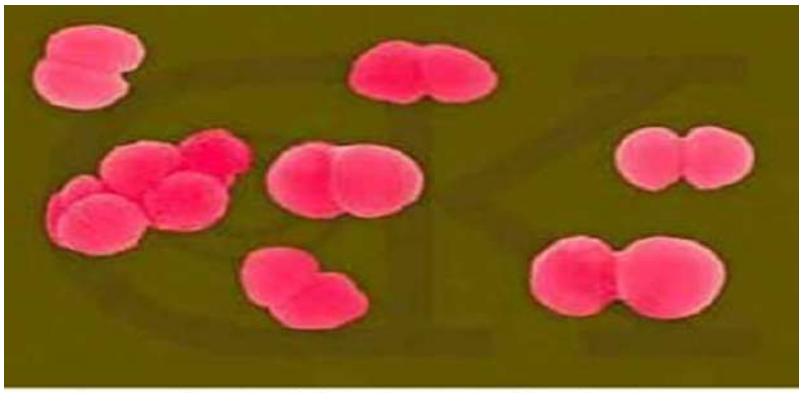
Strept. mutans and other members of viridans group cause **dental caries can infect heart valves during bacteremia causing endocarditis , rheumatic fever.**

Gram-negative cocci, aerobic.

The genus Neisseria consists of G- , aerobic , cocci. Two Neisseria species are pathogenic for humans . *Neisseria gonorrhoeae* (commonly called gonococcus). The causal agent of gonorrhea and *Neisseria meningitis* (commonly called meningococcus) afrequent cause of meningitis .

Neisseria gonorrhoeae, Also known as ***gonococci*** (plural),or ***gonococcus*** (singular), is a species of Gram-negative coffee bean-shaped diplococci bacteria , **frequently observed**

within the polymprphnuclear leukocytes.



N. gonorrhoea was first described by Albert Neisser in 1879. responsible for the sexually transmitted infection gonorrhea, usually transmitted during :

1-Sexual contact
2- More rarely vertically (during the passage of baby through an infected birth canal)

Structure : Gonococci are **un encapsulated** (unlike meningococci) , pilited , and non-motile , and they resemble a pair of kidney beans
1- Pili : enhance attachment of organism to host epithelial and mucosal cell surface act as important virulence factors , and also antigenic.
2- **Lipooligosaccharide (LOS)**: have shorter , more highly branched , non-repeat O- antigen side chains than do lipopolysaccharide found in other G-bacteria . The bacterial antibodies in normal human serum are **IgM molecules directed against LOS antigens** . The gonococcus is also **capable of high frequency variation of the LOS antigens presented on the cell surface** .
3- Porin Protein : This bacteria express a single porin type , known as Por B . Different strains express either PorB1A or PorB1B .
4- Opacity proteins : Opacity (Opa) protein (formerly called Pll proteins) are so named due to their tendency to impart an opaque quality to gonococcal colonies .

Pathogenesis:
1- Pili and Opa proteins facilitate adhesion of the gonococcus to epithelial cells of the urethra , rectum , cervix , pharynx ,and conjunctiva , thereby making colonization possible .
2- Both gonococci and meningococci produce an **IgA protease** that cleaves IgA1.

3- To establish infection in human males , the gonococcus must express proteins that facilitate iron acquisition from either transferrin or lactoferrin .

Clinical Significance :

A higher proportion of females than males are generally a symptomatic , and these individuals act as the **reservoir** for maintaining and transmitting gonococcal infection more than one sexually transmitted disease (STD) may be acquired at the same time , such as gonorrhea in combination with syphilis (*Treponema pallidum* infection) , chlamydia , human immune deficiency virus , or hepatitis B virus.

Patients with gonorrhea may therefore , need treatment for more than one pathogen .

1- Genitourinary tract infections.
In male : a yellow , purulent urethral discharge and painful urination .
In female :A greenish , yellow cervical discharge is most common . often accompanied by inter menstrual bleeding .
2- Rectal infections.
3- pharyngitis
4- Ophthalmin neonatorum : occur in new barns .
5- Disseminated infection : bacteria have limited ability to multiply to blood stream , therefore bacteria with gonococci is rare . In contrast , **meningococci multiply rapidly in blood .**
Note: Gonococcal infection is the most common cause of septic arthritis in sexually active adults .

