Ministry of Higher education and scientific research

Baghdad university

College of Science

Department of Biology

Bacterial pathogenesis

دراسات عليا ماجستير

2021-2020

الفصل الدراسي الثاني

تدريسي الماده

ا.د.رسميه عبد ابوريشه

Lect .1 bacterial pathogenesis prof .Dr.Rasmiya Abd

Bacterial Pathogenesis

A pathogen is a microorganism (or virus) that is able to produce disease.

Pathogenicity is the ability of a microorganism to cause disease in another organism, namely the **host** for the pathogen. As implied above, pathogenicity may be a manifestation of a host-parasite interaction. In humans, some of the normal bacterial flora (e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae*,and *Haemophilus influenzae)* are **potential pathogens** that live in a commensal or parasitic relationship without producing disease. They do not cause disease in their host unless they have an opportunity brought on by some compromise or weakness in the host's anatomical barriers, tissue resistance or immunity.

Furthermore, the bacteria are in a position to be transmitted from one host to another, giving them additional opportunities to colonize or infect. There are some pathogens that do not associate with their host except in the case of disease. These

bacteria may be thought of as **obligate pathogens**, even though some may rarely occur as normal flora, in asymptomatic or recovered carriers, or in some form where they cannot be eliminated by the host.

Opportunistic Pathogens

Bacteria which cause a disease in a compromised host which typically would not occur in a healthy host are acting as **opportunistic pathogens**. A member of the normal flora can such as *Staphylococcus aureus* or *E. coli* can cause an **opportunistic infection**, but so can an environmental organism such as *Pseudomonas aeruginosa.* When a member of the normal flora causes an infectious disease, it sometimes referred to as an **endogenous bacterial disease**, referring to a disease brought on by bacteria 'from within'. Classic opportunistic infections in humans are dental caries and periodontal disease caused by normal flora of the oral cavity.

Virulence Factors

In order for a bacterium to be virulent, it must have capabilities that allow it to infect a host. These capabilities arise from physical structures that the bacterium has or chemical substances that the bacterium can produce. Collectively the characteristics that contribute to virulence are called **virulence factors**. The genes that code for virulence factors are commonly found clustered on the pathogen's chromosome or plasmid DNA, called **pathogenicity islands**. These pathogenicity islands can be distinguished by a G+C content that differs from the rest of the genome and the presence of insertion-like sequences flanking the gene cluster. Pathogenicity islands facilitate the sharing of virulence factors between bacteria due to horizontal gene transfer, leading to the development of new pathogens over time.

Often the genes for virulence factors are controlled by quorum sensing, to ensure gene activation when the pathogen population is at an optimal density. Triggering the genes too soon could alert the host's immune system to the invader, cutting short the bacterial infection.

Determinants of Virulence

Pathogenic bacteria are able to produce disease because they possess certain **structural** or **biochemical** or **genetic** traits that render them pathogenic or **virulent**. (The term **virulence** is best interpreted as referring to the **degree of pathogenicity**.)

The sum of the characteristics that allow a given bacterium to produce disease are the pathogen's **determinants of virulence**. Some pathogens may rely on a single determinant of virulence, such as toxin production, to cause damage to their host. Thus, bacteria such as *Clostridium tetani* and *Corynebacterium diphtheriae*, which have hardly any invasive characteristics, are able to produce disease, the symptoms of which depend on a single genetic trait in the bacteria: the ability to produce a toxin.

Other pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, maintain a large repertoire of virulence determinants and consequently are able to produce a more complete range of diseases that affect different tissues in their host.

• **Adherence and Colonization**

○ Bacterial pathogens must be able to grab onto host cells or tissue, and resist removal by physical means (such as sneezing) or mechanical means (such as movement of the cilated cells that line our airway). **Adherence** can involve polysaccharide layers made by the bacteria, such as a capsule or slime layer, which provide adhesion to host cells as well as resistance from phagocytosis. Adherence can also be accomplished by physical structures such as a pilus or flagellum.

Once cells are successfully adhering to a surface, they increase in number, utilizing resources available at the site. This **colonization** is important for pathogen survival and invasion to other sites, which will yield increased nutrients and space for the growing population.

• Invasion

Invasion refers to the ability of the pathogen to spread to other locations in the host, by invading host cells or tissue. It is typically at this point when disease or obvious signs/symptoms of illness will occur. While physical structures can still play a role in invasion, most bacterial pathogens produce a wide array of chemicals, specifically enzymes that effect the host's cells and tissue. Enzymes such as collagenase, which allows the pathogen to spread by breaking down the collagen found in connective tissue. Or leukocidins, which destroy the host's white blood cells, decreasing resistance. Hemolysins lyse the host's red blood cells, releasing iron, a growth-limiting factor for bacteria.

Bacteria in the bloodstream, a condition known as **bacteremia**, can quickly spread to locations throughout the host. This can result in a massive, systemic infection known as **septicemia**, which can result in septic shock and death, as the host becomes overwhelmed by the bacterial pathogen and its products.

Toxins

Toxins are a very specific virulence factor produced by some bacterial pathogens, in the form of substances that are poisonous to the host. **Toxigenicity** refers to an organism's ability to make toxins. For bacteria, there are two categories of toxins, the exotoxins and the endotoxins.

Exotoxins

Exotoxins are heat-sensitive soluble proteins that are released into the surrounding environment by a living organism. These incredibly potent substances can spread throughout the host's body, causing damage distant from the original site of infection. Exotoxins are associated with specific diseases, with the toxin genes often carried on plasmids or by prophages. There are many different bacteria that produce exotoxins, causing diseases such as botulism, tetanus, and diphtheria. There are three categories of exotoxins:

- **Type I: cell surface-active** – these toxins bind to cell receptors and stimulate cell responses. One example is **superantigen**, that stimulates the host's **T cells**, an important component of the immune system. The stimulated T cells produce an excessive amount of the signaling molecule **cytokine**, causing massive inflammation and tissue damage.

- **Type II: membrane-damaging –** these toxins exert their effect on the host cell membrane, often by forming pores in the membrane of the target cell. This can lead to cell lysis as cytoplasmic contents rush out and water rushes in, disrupting the osmotic balance of the cell.

- **Type III: intracellular –** these toxins gain access to a particular host cell and stimulate a reaction within the target cell. One example is the **AB-toxin** – these toxins are composed of two subunits, an **A portion** and a **B portion**. The B subunit is the binding portion of the toxin, responsible for recognizing and binding to the correct cell type. The A subunit is the portion with enzymatic activity. Once delivered into the correct cell by the B subunit, the A subunit enacts some mechanism on the cell, leading to decreased cell function and/or cell death. An example is the tetanus toxin produced by the bacterium *Clostridrium tetani*.

Endotoxins

Endotoxins are made by gram negative bacteria, as a component of the outer membrane of their cell wall. The outer membrane contains lipopolysaccharide or LPS, with the toxic component being the lipid part known as **lipid A**. Lipid A is heat-stable and is only released when the bacterial cell is lysed. The effect on the host is the same, regardless of what bacterium made the lipid A – fever, diarrhea, weakness, and blood coagulation.

A massive release of endotoxin in a host can cause **endotoxin shock**,

Lect 2

Fimbriae, Pili, Flagella as Bacterial Virulence

Main characteristic

- the ability of bacteria to bind to cells from potential host organisms.
- Fimbriated and piliated bacteria agglutinated erythrocytes in a fashion resembling classical hemagglutination and adhered to host epithelial cells
- Moreover, for some strains bacteria-induced **hemagglutination** was inhibited by the addition of the monosaccharide mannose. This suggested that **mannose** is used as a receptor for adherence and that the free mannose functions as a hapten
- For other bacteria-erythrocyte reactions hemagglutination was not inhibited by mannose implying another receptor selectivity in the binding reaction
- fimbriae or pili function as specific adhesive that aid bacterial colonization of mucosal surfaces.

Functions:

1. Fimbriae are known to bind plasma proteins and to initiate proteolytic cascades
2. others are capable of activating calcium influx and signal transduction cascades in host target cells
3. fimbriae have been shown to act as invasion and motility factors
4. bacterial flagella that typically mediate bacterial motility have also function bacteria adherence and in the initiation of proinflammatory responses.

Classification & biosynthesis

All adhesive factors show typically fimbrial in morphology they showed receptor – specific binding abilities. Fimbriae and flagella share the need to a polymer architecturally outside the ordinary bacterial anabolic machinery.

They classified according to:

1. **given assembly pathway**
2. **receptor specificity or antigenic variation**

1.Chaperone Usher pathway

Types & Microorganism example:

The classical common **type-1 fimbriae** that mediate mannose-sensitive hemagglutination, and the **P-blood-group-antigen-binding P-fimbriae**, or **Pap pili**, are produced through the socalled ‘chaperone/usher’ pathway.

Fimbriae that belong to this ‘chaperone-usher’ family come in several different variants, and are not only defined to ***Escherichia coli***.

Function:

1. fimbriae are known to bind plasma proteins and to initiate proteolytic cascades.
2. capable of activating calcium influx and signal transduction cascades in host target cells.
3. fimbriae have been shown to act as invasion and motility factors.

Manufacturing:

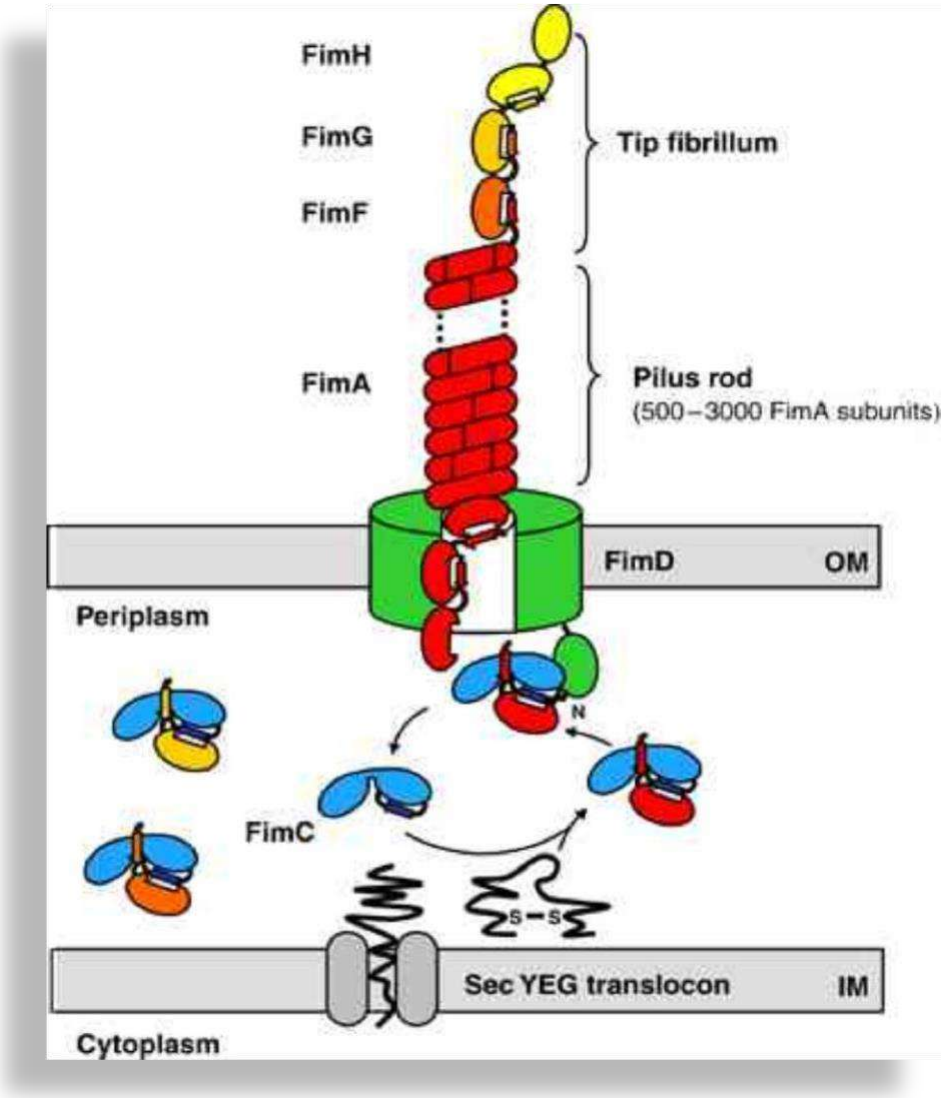
1. Gene clusters that provide the fimbrial subunits, **protein chaperones** and outer membrane anchors for the fimbrial shaft, as well as specific fimbrial regulatory genes code for these fimbriae
2. **nine** '**biosynthetic'** genes and **two** fimbrial **regulatory** genes are included in the *E. coli* **pap gene** cluster responsible for the expression of **P-fimbriae** .
3. The **fimbrial are translocated to the periplasm** through the **housekeeping system**.
4. the **chaperones translocate fimbrial subunits** to the **usher**.
5. then initiates translocation and polymerization of the fimbrial subunits across the outer membrane
6. the P-fimbriae actually were composite fibers, The fimbrial fibers include at least two distinct functions:

a. the constitution of a filament

b. recognition of the receptor.

in addition to the major fimbrial subunit PapA,P-fimbrial filaments were found to contain **minor subunits** , including the **PapE**, P**apF**, **PapK** and **PapG** proteins located at the distal end of the fiber .

The ability to bind the receptor resided in the PapG subunit, whereas other tiplocated Pap proteins functioned as initiators of



fimbrial polymerization and for adapting PapG to the fimbrial shaft.

bind a to share the ability Although all these fimbrial lectin proteins small carbohydrate epitope and to become integrated into the fimbrial filament

the incomplete to Due Fimbrial lectins are interesting candidate antigens for vaccine development. dimeric complexes. structural nature of the adhesin, vaccine trials have been conducted with adhesin-chaperone FimH/FimC The complex provided protection against uropathogenic E. coli in both a murine and a primate cystitis model

2. Type CS1 **Types &**

Microorganism example:

The CSI fimibria forms the prototype of this class that includes several antigenic variants, including the classical **CFA/I fimbriae** of enterotoxigenic *E. coli* (ETEC), and **type II pili** of *Burkholderia cepacia.*

Fimbriae belonging to the class of the CS I fimbrial family are assembled in a manner that phenotypically resembles the 'chaperone-usher' pathway .

Manufacture:

1. The **CSI fimbrial** subunit **CooA** is translocated to the periplasm through a Secdependent path- way
2. then assisted by a protein **CooB** with chaperone-like function.
3. CooA is then fed to a larger transmembrane protein **CooC** concomitant with fimbrial polymerization.
4. polymerization needs the presence of minor fimbrial subunit protein **CooD**, which functions:- both as an *initiator* and the *lectin subunit*.

3.Type Curli pili

- Many enterobacteria are capable of expressing elongated surface organelles, called AgfA fimbriae, with an "aggregative" and chemically robust character
- AgfA fibers appear **not as straight** but rather as twisted, curly structures and hence are referred to as " **Curli** " fimbriae.

Microorganism example:

- Curli fibers of ***E. coli*** and ***Salmonella enterica*** **sv Tyhpimurium** are cooled for by the cfg gene cluster. The cluster consists of two divergently transcribed units that include the csgABC and csgDEFG genes, respectively.
- Although curli fibers are coded for genetic elements comparable in size to the Pfimbrial pap operon, the curli fiber polymerization process is apparently different.
- Interestingly, curli fibers show all the typical characteristic of **amyloid fibers**, such as the binding to the **dye Congo red**.

Manufacture:

- Unlike amyloid formation in human neurodegenerative disorders such as Alzheimer's disease, curli amyloids require a specific assembly machinery. Thus, Vip **the CsgA and CsgB fimbrial subunits appear to be secreted out from the bacteria, where after an interaction between the subunits in the extracellular compartment then leads to polymerization**.

The CsgA subunit occurs in excess in the isolated filament, whereas in vitro both the CsgB subunit and the isolated CsgA subunit are capable of self-polymerization. Thus, as in analogy with type 1, P- and CSI fimbriae the assembly of curli organelles also involves a nucleator component (CsgB), proteins with apparent chaperone functions (CsgE), or a nucleator center (CsgG).

- As with type IV pili, curli fibers have a rather diverse spectrum of receptor targets.
- Curli fibers are reported to mediate binding to mouse small intestinal epithelial cells, in addition to various plasma and extracellular matrix proteins.
- participation of curli in the formation of biofilms.

4.type IV pili

- **Type IV** pili are multifunctional adhesive structures expressed by a number of diverse microorgamism including *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*.
- Related structures have also been identified in *Vibrio cholerae* (toxin-coregulated pili, Tcp) and enteropathogenic *E.coli* (bundle-forming pill, **Bfp**).
- Vip type IV pill are composed primarily of a single protein subunit, termed **pilin**, which are arranged in a helical conformation with 5 subunits per turn. They share unusual amino-terminal **N-methyl Phenylalanine** and immunogenic **Carboxy terminal Di-Sulfade bound**
- type IV pili can be glycosylated and/or phosphorylated depending on the bacterial species. They assembly in cytoplasmic membrane or periplasm. The assembly require **NBP** "Nucleotide-Binding protein" .
- Prepilin peptidase and outer membrane protein are essentially in **protrusion**.

type IV pili may share evolutionary origins with filamentous bacteriophages , and with genes required for bacterial type II protein export and DNA uptake systems; while others **; evolutes through their need to produce Sticky surface-located adhesive organelles**

Type IV pili of *Neisseria* are composed of a major pilus subunit and several other pilusassociated proteins, which have different functions in pilus assembly and adhesion One of these protein is **PilC**, which is associated with the tip and the shaft of the pili and the basal part in the outer membrane

- The ability to express certain types and sets of fimbriae seems overrepresented among urinary tract isolates of *E. coli*.
- The expression of type I fimbriae appears to be both an important:

1. colonization factor
2. factor contributing to the persistence in the bladder epithelium.

- The pattern of mannose binding by the protein FimH is somewhat different among commensal and UTI *E. coli*:

UTI isolates seem capable of binding D-mannose whereas commensals seem to prefer trimannoside structures

P-fimnbriae recognize the core GalαI — 4Galß contained in blood group antigencarrying globoseries glycolipids. the class II G adhesion recognizes most members of GalαI — 4Galßcontaining globoseries glycolipids and has been considered important for kidney infection in persons with a non obstructcd urinary tract.

Beyond Adherence

- Besides mediating adherence to the urinary tract epithelium, type 1 and P-fimbriae have been implicated in the later phases of infection, and in **the generation of innate proinflammatory responses in the infected urinary tract epithelium**.
- **type I fimbriae** appear multifunctional in the pathogenesis of UTI; they:

1. **mediate initial adherence**
2. **invasion**
3. **seem to participate in the formation of an intracellular biofilm.**

Many types of fimbriae, including **type 1**, **type IC** and **P-fimbriae** have all been associated with the induction of proinflammatory responses in epithelial cells

Type I fimbriated *E. coli* induce cytokine expression from both A498 **kidney** epithelial cells as well as in **bladder** cell lines .

- .

Type IV pili in Attachment and Invasion of Pathogenic *Neisseria*

- The important initial interaction between pili of *Neisseria* and its host cell occurs through the receptor molecule **CD46**, a human cell surface protein involved in the regulation of complement activation.
- During initial contact between bacteria and cells, pilus retraction exerts tensile forces upon the plasma membrane.
- The mechanical forces applied to the plasma membrane trigger actin polymerization accompanied by accumulation of phosphotyrosine-containing proteins, which leads to the formation of compact microcolonies.

- type IV pill do not only simply anchor the bacteria at the cell surface, they initiate a **multistep adhesion cascade**,
    1. which starts with a loose adherence and ends with the attachment of bacteria.
    2. Type IV pili also assist in the formation of biofilms that may support further tissue colonization and protect the bacteria against antibodies and antibiotics.

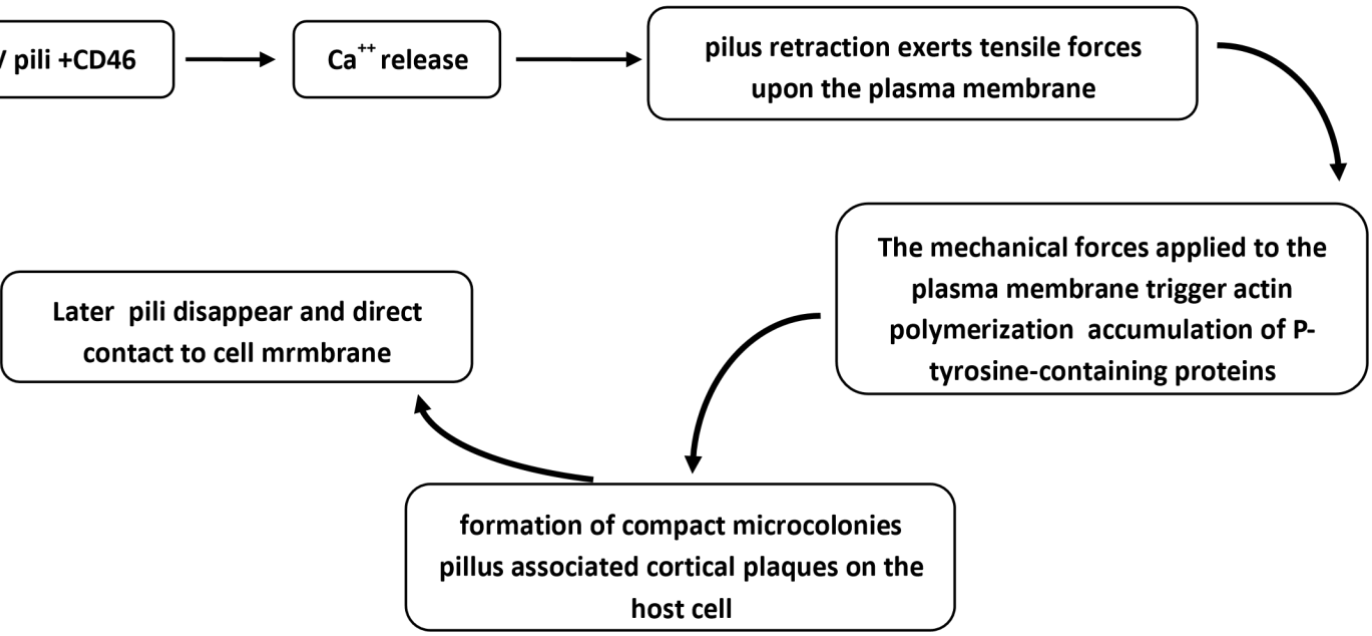
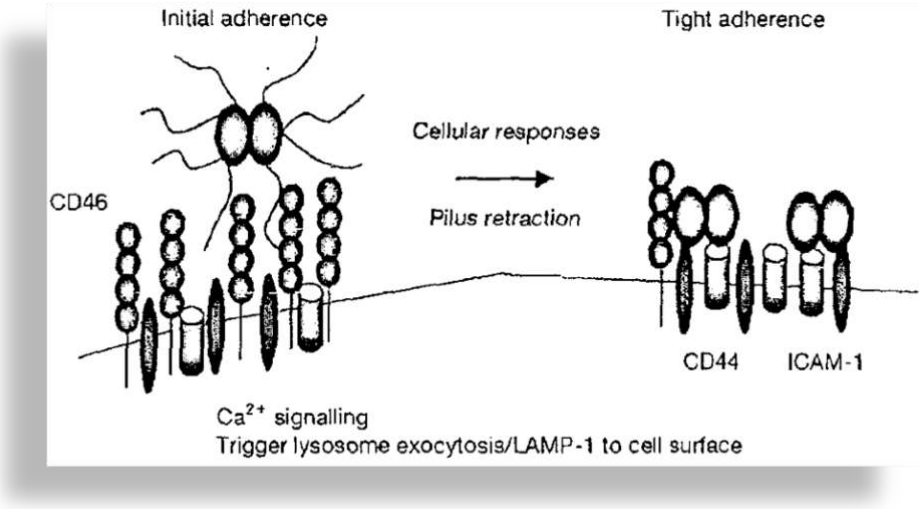