*Laboratory identification :
- In male: finding of numerous neutrophils containing G- diplococcui in a smear of urethral exudate .
- In females : a positive culture is needed to diagnosis gonococcal infection as well as at sites.
If disseminates infection is suspected . Appropriate culture : should be set up as indicated for example of skin lesions , joint fluid , and blood .
1- Growth conditions for culture :

a- Bacteria grows : best under aerobic conditions , and most strains require enhanced CO_2 .
b- Gonococcus utilize glucose but not maltose , lactose ,or sucrose . Meningiococcus utilize both glucose and maltose .

c- All Neisseria genus Oxidase +

2- Selective media : Gonococcus : very sensitive to heating and drying ,**chocolate agar supplemented with several antibiotics that suppress the growth of nonpathogenic** Neisseria and other normal and abnormal flora .

The bacteria on this media appear as **gold standard**.

***Treatment and prevention :**

More than 20 % of current isolates of *N. gonorrhoeae* are resistant's to penicillin , tetracycline, cefoxitin ,and /or spectinomycin.

Penicillinase producing *N. gonorrhoeae* ,however , most organisms still respond to treatment with third generation cephalosporins .

***Prevention:**

Gonorrhea involves evaluating and arrangement of sexual contacts of the patient 1-
Generally using antibiotics as prophylactically in an exposed individual even in the observe of symptoms .

2- Barrier methods

eighth lecture ***Dr. Mouruj Alaubydi and Dr. Jenan AlSaffar***

Neisseria meningitids

Is one of the most frequent causes of meningitis when meningococcus is isolated from **blood or spinal fluid** , its invariably **encapsulated** . The meningococcal polysaccharide capsule is antiphagocytic . Antibodies to capsule carbohydrate are bactericidal . According to the epidemiological classification , this bacteria is classified in to **> 13 serogroups depended on polysaccharide capsule and > 20 serotypes depended on outer membrane proteins** .

Most infections are caused by **serogroups A,B,C,W-135 , and Y(approximately 90% are due to A,B,C**). The meningococcus express Por A and Por B-type porins .

***Epidemiology :** Transmission occurs through **inhalation of respiratory droplets** from a carrier or a patients in the early stages of the disease . In addition to contact with carrier , risk factors for disease include recent viral or mycoplasma upper respiratory tract infection, active or passive smoking , and complement deficiency .

Gastrointestinal gram negative rods

These **G- rods** belong to diverse taxonomic groups, are **facultative** organisms constitute only a fraction of the total microbial flora of the GIT. They contain LPS which is both antigenic and an important virulence factor (endotoxin). Different enteric G- rods cause diseases **within GIT , outside the GIT or in both location** .

- ***Escherichia coli*** :

E. coli is part of the **normal flora** of the colon in human and other animals , but can be pathogenic both within and outside of the GIT . Has **fimbriae or pili that are important for adherence** to host mucosal surface .

-Structural and physiology :

E. coli share many properties with the other enterobacteriaceae , they are all facultative anaerobes . They all ferment glucose , and they all can generate energy by aerobic or anaerobic respiratory , they are **oxidase negative .**

-Clinical significance : Intestinal disease

Transmission of Intestinal disease is commonly by fecal – oral route , food and water serving as vehicles for transmission .

At least five types of intestinal infections that differ in pathogenic mechanisms have been identified

1- **Enterotoxigenic *E. coli*** (ETEC): Is a common cause of travelers diarrhea colonize the small intestine mucosa (pili facilitate the binding of the organism to the mucosa). ETEC cause

a- **Enterotoxin** include : **a heat – stable toxin (ST)and heat- labile toxin (LT)it's like cholera toxin.**

b-Prolonged hyper secretion of chloride ions and water by intestinal mucosal cells , while inhibiting the reabsorption of sodium.

2- **Enteropathogenic *E. coli*** (EPEC) : Are important cause of **diarrhea in infants** . The EPEC attach to mucosal cells in the small intestine by use of bundle – forming pili (BFpA). In addition to destruction of microvilli , are caused by injection of effector proteins in to the host cell by way of a type III secretion system .**EPEC are not invasive and , thus do not cause bloody diarrhea** .

3- **Enterohemorrahagic *E. coli*** (EHEC): Bind to the cells of large intestine , similar to EPEC produce characteristic **lesions called attaching and affecting lesions (A/E). EHEC produce one of two exotoxin (shiga-like toxin 1 or 2) , resulting in sever form of copious , bloody diarrhea (hemorrhagic colitis)in the absence of mucosal invasion or inflammation .** serotype O157:H7 is the most common strain of ***E. coli*** that produce shiga-like toxin . The primary **reservoir of this bacteria is cattle** .Therefore the possibility of infection can be greatly decreased by thoroughly **cooking ground beef and pasteurizing milk .**

4- **Enteroinvasive *E. coli*** (EIEC): cause a dysentery like syndrome with fever and bloody stools. Plasmid encoded virulence factors are nearly identical to those of shigella species in addition EIEC strains produce a hemolysin (HlyA).

5- **Enteroaggregative *E. coli*** (EAEC): Is also cause **Traveler's diarrhea and persistence diarrhea in young children** . Adherence to small intestine is mediated by aggregative adherence .**EAEC produce a heat – stable toxin that is plasmid encoded .**

-Clinical significance: Extraintestinal disease

The source of infection is frequently the patient own flora

1-Urinary tract infection (UTI)

2- Neonatal meningitis

3- Nosocomial (hospital- acquired) infections these include sepsis / bacteremia – endotoxic shock , and pneumonia .

-Lab. identification :

1- Culturing the sample on macConkey and macConkey sorbitol agars.

2-Molecular techniques such as PCR.

1-Its prevented by care in selection , preparation and consumption of food and water .

2-Maintance of fluid and electrolytes balance is of primary importance in treatment .

3-Suitable antibiotics (According to antibiotic sensitivity).

Klebsiella:

Are large ,non-motile ,bacilli ,possess a luxurious capsule (encapsulated), lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It appears as a mucoid lactose fermenter on MacConkey agar. *K. pneumoniae* and *K. oxytoca* are the two strains responsible for most human illnesses. They cause necrotizing labor pneumonia in individuals compromised by alcoholism, diabetes or chronic obstructive pulmonary disease. *K. pneumoniae* also causes UTI and bacteremia particularly in hospitalized patients (nosocomial infection).

Members of the genus *Klebsiella* typically express two types of antigens on their cell surfaces. The first, O antigen, is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties. Both contribute to pathogenicity and form the basis for serogrouping.

Proteus , Providencia , and Morganella

Members of these genera are agents of urinary tracts and other extra intestinal infections . It is common causes **of uncomplicated as well as nosocomial UTI , wound infection, pneumonia and septicemia** . Proteus organisms **produces urease** which colonizes the hydrolysis of urea to ammonia . The resulting **alkaline environment promotes the precipitation of struvite stones containing insoluble phosphate of magnesium and phosphate.** *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora. *Proteus* is also found in multiple environmental habitats, including long-term care facilities and hospitals. This bacteria can colonize both the skin and oral mucosa of both patients and hospital personnel. Infection primarily occurs from these reservoirs.

However, *Proteus* species are not the most common cause of nosocomial infections.

Proteus mirabilis causes 90% of *Proteus* infections and can be considered a community-acquired infection.

Proteus vulgaris and *Proteus penneri* may be isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems.

Providencia pathogens of humans

The genus *Providencia* is a urease-producing gram-negative bacillus and the most important species includes *Providencia stuartii*, *and P. rettgeri* are the most common cause of catheter-associated urinary tract infections, especially in the elderly with long-term indwelling urinary catheters. While *Providencia* species do not routinely cause urinary tract infections or bacteremia, when implicated, the overall mortality rate of bacteremia due to *Providencia* species can be high, especially in the elderly with severe underlying conditions. *P. rettgeri* and *P. stuartii* are commonly found in water, soil, and animal reservoirs, and are opportunistic pathogens in hospitalized patients and elderly residents in a nursing care facility.

Morganella

Are motile, non-lactose fermenting gram-negative bacteria, which share with Proteus the capacity for urease production and presence of phenylalanine deaminase. They can be separated from Proteus species by the lack of swarming activity or gelatin liquefaction or H2S production. Commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora. Despite its wide distribution, it is an uncommon cause of community-acquired infection and is most often encountered in postoperative and other nosocomial settings.