Fig. Initial adherence of type IV piliated *Neisseria* involves initial contact with cell surface receptors followed by cell signaling leading to tight adherence and invasion of host cells. Failure in the pilus retraction events and/or host cell signalling leads to lost or changed adherence patterns, and a loss of ability to enter and invade host cells.



Binding of fimbriae to another tissues

primary role of fimbriae might be to mediate adhesion and subsequent events through binding to specific structures on host (**epithelial**) cells, it has recently become evident that fimbriae can also bind various **connective tissue proteins**, as well as **plasma** and **serum** proteins.

- The F17 fimbriae occur characteristically in *E.coli* isolates causing diarrhea and septicemia in newborn.
- F17 fimbriae mediate binding to the calf intestinal epithelium, which suggests a role for F 17 fimbria in the intestinal colonization. In addition, the Fl7 fimbria is capable of binding **plasminogen** and the extracellular matrix protein **laminin**.
- Binding to laminin is inhibited by the receptor N-acetyl-D-glucosamine, indicating that carbohydrate receptors on the extracellular matrix protein are recognized by the minor fimbrial lectin protein GafD.
- fimbriae may assist bacteria during tissue dissemination by directing them to extracellular matrix proteins, and by coating them with proteolytically active proteins that enable the bacteria to penetrate through the tissue.

- **Pili and motility**

- **Twiching motility** is flagella-independent movements of bacteria. Twitching motility occurs in a wide range of bacteria, and has been well studied in ***N. gonorrhoeae*** and ***P. aeruginosa***. it occurs on solid, wet surfaces and is mediated by **type IV pili.**
- Type IV pill serve as an initial bridge between bacteria and cells, and twitching motility allows bacteria to spread in the infected tissue.
- Twitching motility has been shown to be important for infection by ***P. aeruginosa*** as well as **for biofilm formation**, which appears to be involved in chronic infection.
- PilT, an ATPase associated with various cellular activities , seems to act as a molecular motor.

- **Flagella as adhesins and virulance factors**

- Like fimbriae, flagella are protein polymers, each flagellum consisting of thousands of flagellin monomers .
- These filaments are connected to the cell surface through the **'hook'** structure, and the **basal** structure that forms the rotation device and that traverses the bacterial cell wall.
- role of flagella is to **ensure motility**, either as **swimming** movement in liquid medium or as **swarming** on solid surface these are also applied in bacterial virulence . For example, flagella-mediated motility acts as a virulence function for *V cholerae* . For *V cholerae* and *H. pylori*, the role of flagella as virulence factors is also supported through which show an up regulation of motility genes in infecting bacteria.

For *P. mirabilis*, the swarming state involves a transition to a hyper flagellated state and an up regulation, the expression of selected virulence functions.

Vibrio parahaemolyticus and *Aeromonas* spp., apply two separate sets of flagella: **polar** and **lateral** sets .The different flagellar sets expressed by *Aeromonas* primarily associate with a **shift in motility**, the lateral set being used for swarming.

for *P mirabilis*, the switch to a swarming phenotype reflects a more fundamental alteration in the expression of the bacterial virulence potential.

The flagellar assembly pathway is related to the contact-dependent, so-called **type III protein secretion pathway** that is applied by many pathogens, like *Yersiniae*, for the translocation of bacterial virulence protein into host cell.

Lect 3

Gram positive

Adhesins

In the process of bacterial infection, adhesion to host tissues represents an initial and essential step. Adhesion allows the pathogen to attach to and colonize specifie sites of the body, thereby withstanding eradication through cleansing mechanisms such as excretion and peristalsis. Once attached to the target tissue, bacteria may either remain extracellular, multiply, and eventually spread into deeper tissue, or trigger their own uptake by host cells, resulting in an intracellular location that may allow the pathogen to persist or further spread within the cellular or subcellular compartment. Bacterial surface components that mediate adherence are called adhesi4ns. Among gram-positive pathogens, surface proteins represent the largest group of adhesins, although other factors such as polysaccharides and lipids may also display adhesive functions. F Targets mr-tfiéöGF%bial adTiGs are host molecules found on mucosal surfaces, skin, and wounds. Depending on the strength of this interaction, adhesins allow the pathogen loosely associate with or intimately bind to specific cells or tissues. Most gram-positive pathogens express multiple adhesins that may bind to either the same or distinct target molecules. Multiple adhesins of one pathogen are likelv to be involved in different stages of an infection, expresse Ider different environmentally determined conditions, and may display a redun t function. In the present article, adhesions of pathogenic gram-positive bac

••a belonging to the genus Streptococcus, Staphylococcus and Listeria, a w as the most important host molecules targeted by these adhesins are r viewe .

The Extracellular Ma trix Major Target for Pathogens

Many adhesins function by ecifically reco ng and •nding to various components found in the extacelluar matrix MA o ost the -major structural support for cells and tissues and is respo i Oorm aining the strength and elasticity of the body. Thu it is pre and frequently exposed in cases such as trauma d injury. Ration tha render its constituents ideal targets for many adhesins. e follo •n gives a short overview on the mqjor ECM components, the' struct re eir basic function.

C O llagens

Collagens are the most abundant proteins in the mammalian body and it is well recognized that collagens fulfill an important structural role in the ECM in a

number of tissues. More than 25 distinct collagen types have been identified, in which identical or distinct a chains fonn a triple helix. Collagens can be divided into fibril-forming interstitial collagens (e.g. types I, Il, Ill, V, and XI) and nonfibril-forming collagens such as type IV, VI, and X . Type I collagen is found in tendons and muscle, while type Il collagen is the major constituent of cartilage. , bacterial binding to collagens such as cartilage collagen and basement membrane collgen , represent important adhesion mechanisms among pathogens .

Fibronectin

Fibronectin, which exists both as a soluble protein in plasma and as a fibrillar polymer in the ECM, is a large glycoprotein involved in cell adhesion, migration, and differentiation. Fibronectin exists as a dimer composed of two 250-kD subunits which are carboxy-terminally linked via a pair of disulfide bonds. Fibronectin is an ideal target for many pathogens due to its wide presence in exudates, blood, wounds, as well as on the surface of cells.

Laminin

Laminin is a 900-kD glycoprotein and is a major component ofthe basement membrane. Its macromolecul@t• s ucture is formed by assembly of three distinctpolypeptide chains, nd Y . In the case of epithelial and endothelial injury, basement membrane components such as laminin are likely to be exposed and may serve as target structures for bacterial colonization of damaged tissue.

Elastin

Elastin is the major ECM protein of lung, skin and large arteries such as the aorta, imparting characteristics oféxtensibility and elastic recoil . Elastin is formed by polymerization and cross-linking of its precursor tropoelastin. ₄Elastin serves as a target for pathogenic staphylococci, which use this molecule for adhesion to host tissue.

Vitronectin

Vitronectin is a multifunctional 75-kD glycoprotein present in blood and the ECM. It binds collagen, plasminogen and the urokinase receptor, and stabilizes the inhibitory conformation of plasminogen activation inhibitor-I, thereby regulating the proteolytic degradation of the ECM. , vitronectin is an ideal target for adhesins of pathogens due to its presence in the ECM, in blood, and at sites of tissue injury.

Fibrinogen

Fibrinogen is a 340-kD plasma glycoprotein composed of six polypeptide chains, two A alfa, two B beta, and two Y chains that form a dimer. In the vascular system, fibrinogen mediates platelet adherence and aggregation at

sites of trauma and injury, thereby acting as an important clotting factor .. Many gram-positive pathogens have evolved distinct factors that specifically bind fibrinogen, evoking bacterial adhesion, aggregation, and evasion of phagocytosis..

Glycosaminoglycans

Glycosaminoglycans are polysaccharide chains covalently linked to a protein core to form proteoglycans. Being composed of distinct repeating disaccharide units, these molecules can be divided into different classes such as heparin sulfate, dermatan sulfate, and chondroitin sulfate. . Glycosaminoglycans may mediate adherence and entry of pathogens including bacteria, viruses and parasites .

Streptococcal Adhesins

Streptococcus pyogenes S. pyogenes, the group A Streptococcus, is an important human pathogen that causes localized infections of the respiratory tract and the skin, but also insevere invasive diseases, such as sepsis and toxic shocklike syndrome. Severe nonsuppurative sequelae such as acute rheumatic fever and glomerulonephritis may follow primary group A streptococcal infection. S. pyogenes initiates infection by interacting specifically with host molecules present on mucosal surfaces or skin. A variety of different adhesins that. either bind to identical or distinct target molecules are expressed by S. pyogenes . Among the large number of bacterial factors that bind to host molecules, only those for which adhesive properties were clearly demonstrated are herein termed adhesins. S. pyogenes possesses at least nine distinct fibronectin-binding

large a in occur these of Some adhesins. of number

FBP54 whereas others such as Ml or M3 serotypes, such as SfbI protein or] protein are exclusively expressed by Ml M3 serotypes, , HA may act as an adhesin itself but may also mask binding interactions o other streptococcal surface molecules, depending on the type of the M serotype or tissue

Laminin, another constituent of the ECM, also represents a target for S. pyogenes. Two laminin-binding proteins are known, Lbp that has adhesive properties for epithelial cells and SpeB, the secreted cysteine protease which also displays glycoprotein-binding activity .