*** Shigella**

Shigella species cause shigellosis (bacillary dysentery) Shigella are non-motile , **unencapsulated , and Lac -.** Most strain do not produce gas in a mixed – acid fermentation of glucose . **Human are the only natural host for shigella species**

Epidemiology :

1- Spread from person to person , with contaminated stools .
2- Flies and contaminated food or water can also transmitted the disease .
3- 10-100 viable organisms are sufficient to cause disease .

4- 40 serotypes organized in to four groups(A,B,C,and D) based on the polysaccharide O antigens.

The most prevalence species are

1- *Shigella dysenteriae* type 1 produce shiga toxin which is similar to shiga – like toxins 1 and 2 produced by *E. coli* . Shiga and shiga- like toxins are capable of resulting in susceptible individuals.
2- *Shigella sonni* (group D)
3- *Shigella flexneri*

Pathogenesis and clinical significance

*Shigella i*nvade and **destroy the mucosa of the large intestine** . Plasmid – encoded virulence genes that encode a type III secretion system . This plasmid encodes proteins that allow the shigella to polymerize actin at one pole . **This virulence plasmid is also possessed by EIEC . An exotoxin (shiga toxin)with enterotoxic and cytotoxic properties** .It's may cause

1- Hemorrhagic colitis
2- Classic bacillary dysentery characterized by diarrhea with blood , mucus.
3- May lead to severe dehydration and sometime death.

Laboratory identification

Organisms can be cultured from stools using differential , selective Hektoen agar

Treatment:

1- Antibiotics (Ciprofloxacin or azithromycin)
2- Water and food supply and personal hygiene .
3- Vaccination .

Enterobacteriaceae

Escherichia coli Escherichia organisms are gram-negative bacilli that exist singly or in pairs. E coli is facultative anaerobic . They are either nonmotile or motile by peritrichous flagella. E coli is a major facultative inhabitant of the large intestine. E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or from the individuals handling the child. In the bowel, E. coli adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract.

Morphology

E. coli is a straight gram-negative rod non-capsulated nonsporulating bacterium Cells are typically rod-shaped, motile by peritrichous flagella. E. coli is the most abundant facultative anaerobe E. coli ferments lactose, a property that distinguishes it from the two major intestinal pathogens, Shigella and Salmonella.

ANTIGENIC STRUCTURE

It has three antigens that are used to identify the organism in epidemiologic investigations: the O, or cell wall, antigen; the H, or flagellar, antigen; and the K, or capsular, antigen. Because there are more than 150 O, 50 H, and 90 K antigens.

Pathogenesis

The source of the E. coli that causes urinary tract infections is the patient's own colonic flora that colonizes the urogenital area. The source of the E. coli that causes neonatal meningitis is the mother's birth canal; the infection is acquired during birth. In contrast, the E. coli that causes traveler's diarrhea contaminated with human feces. E. coli has several clearly identified components that contribute to its ability to cause disease: pili, a capsule, endotoxin, and three exotoxins (enterotoxins), two that cause watery diarrhea and one that causes bloody diarrhea and hemolytic–uremic syndrome.

Pathogenesis and Clinical Findings

The clinical manifestations of infections with E coli and the other enteric bacteria depend on the site of the infection and cannot be differentiated by symptoms or signs from processes caused by other bacteria. 1. Urinary

tract infection—E coli is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Most of the urinary tract infections that involve the bladder or kidney in an otherwise healthy host are caused by a small number of O antigen types that have specifically elaborated virulence factors that facilitate colonization and subsequent clinical infections. These organisms are designated as uropathogenic E coli. Typically, these organisms produce hemolysin, which is cytotoxic and facilitates tissue invasion. 2. E coli–associated diarrheal diseases—E coli that cause diarrhea are extremely common worldwide. These E coli are classified by the characteristics of their virulence properties , and each group causes disease by a different mechanism—at least six of which have been characterized. a. a. Enteropathogenic E. coli (EPEC) E coli (EPEC) are an important cause of diarrhea in infants. b. Enteroinvasive E.coli (EIEC) produce a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. Similar to Shigella, EIEC strains are non lactose or late lactose fermenters and are nonmotile. EIEC produce disease by invading intestinal mucosal epithelial cells

c. Enterohemorrhagic E. coli (EHEC) which causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. d. EnteroaggregativeE. coli (EAEC) So named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAEC are noninvasive. They produce a hemolysin and an ST enterotoxin similar to that of ETEC.

e. Adherent-Invasive E. coli (AIEC) AIEC are able to invade intestinal epithelial cells and replicate intracellularly. It is likely that AIEC are able to proliferate more effectively in hosts with defective innate immunity.