S.agalactiae

S. agalactiae, the group B streptococcus, is a gram-positive commensal of the human vagina, but also the major cause of neonatal sepsis and meningitis. S. agalactiae may also cause serious infections in immunocompromised adults. Compared to S. pyogenes, the number of adhesins identified so far is relatively small . The host molecules known to be targeted by S. agalactiae ar s fibronectin , lamjnin , and cytokeratin 8 . The only nown fibronectin-binding

factor of group B streptococci is C5a peptidase (ScpB), a large serine protease that is secreted but also attached to tfre s eptococcal surface. Purified recombinant ScpB was demonst atedfto bind to immobilized fibronectin , as well as to HE A549 cell a surface-associated lipoprotein belonging to the-ffål family of protei was shown to meiliate attachment of group B streptococci to laminin .

Whether Lmb indeed acts as an adhesin remains to be determined. Other data suggest a direct role for the alpha C rotein in adherence to cervical epithelial cell The alpha C protein IS the prototype for a family oflong tandem repeatcontaining surface proteins that also include R28 of S. pyogenes and Esp of

Enterococcusfaecalis. The cellular receptor for alpha C protein is, as in the case of R28, still unknown. The molecular nature of another streptococcal adhesion that binds to cytokeratin 8 , a molecule potentially important for colonjzation of keratinized epithelium or damaged cells, also remains to be identified.

S. pneumoniae

S. pneumoniae, the pneumococcus, is a natural colonizer of the nasopharyngeal epithelium and has the ability to penetrate the epithelial barrier, to translocate into deeper tissue, where it can cause severe infections such as pneumonia, meningitis and sepsis. Although binding to lamiuin, type collagen, and vitronectin was described over a decade ago , only three adhesins that bind to other target molecules have been identified in this streptococcal species.

Table 1.

Table 1 Streptococcal adhesins

Re	Target cells, tissue	Ligand molecule	Adhesin	rence no.
	and pharynx		S. pyogenes	2
	lung epithelial cells.		SfbVFl	3
	endothelial cells, collagen			3
		n.d. matrix FBP54	F2/PFBP	. 3
	cells epithelial	buccal epithelial cells	FbaB	Fba
	cells epithelia} cells protein epithelial	Ml cells H epithelial	Protein	
		cells, epithelial	LTA	
	n.d.	macrophages collagen matrix cpa	M3 protein	
	collagen matrix,	fibronectin, collagen	HA capsule	
	keratinocytes epithelial cells. Lbp	M proteins keratinocytes	protein	
	fibroblast cells	fibronectin fibronectin fibronectin fibronectin		
	fibronectin, M proteins fibronectin fibrouectin, macrophage scavenger receptor type and		speB	
		epithclial cclls	IV	
	n.d.	collagen, fibronectin type I collagen type I and	R28	
	epithelial cervical	IV collagen,	SclA/Scl I	
	cells pharyngeal cells	CD46 glucosaminoglycans,	SciB/Sc12	
	fibroblast cells	M proteins laminin laminin, glycoproteins n.d.	S. agalactiae	
		n.d.	scpB	
	lung and npharynx		Lmb	
	cervical	epithelial cells protein S. fibronectin	Alpha C	42
		n.d. laminin pneumoniae epithelial cellsn.d.		
	plgR•expressing cells		SpsAJCbpÆPspC	
	and endothelial epithelial cells	Phosphorylcholine SC, sIgA, factor H PA eeeptor		
	n.d.	fibronec	PavA	

n.d. Nol determined.

96

Table 2. S. aureus adhesins

Adhesin	Ligand molecule	Target cells, tissue	Reference no.
FnbpA	fibronectin, fibrinogen	epithelial cells, endothelial cells, mammary glands, T lymphocytes	65–75
FnbpB	fibronectin	epithelial cells, endothelial cells, mammary glands	67, 69–75
Ebh	fibronectin	?	85
Cna	collagen	cartilage	86–94
ClfA	fibrinogen	thrombi, implanted biomaterial	95–100
ClfB	fibrinogen, cytokeratin	thrombi, implanted biomaterial, keratinocytes, nasal epithelial cells	101–102
SasG	?	nasal epithelial cells	103, 105
Pls	?	nasal epithelial cells	104, 105
Bbp	bone sialoprotein	bone tissue	106
Spa	v WF	damaged endothelium	108
vWbp	v WF	?	109
Map/Eap	fibronectin, fibrinogen, vitronectin, bone sialo-protein, thrombospondin, collagen, osteopontin ICAM-1	epithelial cells, fibroblast cells	110–116
Emp	fibronectin, fibrinogen, vitronectin, collagen	?	117
EbpS	elastin	?	118–120
PIA	?	biofilm formation, cell-cell adhesion	121
Capsule	?	epithelial cells, endothelial cells	122

Another fibronectin-binding protein of S, is IEbh, a large 1,1megadaltön surface-associated protein that has been shown .10 bind soluble and immobil€zed fibronectin [851. The role of Ebb in cell adherence is, however, still undefined*

CÅa, the collagen-binding factor of' S. aureus is an important adhesin which medi?tes attachment to collagen substrates and collagenous tissues [86, 87]. In addition to this, Cna is t!ble to mediate adherence to cartilage, a poten lially important mechanism clunng septic arthritis 188, 891 and/or osteomyelilis [90]. The ligand-binding domain of Cna was identified to be located on a

168-atnino-acid-long segment within the amino-terminal A domain of the protein

102

To date, the best-studied adhesin of S. pneumoniae is SpsA. also named CbpA or PspC . SpsA binds to human secretory lgA , mediates adherence to activated human cells , and uses the human polymeric immunoglobulin receptor as a terminal receptor on the surface of host cells for adherence and translocation . In addition to these properties, SpsA is a protective antigen that also binds to factor H , suggesting a multifunctional role for this adhesin.

Staphylococcal Adhesins

S. aureus is an important opportunistic pathogen ofhumans and animals. The spectrum of diseases ranges from superficial skin infection to serious infections such as endocarditis, septic arthritis, and community-acquired and nosocomial sepsis. Besides this, S. aureus is a major cause of infections originating from catheters and implanted synthetic mew evices.

Many S. *aureus* isolates have the ability to bil nectin Most strains express FnbpA and Fnbnp , two related fibronectin-binding proteins encoded by closely linked genes . These two proteins were shown to bind soluble and immobilized fibronectin via their carboxy-terminal repeat region, whereas FnbpA was also shown to bind fibrinogen via its amino-terminal A domain . In vitro infection experiments employing distinct cell types as well as isogenic S. *aureus* strains either expressing or lacking one or both Fnbps revealed that fibronectin-coated devices, human epithelial cells, endothelial cells, and T lymphocytes are targets for Fnbp-mediated adhesion . As in the case for the S. *pyogenes* fibronectinbinding proteins SfbIIF I and Ml underlying color interaction and Binding to fibronectin.

Other Gram positive adhesin

Listeria monocytogenes is Gram positive food borne humen pathogen that causes listeriosis . This bacteria sever invasive which disseminate to the fetoplacental, and to the central nervous system. Although the overall of listeriosis cases was low .these bacteria have factors for interaction with tissues the two important factors was Internalin A and B (Inl A and InlB).

Which also consider as invasive factor ,the receptor for InlA was E.Cadherin in human intestine , the specific infection of listeria aside according to single amino acid of asurafce of E.cadherin the finding amino acid proline at position 16 of this receptors give specific for interaction with human E cadherin while can't interact with rat and mouse receptor which contains glutamic acid residue at position 16 thus was not susceptible to listeriosis.

Recent studies demonstrated aautolysinalso may work as adhesins in Gram positive also may play as adhesins the first autolysin was shown act as an adhesin was AtlE of S.*epidermidis*

. AtlE was suggested to playa role in the attachment to polystyrene surfaces and to vitronectin thereby contributing to biofilm formation of S. epidermidis 0TTmplanted polymers. Aas, an orthologous autolysin of Staphylococcus

saprophyticus, mediates adhesion and binds to fibronectin mediate bacterial attachment wa . Adhesive properties were localized within the noncat$ic carboxy-terminal cell wallanchoring domain, composed of so-called GW modules, short dipeptide repeats containing the amino acid residues glycine and tryptophane . Linkage of GW modules to LTA, as well as to glycosaminoglycans, anchor GW module-containing proteins to the surface of gram-positive bacteria . GW modules are found within all adhesive autolysins described herein, but also in eight other listerial proteins including InlB Thus, to define the adhesive properties of the yet uncharacterized module-containing proteins will be a future goal. Interestingly, Cwp66 of Clostridium difficile, the first identified adhesin of this gram- Ive spore-forming pathogen belonging to the genus clostridia, exhibits homology to the catalytic domain of CwLB the autolysin of *Bacillus subtilis* In contrast to the above-descri dhesive autolysins, Cwp66 lacks repetitive GW modulesbut may be linked to the gram-positive cell wall via an alternative mechanism, explaining its surface localization It is important to mention that a variety of adhesins,

Lect **4** biofilm

Introduction

Biofilms are complex communities of surface-attached microorganisms, comprised either of a single or multiple species. Over the past few decades, there has been a growing realization that bacteria in most environments are not found in a unicellular, planktonic (free-living) form such as those typically studied in the laboratory, but exist predominantly in multi-cellular surface attached communities called biofilms. Biofilm development is a series of complex but discrete and well-regulated steps. The exact molecular mechanisms differ from organism to organism, but the stages of biofilm development are similar across a wide range of micro-organisms. The sequential stages of biofilm development (Figure 1):

(1) Microbial attachment to the surface.

(2) Adhesion, growth, and aggregation of cells into **microcolonies** (relatively small groups of bacteria).

(3) Maturation and dissemination of progeny cells for new colony formation.

Microbial Attachment

Direct contact of the microorganism with the surface is required for attachment and subsequent colonization. The ability of a cell to perform this “initial attachment event” is controlled by both environmental factors, including nutrient levels, temperature, pH, and genetic factors, including the presence of genes encoding motility functions, environmental sensors, adhesins, etc. The mere "touch" of the cell wall with the biomaterial alters the microorganism's phenotypic expression to begin production of a sticky adhesin that attaches the cell to the surface.