3. Sepsis—When normal host defenses are inadequate, E coli may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to E coli sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infection and often the major clone associated with invasion is E coli O25b/ST131.

4. Meningitis E.coli and group B streptococci are the leading causes of meningitis in infants. Approximately 80% of E coli from meningitis cases have the K1 antigen.

Klebsiella

Klebsiella is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule.

The members of the genus Klebsiella are a part of the human and animal's normal flora in the nose, mouth and intestines. The species of Klebsiella are all gram-negative and non-motile. They tend to be shorter and thicker when compared to others in the Enterobacteriaceae family. The cells are rods in shape and generally measures 0.3 to 1.5 µm wide by 0.5 to 5.o µm long. They can be found singly, in pairs, in chains or linked end to end. Klebsiella can grow on ordinary lab medium and do not have special growth requirements, like the other members of Enterobacteriaceae. The species are aerobic but facultatively anaerobic. Their ideal growth temperature is 35° to 37°, while their ideal pH level is about 7.2.

.List of species of the genus Klebsiella

 K. granulomatis K. oxytoca K. michiganensis K. pneumoniae (type-species) o K. p. subsp. ozaenae o K. p. subsp. pneumoniae o K. p. subsp. Rhinoscleromatis

the medical importance of the genus Klebsiella (family Enterobacteriaceae) led to its being subdivided into three species corresponding to the diseases they caused: K. pneumoniae , K. ozaenae, and K. rhinoscleromatis.

Morphology

Klebsiella bacteria tend to be rounder and thicker than other members of the Enterobacteriaceae family. They typically occur as straight rods with rounded or slightly pointed ends. They can be found singly, in pairs, or in short chains. Diplobacillary forms are commonly found in vivo Typical colonies are large and often slimy in appearance becase of capsule formation colonies are pink on MacConkey agar.Species of Klebsiella are all gram-negative and non-motile. They tend to be shorter and thicker when compared to others in the Enterobacteriaceae family.

CULTURE

Members of the genus produce a prominent capsule, or slime layer, Typical colonies are large and often slimy in appearance becuase of capsule formation colonies are pink on MacConkey agarThey have no specific

growth requirements and grow well on standard laboratory media, but grow best between 35 and 37 °C and at pH 7.2. The species are facultative anaerobes, and most strains can survive with citrate and glucose as their sole carbon sources and ammonia as their sole nitrogen source.

Klebsiella in humans klebsiella organisms can lead to a wide range of disease states, notably . , Pneumonia. Urinary tract. Infections. Septicemia. Meningitis. Diarrhea. soft tissue infections

K. pneumoniae is the most common cause of nosocomial respiratory tract and premature intensive care infections, and the second-most frequent cause of Gram-negative bacteraemia and urinary tract infections.

The ability of K. pneumoniae to colonize the hospital environment, including carpeting, sinks, flowers, and various surfaces, as well as the skin of patients and hospital staff, has been identified as a major factor in the spread of hospital-acquired infections.

PROTEUS

Proteus species produce infections in human 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. In the past, there were four medically important species of Proteus.

Proteus volgaris Proteus mirabilis Proteus morganii Proteus rettgeri

However, molecular studies of DNA relatedness showed that two of the four were significantly different. These species have been renamed: Proteus morganii is now Morganella morganii, and Proteus rettgeri is now Providencia rettgeri. In the clinical laboratory, these organisms are distinguished from Proteus vulgaris and Proteus mirabilis on the basis of several biochemical tests. Proteus species produce urease, resulting in rapid hydrolysis of urea with liberation of ammonia. Thus, in urinary tract infections with Proteus species, the urine becomes alkaline, promoting stone formation and making acidification virtually impossible. The rapid motility of Proteus may contribute to its invasion of the urinary tract.

Macroscopic morphology

Typical colonies are thin ,moist and spreading on blood agar . Small colorless colonies are produced on MacConkey agar. These organisms usually are highly motile and produce a ―swarming‖ overgrowth on blood agar, which can frustrate efforts to recover pure cultures of other organisms. Growth on blood agar containing phenylethyl alcohol inhibits swarming, thus allowing isolated colonies of Proteus and other organisms to be obtained. They produce non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. P. vulgaris and P. mirabilis produce H2S, which blackens the butt of TSI agar.