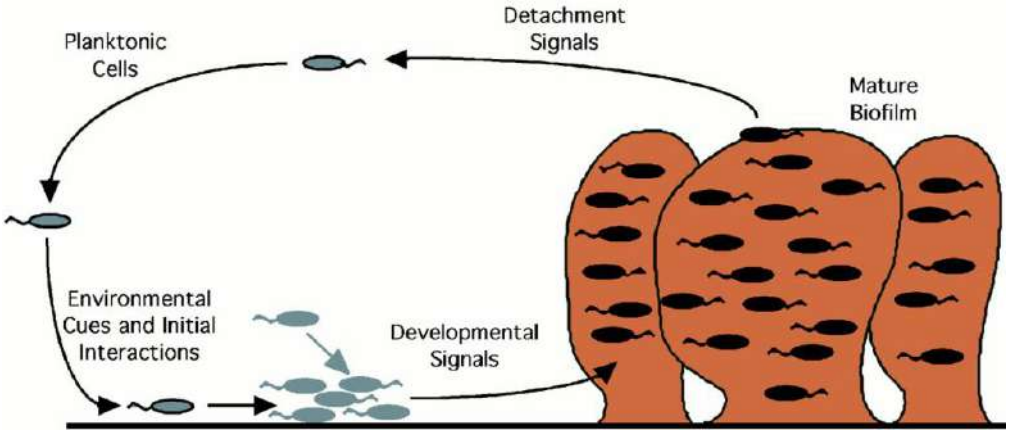


Figure 1. Schematic diagram of biofilm formation and growth

The matrix of host products serves as scaffolding for the simultaneously developing biofilm and provides receptor-binding sites for newly arriving bacteria. However, this attachment is depending on the

type of host matrix. For example, whole blood promotes *Pseudomonas aeruginosa* biofilm formation, while plasma proteins such as fibrinogen and fibronectin enhance *Staphylococcus aureus* binding but inhibits *S. epidermidis* and Gram-negative bacteria adherence.

Adhesion and Microcolony Formation

The attachment of a small number of bacterial cells is all that is needed to initiate biofilm formation anywhere along the system. Within a few seconds, the progression of phenotypic changes in the bacteria remarkably alters protein expression to further produce species-specific adhesions that irreversibly anchor the cell to the surface. Type IV pili are involved in a type of surface-associated motility called **twitching**, and this twitching might be required for the aggregation of cells into microcolonies.

Within as few as 12 minutes, the adherent cells **upregulate** genes that direct production of accumulation proteins and polysaccharides, which firmly attach the cells to the substratum and to each other as they undergo exponential binary division.

After initial attachment, the cells begin to grow and spread as a **monolayer** on the surface. As the cells continue to divide, the daughter cells spread outward and upward from the attachment point to form cell clusters. The production of **exopolysaccharides** (EPS) or "slime" embeds the aggregating cells to form **microcolonies**. Typically, the microcolonies are composed of 10% to 25% cells and 75% to 90% EPS matrix (Figure 2), with a consistency similar to a viscous polymer hydrogel.

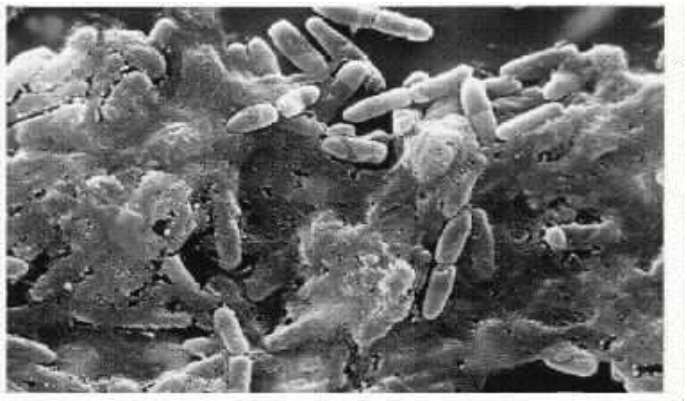


Figure 2: Electron micrograph of interior surface of a vascular catheter removed from a patient showing growth of a bacterial biofilm of *P. aeruginosa*

EPS of biofilm provide certain degree of shelter of homeostasis to the bacteria residing in biofilm. The EPS matrix also has the potential to physically prevent the access of certain antimicrobial agents into the biofilm by acting as an anion exchanger. It restricts the diffusion of compounds from surroundings into the biofilm.

The polysaccharide that comprise the matrix give a three dimensional shape to the mature biofilm and provide structural support. The matrix enables the bacterial cells to remain close to the surface and to easily attach to one another

The structural organization is mainly influenced by hormone-like regulatory signals produced by the biofilm cells themselves in reaction to growth conditions. This interactive network of signals allows for communication among the cells, not only controlling colony formation but also regulating growth rate, species interactions, toxin production, and invasive properties.

Cellular density typically increases to a steady state i 1-2 weeks, depending on the species and local environmental conditions. Adjoining

microcolonies are connected by **water channels** that serve as a primitive circulatory system for delivery of nutrients and removal of wastes.

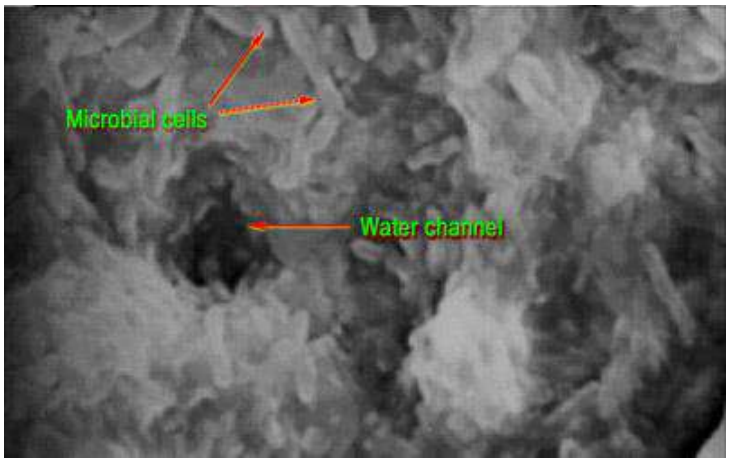


Figure 2: Water channels. 5000X

The thickness of the biofilm is variable (13-60 µm) and uneven, as determined by the balance between growth of the biofilm and detachment of cells. Depending on the initial number of attached organisms, the multilayered cell clusters develop as patchy networks or form a contiguous layer over the surface of the catheter.

Dispersal and Dissemination of Biofilm Cells

The formation of biofilm is a universal strategy for microbial survival. In order to colonize new surfaces and to prevent density-mediated starvation within the mature biofilm, the cells must detach and disseminate. However, those released in clumps retain antibiotic resistance and may embolize at a distant anatomic site to develop **metastatic** infections such as endocarditis or osteomyelitis.

Studies have shown that Dispersal occurs when the organisms respond to chemical substances secreted by them such as signalling

molecules, proteins and degradative enzymes and oxidative or nitrosative stress-inducing molecules such as nitric oxide (NO) produced as a result of metabolic processes within a biofilm. It has also been shown that alginate lyase; a degradative enzyme produced by biofilm organisms cleaves the polymer matrix into short oligosaccharides. The cleavage antagonises the attachment characteristics of alginate leading to increased Dispersal of biofilm organisms

Dispersal is accomplished by shedding, detachment, or shearing:

1. **Shedding**: occurs when daughter cells from actively growing bacteria in the upper regions of the microcolonies are released from the cell clusters. Due to increased cell density a programmed set of events (cell-cell signaling) within the biofilm leading to a local hydrolysis of the extracellular polysaccharide matrix (alginase in the case of *P. aeruginosa*), and conversion of a subpopulation of cells into motile planktonic cells, which leave the biofilm.
2. **Shearing**: biofilms are exposed to variable flow rates and shear forces. When the shear force of the infusion exceeds the tensile strength of the viscous biofilm, fragments break away.
3. **Detachment**: Clumps or fragments of detached biofilm may contain thousands of cells, but they leave behind an adherent layer of cells on the surface to regenerate the biofilm.

Staphylococcus aureus tends to release large clumps of biofilm; an alternative dispersal strategy to that of *P. aeruginosa* which sheds only single cells or small clumps.

Biofilm formation in mixed culture

It has been demonstrated that cell-to-cell communication through quorum sensing mechanisms may occur not only within species of microbes but also between them. It was demonstrated that the biofilm-forming capabilities of *P. aeruginosa* and MRSA within a CAUTI model are significantly enhanced when these organisms are grown together as a mixed culture, compared to growth as monocultures.

Furthermore, production of exotoxin A by *P. aeruginosa* is substantially increased when grown in a mixed culture MRSA biofilm. *P. aeruginosa* displayed slow progressive biofilm growth in the monoculture CAUTI model. This contrasted to the growth of the MRSA monoculture which showed a high rate of initial attachment followed by a decrease in biofilm area coverage due to large detachment events.

Biofilm resistance to antimicrobial agents

Biofilms growing in natural and industrial environments are resistant to bacteriophage, to amoebae, and to the chemically diverse biocides used to combat biofouling in industrial processes. Of importance with respect to medicine, sessile bacterial cells can withstand host immune responses, and they are much less susceptible to antibiotics than their nonattached individual planktonic counterparts. It is likely that biofilms evade antimicrobial challenges by multiple mechanisms:

1. The failure of an agent to penetrate the full depth of the biofilm. Polymeric substances like those that make up the matrix of a biofilm are known to retard the diffusion of antibiotics, and solutes in general diffuse at slower rates within biofilms than they do in water. Antibiotics have been shown to penetrate biofilms readily in some

cases and poorly in others, depending on the particular agent and biofilm.

2. Some of the cells in a biofilm experience nutrient limitation and therefore exist in a slow-growing or starved state. Slow-growing or non-growing cells are not very susceptible to many antimicrobial agents. Such heterogeneity of biofilms constitutes an important survival strategy because at least some of the cells, which represent a wide variety of different metabolic states, are almost certain to survive any metabolically directed attack.
3. Some of the cells in a biofilm adopt a distinct and protected biofilm phenotype. This phenotype is not a response to nutrient limitation; it is a biologically programmed response to growth on a surface.
4. Multidrug efflux pumps could be up-regulated on expression of a biofilm phenotype. Whilst this is an appealing and simple explanation, because of its ability to explain the breadth of agents to which biofilms are resistant, recent work has suggested that this is not the case.
5. Alternative hypotheses attempt to explain the diversity of agents by invoking a common cause of death for which singular resistance mechanisms could be applied. It is therefore suggested that an altruistic majority of sublethally damaged cells in a population commit suicide (apoptosis), thereby providing some protection to the survivors. A proportion of cells (persisters) is suggested to be defective, or repressed, in their suicide response, and survive.
6. A second explanation of the presence of persisters is that the general stress response, well known to include the adoption of a viable, non-culturable state of quiescence, is up-regulated in small pockets of the

biofilm community, where nutrients are particularly scarce. Such quiescent cells noted for their resistance towards the metabolically acting biocides would potentially have their dormancy broken after treatment by the replenished supply of nutrients caused by the death of the majority.

7. A more recent hypothesis suggests that extracellular signals, 'alarmones', released from killed cells might prime recipients into a state of resistance. Thus, in biofilm communities deep lying cells might be alerted into a resistant state by the premature death of peripheral cells. It is equally possible that 'alarmones', in this context, are merely the post-treatment 'wake-up' call to a previously quiescent subset of cells.