Pathogenesis & Epidemiology

The organisms are present in the human colon as well as in soil and water. Their tendency to cause urinary tract infections is probably due to their presence in thecolon and to colonization of the urethra, especially in women. The vigorous motility of Proteus organisms may contribute to their ability to invade the urinary tract. Production of the enzyme urease is an important feature of the pathogenesis of urinary tract infections by this group. Urease hydrolyzes the urea in urine to form ammonia, which raises the pH, producing an alkaline urine. This encourages the formation of stones (calculi) called ―struvite‖ composed of magnesium ammonium phosphate. Stones in the urinary tract obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone. Because alkaline urine also favors growth of the organisms and more extensive renal damage, treatment involves keeping the urine at a low PH.

tenth lecture *Dr. Jenan Al saffar and Dr. Mouruj Alaubydi*

PSEUDOMONAS

The genus Pseudomonas comprises more than 140 species but only one of these is pathogenic to man .i.e Pseudomonas pyocynea (Ps. aeruginosa). *P aeruginosa* is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses, especially in individuals with neutropenia.

Pseudomonas aeruginosa causes infections (e.g., sepsis, pneumonia, and urinary tract infections) primarily in patients with lowered host defenses. It also causes chronic lower respiratory tract infections in patients with cystic fibrosis, wound infections (cellulitis) in burn patients and malignant otitis externa in diabetic patients. It is the most common cause of ventilator-associated pneumonia.

Morphology and Identification

A. Typical Organisms

P aeruginosa is motile and rod shaped, measuring about 0.6 ×2 µm It is Gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains..

B. Culture

P aeruginosa is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like or corn taco–like odor. Some strains **hemolysis blood**. ***P aeruginosa*** **forms smooth round colonies with a fluorescent greenish color. It often produces the nonfluorescent bluish pigment pyocyanin, which diffuses into the agar. Other Pseudomonas species do not produce pyocyanin.** Many strains of ***P.aeruginosa*** **also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar . Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin**. **P aeruginosa** in a culture can produce multiple colony types . ***P aeruginosa*** from different colony types may also have different biochemical and enzymatic activities and different antimicrobial susceptibility patterns

C. Growth Characteristics

P aeruginosa grows well at 37–42°C; its growth at 42°C helps differentiate it from other **Pseudomonas species** that produce fluorescent pigments. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose. Identification is usually based on colonial morphology, oxidase positivity, the presence of characteristic pigments, and growth at 42°C. Differentiation of P aeruginosa from other pseudomonads on the basis of biochemical activity requires.

Antigenic Structure and Toxins

Pilli (fimbriae) extend from the cell surface and promote attachment to host epithelial cells. **An exopolysaccharide, alginate, is responsible for the mucoid colonies seen in cultures from patients with CF**. **Lipopolysaccharide,** which exists **in multiple immunotypes**, **is responsible for many of the endotoxic properties of the organism. *P aeruginosa* can be typed by lipopolysaccharide immunotype and by pyocin** (**bacteriocin) susceptibility**. Most ***P aeruginosa*** isolates from clinical infections produce **extracellular enzymes, including elastases, proteases, and two hemolysins (a heat-labile phospholipase and a heat-stable glycolipid). Many strains of *P aeruginosa* produce exotoxin A, which causes tissue necrosis Diagnostic Laboratory Tests**

A. Specimens

Specimens from skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

C. Culture

Specimens are plated on blood agar and the **differential media (MacConkey,s or EMB agar) .** It is **oxidese - positive . A typical metallic sheen of the growth on TSI agar coupled with the blue – green pigment on ordinary nutrient agar** .***P aeruginosa*** does not ferment lactose and is easily differentiated from the lactose fermenting bacteria.