The role of biofilms in pathogenesis

Biofilms can be found almost anywhere and may impact human health both positively and negatively. One example of a positive effect includes the biofilms of commensal bacteria such as *Staphylococcus epidermidis*, which can impede the colonisation of potentially pathogenic bacteria through the stimulation of host-cell immune defences and the prevention of adhesion. However, biofilms are more often associated with many pathogenic forms of human diseases and plant infections. One common example is cystic fibrosis, the most frequently passed genetic disorder in Western Europe. Cystic fibrosis (CF) patients suffer from chronic *P. aeruginosa* infections. When infecting the CF lung, *P. aeruginosa* undergoes a

characteristic transition from an acute virulent pathogen to a CF-adapted pathogen, allowing it to persist in the lung for years or even decades. This is due to the overproduction of the matrix polysaccharide alginate, leading to the formation of a mucoid biofilm that tolerates antibiotics, components of both the innate and adaptive immune response, and resists phagocytosis. The persistence of these mucoid biofilms within the CF lung leads to the development of a distinct antibody response. This prompts chronic inflammation mediated by granulocytes, and results in severe damage to the lung tissue of CF patients . A second example for biofilms in human health is dental plaque potentially leading to dental caries. The consumption of fermentable carbohydrates such as sugary treats or drinks causes an increase in the production and secretion of organic acids by the bacteria found in dental plaque. If left untreated, the increased acidification of the biofilm leads to the demineralisation of the enamel and the formation of dental caries

Lect .5

Colinization and invasive by pathogenic bacteria

INVASION

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.

The extracellular proteins produced by bacteria which promote their invasion are not clearly distinguished from some extracellular protein toxins ("exotoxins") which also damage the host. Invasins usually act at a short range (in the immediate vicinity of bacterial growth) and may not actually kill cells as part of their range of activity; exotoxins are often cytotoxic and may act at remote sites (removed from the site of bacterial growth). Also, exotoxins typically are more specific and more potent in their activity than invasins. Even so, some classic exotoxins (e.g. diphtheria toxin, anthrax toxin) may play some role in colonization or invasion in the early stages of an infection, and some invasins (e.g. staphylococcal leukocidin) have a relatively specific cytopathic effect.

Once adhered to a host surface, some pathogens gain deeper access into the host to perpetuate the infection cycle. This pathogenic principle,termed invasion,can be divided into two types: extracellular and intracellular. Extracellular invasion occurs when a microbe breaks down the barriers of a tissue to disseminate in the host while remaining outside of host cells.

This is a strategy used by group A β-haemolytic streptococcus and *S aureus*.[13] These species secrete several enzymes that degrade host cell molecules:hyaluronidase (cleaves proteoglycans in connective tissue), streptokinase and staphylokinase (breaks down fibrin clots), lipase (degrades accumulated host oils), and nuclease (digests released RNA and DNA). The haemolysins (which punch holes in host cells) expressed by these species lyse not only erythrocytes but other

cell types as well and may also contribute to their spread in host tissues. *Pseudomonas aeruginosa* secretes an enzyme, elastase, which degrades extracellular molecules and aids tissue invasion associated with keratitis, burn tissue necrosis, and cystic fibrosis. Extracellular invasion allows these pathogens access to niches in tissues where they are able to proliferate, disseminate to other sites in the body, express toxins, and initiate inflammatory responses. There is a growing body of evidence that suggests that extracellulary invading pathogens may also enter host cells and use both the extracellular and intracellular pathways during infection. Intracellular invasion occurs when a microbe actually penetrates the cells of a host tissue and survives within this environment. A number of Gram negative, Gram positive, and mycobacterial pathogens have been shown to have the ability to enter host cells,[1]and both phagocytic and nonphagocytic cell types can serve as targets for invasion. Some pathogens have an obligate intracellular lifecycle which absolutely requires a mammalian cell for growth. These include *Chlamydia* spp, *Rickettsia* spp, and *Mycobacterium leprae*.[13] Other pathogens are facultatively intracellular, using their ability to enter and survive within host cells as a means of proliferation or spreading to other tissues.

A major advance in bacterial pathogenesis in recent years has been the identification of genes that allow pathogens to invade host non-phagocytic cells. Remarkably, these invasion genes, present in several different pathogens, were found to encode an evolutionarily related type III protein secretion pathway that serves to inject signalling proteins from the microbe into the host cell.

The injected proteins then activate host cell signalling pathways that cause the host cell to internalise the microbe. These entry mechanisms are well characterised in *Salmonella* spp and *Shigella* spp. A common outcome of type III secretion signalling is the rearrangement of host cell actin such that the cytoskeleton is recruited to engulf the invading microbe. Both *Salmonella* and *Shigella* engage actin regulatory proteins, called Rho GTPases,to "switch on"the actin rearrangement pathway to form nodes of actin underneath the invading pathogen. This type of interaction highlights the phenomenon of biochemical crosstalk between host and pathogen that is essential for penetration of host cells.

Spreading Factors lect 6

"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.

Hyaluronidase. is the original spreading factor. It is produced by streptococci. staphylococci, and clostridia. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing hyaluronic acid.

Collagenase is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

Neuraminidase is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

Streptokinase and **staphylokinase** are produced by streptococci and staphylococci, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Enzymes that Cause Hemolysis and/or Leucolysis

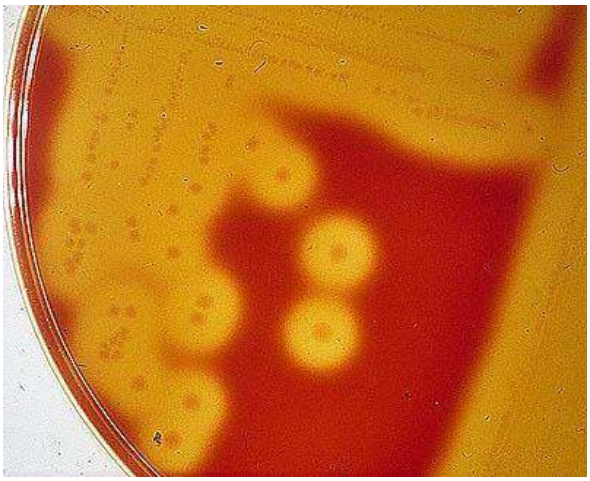
These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane. They may act as **lecithinases** or **phospholipases**, and if they lyse red blood cells they are

sometimes called **hemolysins**. **Leukocidins**, produced by staphylococci and **streptolysin** produced by **streptococci** specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

Phospholipases, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

Lecithinases, also produced by *Clostridium perfringens,* destroy lecithin (phosphatidylcholine) in cell membranes.

Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.



Beta-hemolytic Streptococcus. This is the characteristic appearance of a blood agar plate culture of the bacterium. Note the translucency around the bacterial colonies, representing hemolysis of the red cells in the culture medium due to production of a diffusible hemolysin (streptolysin).

Staphylococcal coagulase

Coagulase, formed by *Staphylococcus aureus*, is a cell-associated and diffusible enzyme that converts fibrinogen to fibrin which causes clotting. Coagulase activity is almost always associated with pathogenic *S. aureus* and almost never associated with nonpathogenic *S. epidermidis,* which has led to much speculation as to its role as a determinant of virulence. Possibly, cell bound coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface. Or a staphylococcal lesion encased in fibrin (e.g. a boil or pimple) could make the bacterial cells resistant to phagocytes or tissue bactericides or even drugs which might be unable to diffuse to their bacterial target.

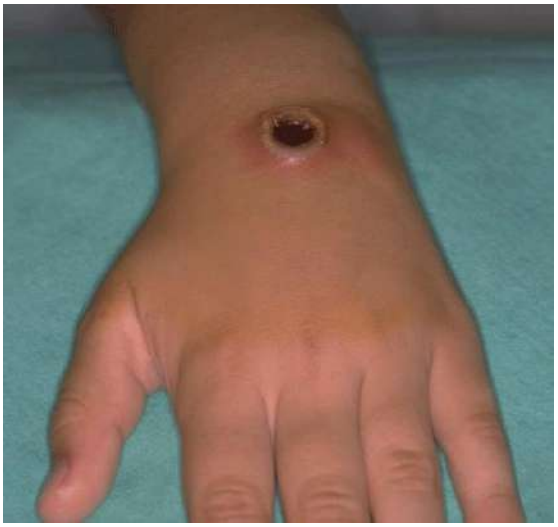
Extracellular Digestive Enzymes

Heterotrophic bacteria, in general, produce a wide variety of extracellular enzymes including **proteases**, **lipases**, **glycohydrolases**, **nucleases**, etc., which are not clearly shown to have a direct role in invasion or pathogenesis. These

enzymes presumably have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly.

Toxins With Short-Range Effects Related to Invasion

Bacterial protein toxins which have adenylate cyclase activity are thought to have immediate effects on host cells that promote bacterial invasion. One component of the anthrax toxin (**EF** or **Edema Factor**) is an **adenylate cyclase** that acts on nearby cells to cause increased levels of cyclic AMP and disruption of cell permeability. One of the toxins of *Bordetella pertussis,* the agent of whooping cough, has a similar effect. These toxins may contribute to invasion through their effects on macrophages or lymphocytes in the vicinity which are playing an essential role to contain the infection. For example, since they use ATP as a substrate, they may deplete phagocyte reserves of energy needed for ingestion. Edema is seen as a pathology because the increase in cAMP in affected cells disrupts equilibrium.



Gelatinous edema seen in a cutaneous anthrax lesion. .

The following table summarizes the activities of many bacterial proteins that are noted for their contribution to bacterial invasion of tissues.

TABLE 3. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	*Bacteria Involved*	*Activity*
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue

Collagenase	*Clostridium* species	Dissolves collagen framework of muscles
Neuraminidase	*Vibrio cholerae* and *Shigella dysenteriae*	Degrades neuraminic acid of intestinal mucosa
Coagulase	*Staphylococcus aureus*	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	*Staphylococcus aureus*	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	*Streptococcus pyogenes*	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	*Clostridium perfringens*	Destroy lecithin in cell membranes
Phospholipases	*Clostridium perfringens*	Destroy phospholipids in cell membrane
Anthrax EF	*Bacillus anthracis*	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	*Bordetella pertussis*	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

a

Invasion of epithelial cells

The polarized epithelium of the mammalian host plays a significant role in resistance to infection. Cell–cell and cell–matrix junctional complexes important for the development of a polarized epithelium and an intact barrier are depicted in Fig. 1.

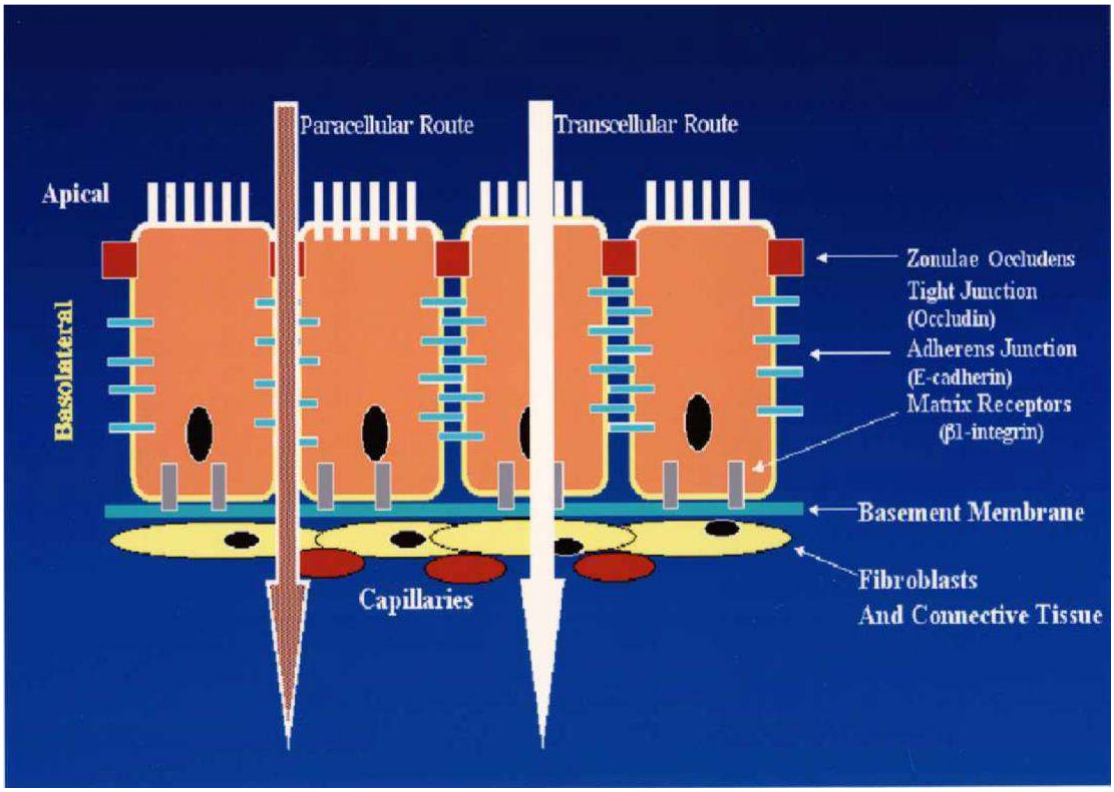


Figure 1: Schematic diagram depicting cell junctions and routes of bacterial invasion in a simple polarized epithelium.

In order to gain access to deeper tissues and/or cause disease, bacterial pathogens must first breach the epithelial barrier. The epithelial barrier is the first line of defense against the entry of most human pathogens. Knowledge of microbial virulence factors involved in the utilization or destruction of the structures that keep the integrity of the epithelium is crucial for our further understanding of infections. In addition to direct trauma, bacterial invasion through epithelial cells can occur via 1) Transcellular route and 2) Paracellular route.

I- Invasion through transcellular route

This route utilizes two mechanisms; anchored and anchorless.

A: Anchored mechansim

- **Fibronectin-binding proteins**

Several Gram-positive bacteria, including streptococci and staphylococci, specifically bind to fibronectin using several different adhesins with highly homologous recognition motifs. These adhesins are anchored on the bacterial surface via an LPxTG (Leu-Pro x Thr-Gly) motif.

Fibronectin binding is involved in both adherence and invasion. The mechanism involved is illustrated by the streptococcal fibronectin binding protein I (SfbI protein), a major adhesin of *Streptococcus pyogenes*. The SfbI protein contains all the features required for LPxTG-mediated anchoring. Additionally, it contains two fibronectin-binding domains, a 37 amino acid **repetitive** domain and a 30 amino acid region designated the **spacer** domain. The repetitive domain binds to the 30 kDa amino-terminal fragment of fibronectin, allowing the bacteria to adhere to eukaryotic cells. This interaction causes the spacer domain to bind to the 45 kDa fragment of fibronectin, thereby trigging the integrin-mediated uptake of streptococci by eukaryotic cells.

.

- **Rocketing**

Listeria monocytogenes is a food- and water-borne pathogen that causes infections ranging from gastroenteritis to septicemia. It is an intracellular pathogen that employs a particularly interesting mechanism for migration through host tissue by a process known as “rocketing.

The bacterium requires at least two surface factors for entry into cultured cells; Internalin A (InlA) and internalin B (InlB). InlA binds to the E-cadherin receptor on epithelial cells, while InlB interacts with the complement receptor gC1qR, glycosaminoglycans, and the tyrosine

kinase receptor. Activation of signal transduction cascades promotes phagocytosis and the bacterium is enveloped in a phagocytic vesicle.

B- Anchorless mechanism α-enolase (Eno) is one of the key enzymes in the glycolytic cycle, and is located in the pneumococcal cytoplasm. Eno is secreted by an as-yet unknown mechanism and can reassociate by interacting with receptors on encapsulated and unencapsulated pneumococci (figure 4). This reassociation has important biological consequences. Surface-associated Eno binds to plasminogen and subsequently facilitates pneumococcal penetration through biological membranes by activating plasminogen during the invasive infection process.

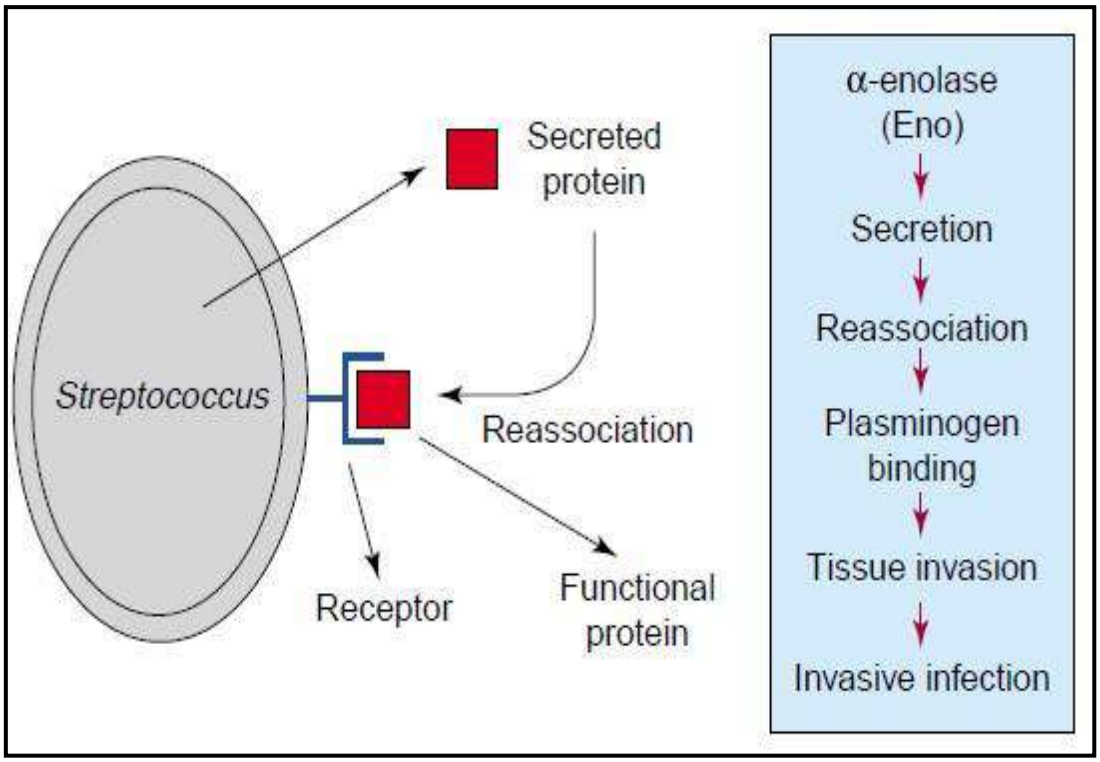


Figure 4: Enolase mechanism

II- Paracellular route

Clotridium difficile

Toxin A of *Clotridium difficile* disrupts the actin cytoskeleton. This finding suggested that the effect of toxin A on epithelial barrier function resulted from alterations of the actin cytoskeleton, which indirectly altered tight junction function. Furthermore, *C. difficile* toxin B induces a redistribution of filamentous actin.

Streptococcus pyogenes

Streptococcus pyogenes infections are occasionally complicated by severe invasive infection; which occurs via the paracellular pathway and is mediated by CD44 cell signaling. The mechanism by which this occurs is through induction of cytoskeletal rearrangements including membrane ruffling, disruption and opening of intercellular junctions that allow tissue penetration.

Lect 7 Capsule And its role in bacterial pathogenesis

INTRODUCTION

Capsules are the outmost structures of bacterial and fungal cells, which are physically associated with the cell surface and cannot be readily washed off. In contrast, slime polysaccharides are released into the medium. Both capsular and slime polysaccharides are negatively charged and the differentiation between them is not strict.

Thus, the mucus polysaccharide (colanic acid or M antigen) produced by many bacteria at low temperature is usually considered to be a capsule. The extracellular polysaccharide (alginate) of Pseudomonas aeruginosa, which is an important pathogenic factor in chronic pulmonary infection of cystic fibrosis patients], may also be regarded as a capsule.

When growing on agar media, encapsulated bacteria usually form colonies with distinct appearance and morphology. In some bacteria, the colonies of encapsulated bacteria are referred to as ‘smooth’ colonies, whereas those of unencapsulated bacteria take on ‘rough’ morphology (relatively small in size and dry in texture)ia .

Other studies on the pneumococcal capsule also indicated that the capsular polysaccharides (CPSs) are able to stimulate protective immunity against the infections of the homologous bacteria . These findings laid a foundation for the current CPS based vaccines for some encapsulated

TABLE 3.1 Pathogenic Bacteria with Capsules

Organism	Number of Serotypes	Chemical Nature	Reference
Acinetobacter baumannii	UD[1]	Polysaccharide	[16,17]
Campylobacter jejuni	47	Polysaccharide	[18,19]
Escherichia coli	80	Polysaccharide	[20]
Haemophilus influenzae	6	Polysaccharide	[21]
Klebsiella pneumoniae	82	Polysaccharide	[22]
Neisseria meningitidis	13	Polysaccharide	[23]
Pasteurella multocida	4	Polysaccharide	[24]
Pseudomonas aeruginosa	1	Polysaccharide	[25]
Salmonella enterica serovar Typhi	1	Polysaccharide	[26]
Streptococcus pneumoniae	94	Polysaccharide	[27,28]
Streptococcus agalactiae	9	Polysaccharide	[29]
Streptococcus pyogenes	1	Polysaccharide	[30]
Streptococcus suis	35	Polysaccharide	[31]
Staphylococcus aureus	11	Polysaccharide	[32]

Staphylococcus haemolyticus	1	Polysaccharide	[33]
Bacillus anthracis	1	Polyglutamate	[34]

1 Fregolino et al. identified two different CPS structures from

Acinetobacter baumannii isolates but CPS typing has not been established in this species; UD, undefined.

BIOLOGICAL FUNCTIONS OF CAPSULES

Capsules as virulance factors

Capsules have been well recognized as essential virulence factors for bacterial pathogens during infection and invasion processes in mammalian hosts. In 1891, some scientists observed severe attenuation of S. *pneumoniae* clinical isolates in animal models after the bacteria become unencapsulated . ,

. The importance of capsules in bacterial virulence has been universally demonstrated in all other encapsulated pathogens; the loss of the capsules in all encapsulated bacteria known thus far leads to attenuation of virulence to various extents. The importance of capsules in bacterial virulence varies in different niches of mammalian hosts during infection. Capsules provide a protective shield

against host immune recognition, leading to immune evasion and survival of the encapsulated bacteria in the niches where the host immune factors are abundantly present, such as the bloodstream during systemic infections . This principle is exemplified by the fact that many pathogens enhance their encapsulation during systemic infections . However, capsules may become an 'inconvenient' obstacle when bacteria need to interact with the mucosal surfaces of mammalian hosts for colonization in the early stage of infection. The colonization process requires the exposure of surface molecules for adhesive interactions .