Culture is the specific test for diagnosis of ***P aeruginosa*** infection. The diagnosis is confirmed by biochemical reactions**.**

Epidemiology and Control

P. aeruginosa is found chiefly in soil and water, although approximately 10% of people carry it in the **normal flora of the colon. It is found on the skin in moist areas and can colonize the upper respiratory tract of hospitalized patients. Its ability to grow in simple aqueous solutions has resulted in contamination of respiratory therapy and anesthesia equipment, intravenous fluids, and even distilled water**. ***P. aeruginosa*** is **primarily an opportunistic pathogen that causes infections in hospitalized patients** (**e.g., those with extensive burns), in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed; in those with neutrophil counts of less than 500/mL; and in those with indwelling catheters. It causes 10% to 20% of hospital-acquired infections and, in many hospitals, is the most common cause of gram-negative nosocomial pneumonia, especially ventilator-associated pneumonia**.

P aeruginosa **is primarily a nosocomial pathogen**, and the methods for control of infection are similar to those for other nosocomial pathogens. Because Pseudomonas thrives in moist environments, special attention should be paid to sinks, water baths, showers, hot tubs, and other wet areas. For epidemiologic purposes, strains can be typed using molecular typing techniques.

Pathogenesis

Is based on multiple virulence factors: endotoxin, exotoxins, and enzymes. Its endotoxin, like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock. The best known of the exotoxins **is exotoxin A, which causes tissue necrosis**. **It also produces enzymes, such as elastase and proteases, that are histotoxic and facilitate invasion of the organism into the blood stream. Pyocyanin damages the cilia and mucosal cells of the respiratory tract.**

Clinical Findings

***P. aeruginosa* can cause infections virtually anywhere in the body**,

1-urinary tract infections,**2-pneumonia** (especially **in cystic fibrosis** patients), and **3-wound infections** (**especially burns**) predominate. **It is an important cause of hospital acquired pneumonia**, especially in those undergoing mechanical ventilation (ventilator-associated pneumonia). From these sites, the organism can **enter the blood**, **causing sepsis**. The bacteria can spread to the skin, where they **cause black,necrotic** lesions . Patients with ***P. aeruginosa* sepsis** have a mortality rate of greater than 50%. It is an important cause of endocarditis in intravenous drug users. Necrotic skin lesion caused by **Pseudomonas aeruginosa**. **Severe external otitis (malignant otitis externa) and other skin lesions (e.g. folliculitis) occur in users of swimming pools and hot tubs (hot tub folliculitis) in which the chlorination is inadequate.**

PROTEUS

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promoting stone formation and making acidification virtually impossible. The rapid motility of Proteus may contribute to its invasion of the urinary tract.

Laboratory Diagnosis

The spreading nature and fishy odour of the growth on ordinary media (blood agar or nutrient agar) will cover the colonies of any other bacteria may be present in the culture., combined with the appearance of pale colonies on MacConkey

Macroscopic morphology

Typical colonies are thin ,moist and spreading on blood agar . Small colorless colonies are produced on MacConkey agar. These organisms usually are **highly motile and produce a "swarming**" overgrowth on blood agar, which can frustrate efforts to recover pure cultures of other organisms.

Growth on blood agar containing phenylethyl alcohol inhibits swarming, thus allowing isolated colonies of **Proteus** and other organisms to be obtained. They produce non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar.

P. vulgaris and P. mirabilis produce H2S, which blackens the butt of TSI agar, whereas neither **M. morganii** nor ***P. rettgeri*** does. ***P. mirabilis*** is indole-negative, whereas the other three species are indole-positive . These four medically important species are urease-positive.

Pathogenesis & Epidemiology

The organisms are present in the human colon as well as in soil and water. Their tendency to cause urinary tract infections is probably due to their presence in thecolon and to colonization of the urethra, especially in women. The vigorous motility of Proteus organisms may contribute to their ability to invade the urinary tract. Production of the enzyme urease is an important feature of the pathogenesis of urinary tract infections by this group. Urease hydrolyzes the urea in urine to form ammonia, which raises the pH, producing an alkaline urine. This encourages the formation of stones (calculi) called "struvite" composed of magnesium ammonium phosphate. Stones in the urinary tract obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone.

Because alkaline urine also favors growth of the organisms and more extensive renal damage, treatment involves keeping the urine at a low PH.

Clinical Findings

The signs and symptoms of urinary tract infections caused by these organisms cannot be distinguished from those caused by E. coli or other members of the Enterobacteriaceae. Proteus species can also cause pneumonia, wound infections, and septicemia. P. mirabilis is the species of Proteus that causes most community and
hospital-acquired infections, but P. rettgeri is emerging as an important agent of nosocomial infections.