Under these conditions, the capsules are commonly down-regulated to minimize their physical interference with bacterial adhesion . Some studies. demonstrate that a mutant of type 3 S. pneumoniae producing 20% of the parental amounts of capsule remains fully competent for colonizing the nasopharynx of mice, but the virulence of the same strain is severely decreased when directly inoculated into the bloodstream .

Certain capsule types within a single species appear to contribute more to the virulence traits than others. This notion is consistent with the fact that some CPS types are more prevalent in the clinical cases of diseases for a number of bacteria. As an example, most of the N. meningitidis clinical isolates possess six of the 13 serogroups (A, B, C, Y, W135 and X) . Although 94 CPS types have been identified in S. pneumoniae, the majority of invasive disease cases in children were caused by the pneumococci of seven CPS types (4, 6B, 9V,14,18C,19F, and 23F) before the introduction of the PCV7 conjugate vaccine . Along the same lines, serotype 5 and 8 isolates of S. aureus capsule account for the majority of isolates recovered from humans .

. The differential impact of capsules on bacterial virulence has been experimentally demonstrated in animal models. some studies show that pneumococcal strains expressing type 3 and 6 capsules are significantly more virulent than those producing type 14, 19 and

23 capsules in a systemic infection mouse model

In a similar manner, capsule type K1 and K2 isolates of K. *pneumonias* strains are especially virulent in a mouse peritonitis model, but the strains expressing other CPS types have little or no virulence]. It is possible that certain CPS structures are more competent in protecting bacteria against host immunity, facilitating adherence/ colonization, or promoting dissemination during infections. The precise mechanisms for this disparity are largely unknown.

However, there is increasing evidence that soluble capsular polysaccharide released from encapsulated microbes can also contribute to virulence through immunomodulatory effects. For example, the capsular polysaccharide of C. neoformans has been documented to mediate numerous untoward effects on immune cells from causing alterations in cytokine production to interfering with leukocyte migration . Like toxins, antibody responses to capsular polysaccharides, when they occur or are induced by immunization, often render the host immune to disease by the relevant organism. In this regard, effective polysaccharidebased vaccines have been generated against S*. pneumoniae*, *N*. *meningitides*, *H*. *influenzae* and C. neoformans, among other microbes. Like toxins, capsules are not necessary for viability in vitro and can be shown to be important for virulence by generating mutants and comparing the virulence of non-encapsulated and wild-type strains. Although many microbial capsules are composed of polysaccharides, some are composed of cross-linked amino acids. In this regard, the capsule of Bacillus anthracis is composed of poly-gamma-Dglutamic acid and functions to interfere with phagocytosis. However, like the experience with polysaccharide capsules, antibodies to gamma-D-glutamic acid are opsonic and protective against B. *anthracis* in murine models of anthrax.

Despite the structural and immunological diversity of capsules, they all perform the same principal function of protecting the bacteria from immune recognition and phagocytic killing]. Certain physical properties of capsules (e.g. hydrophobicity and negative charge) hinder the recognition and binding of phagocytes to encapsulated cells . In particular, capsules are important for evading the complement-mediated opsonophagocytosis .Also capsule work as modulating and evasion of complement

Pneumococci can degrade C3b without host proteins , and Group A and B streptococci express a C5a peptidase, which inhibits leukocyte recruitment). Other strategies complement inhibition involve microbial determinants that bind or mimic ligands of human regulators of complement activation (RCA), such as Factor H, CD55 (decay accelerating factor, DAF), CD21 (CR2) and CD46 (MCP) .The nature of these determinants is diverse, but they have in common that they are surfaceexposed molecules. Examples of microbial components that affect complement system activation and regulation are: sialic acid residues on gonococci and in the capsule of type B Streptococcus

capsule

mediated inactivation of C3b ;pneumococcal surface protein C (PspC) that binds Factor H; pneumococcal surface protein A (PspA) that inhibits the activation of the alternative complement pathway

8; **Lec**

Bacterial iron transport system and its role in pathogenesis

Iron is an essential nutrient for bacterial growth, replication, and metabolism. Humans store iron bound to various proteins such as hemoglobin, haptoglobin, transferrin, ferritin, and lactoferrin, limiting the availability of free iron for pathogenic bacteria. However, bacteria have developed various mechanisms to sequester or scavenge iron from the host environment .

. Iron can be taken up by means of active transport systems that consist of bacterial small molecule siderophores, outer membrane siderophore receptors, the TonB-ExbBD energy-transducing proteins coupling the outer and the inner membranes, and inner membrane transporters. Some bacteria also express outer membrane receptors for iron-binding proteins of the host and extract iron directly from these for uptake. Ultimately, iron is acquired and transported into the bacterial cytoplasm. The siderophores are small molecules produced and released by nearly all bacterial species and are classified according to the chemical nature of their iron-chelating group (ie, catechol, hydroxamate, α-hydroxyl-carboxylate

There are numerous **iron uptake** pathways in Gram-negative **bacteria** which include **iron uptake** from transferrin, siderophores, or heme. All of these **uptake** pathways require an outer membrane receptor, periplasma Binding protein PBP, and an inner-membrane ABC transporter. Not all **bacteria** have all three systems; but some have more than one type.

Sidrophore

Siderophores (Greek: "iron carrier") are small, high-affinity iron-chelating compounds that are secreted by microorganisms such as bacteria and fungi and serve primarily to transport iron across cell membranes, although a widening range of siderophore functions is

now being appreciated. Siderophores are among the strongest soluble Fe^{3+} binding agents known.

Iron acquisition by pathogens

The problems that bacteria face in acquiring sufficient iron from their surroundings are particularly acute for pathogens. The host specifically limits iron availability as part of its innate defence against invading cellular micro-organisms. Mammals employ iron-binding proteins (transferrin, lactoferrin) to reduce the levels of free extracellular iron to around 10^{-18} M levels insufficient to allow bacterial growth . In addition, the host produces proteins that bind haem and haemoglobin (e.g. haemopexin and haptoglobin) and consequently limit the availability of haem as an iron source for pathogenic bacteria. Pathogens often use low environmental iron levels as a signal for the induction of virulence genes . For instance, the Shiga-like toxin I of enterohaemorrhagic *E. coli* is induced by iron starvation . Pathogens are able to counter the iron restriction imposed by their hosts through the use of siderophores. Siderophores can compete with host iron-binding proteins and indeed some siderophore-based transport systems (such as the plasmid pColV-K30-encoded aerobactin system found in *E. coli* ColV strains are known to be required for effective host colonisation. However, it is common for pathogens to acquire iron directly from host iron-binding proteins by using receptor-mediated transport systems specific for host–iron complexes.

Iron transport related with virulance

The abilities of bacterial pathogens to adapt to the environment within the host are essential to their virulence. Microorganisms have adapted to the iron limitation present in mammalian hosts by evolving diverse mechanisms for the assimilation of iron sufficient for growth. In addition, many bacterial pathogens have used the low concentration of iron present in the host as an important signal to enhance the expression of a wide variety of bacterial toxins and other virulence determinants. The molecular basis of coordinate regulation by iron has been most thoroughly studied in Escherichia coli. In this organism, coordinate

regulation of gene expression by iron depends on the regulatory gene, fur. Regulation of gene expression by iron in a number of pathogenic organisms is coordinated by proteins homologous to the Fur protein of E. coli. Additional regulatory proteins may be superimposed on the Fur repressor to provide the fine-tuning necessary for the precise regulation of individual virulence genes in response to iron and other environmental signals. Studies of the mechanisms of regulation of iron acquisition systems and virulence determinants by iron should lead to a better understanding of the adaptive response of bacteria to the low-iron environment of the host and its importance in virulence

understanding of the adaptive response of bacteria to the low-iron environment of the host and its importance in virulence.

The relationship of iron transport to virulence is usually not easy to establish since bacteria normally Express several iron transport system .Knocking out one of system by mutation might not result in conversion of pathogenic strains to nonpathogenic since other iron transport system takeover the iron supply.for example a pathogenic *E.coli* strains may transport ferrus Fe++ or ferric Fe+++ by the sidrophore,aerobactin,salmochelin ,citrate,ferrichromeand heme.

Regulation of iron uptake

The Ferric uptake regulator (Fur) protein is a global iron regulator found in most prokaryotes. Although the Fur protein is involved in a variety of metabolic pathways, it is specifically known for the regulation of several iron responsive genes. Not only absence of iron or decreased levels effect on bacterial metabolism also increases levels caused damage for bacterial DNA and protein etc.under aerobic conditions because release of some toxic radicals which destroy bacterial structures. Thus bacteria used Fur system for regulation of iron uptake

Bacterial secretion systems systems **lect .9**

Are protein complexes present on the cell membranes of bacteria for secretion of substances. Specifically, they are the cellular devices used by pathogenic bacteria to secrete their virulence factors (mainly of proteins) to invade the host cells. They can be classified into different types based on their specific structure, composition and activity. Generally, proteins can be secreted through two different processes. One process is a one-step mechanism in which proteins from the cytoplasm of bacteria are transported and delivered directly through the cell membrane into the host cell sec independents . Another involves a two-step activity in which the proteins are first transported out of the inner cell membrane, then deposited in the periplasm, in manner called sec .dependent finally through the outer cell membrane into the host cell.There 8 secretion system utilizes by different types of bacterial in Gram positive and negative.

Type 1 **Secretion**

The type I secretion mechanism involves the one-step translocation of a secretion substrate in a Sec-independent manner . mechanism is employed for secretion of diffusible toxins to extracellular space. The type I mechanism has been demonstrated for the secretion of *hemolysin (HIYA) in E. coli, as well as for the secretion ofBordetella pertussisa-dfiYIE$IäE¯üTae mosa protease . In each instance, the toxin is recruited to a translocation comp ex that assembles upon association with the substrate. The type I secretion system is relatively simple in architecture, consisting of only three factors, each required for transport of the substrate. A characteristic f.eature of the system is the presence of an ATP-binding cassette (ABC) protein transporter .

Type Il Secretion

The GSP represents the primary route for translocation of polypeptides to the extracellular space among gram-negative bacteria. The type Il secretion mechanism represents the archetype for protein secretion in the GSP, and has been designated the main terminal branch. The type Il pathway is associated with the secretion of virulence factors in several bacterial pathogens. Alternate GSP branches include the secretion of autotransporters (type V secretion), the chaperone/ushermediated assembly of P or type I pili in E. coli, the assembly of type IV pili in P aeruginosa and Neisseria gonorrhea, and the assembly of curli in E. coli . The factors required for extrusion of filamentous bacteriophage from the bacterial envelope also share conserved components with the GSP . One common feature associated with all ofthese strategies is the requirement for the Sec-mediated translocation of secretion substrates to the periplasm. II pathway

therefore represents a two-step translocation process, incorporating distinct secretion reactions for translocation across the inner and outer membranes. The type IIdependent secretion of pullulnase which is lipoprotein of alfa amylase family degradation of complex carbohydrates in *Klebsiella oxytoca* is a wellstudied example of this secretion

Type Ill Secretion

The delivery of polypeptides from the bacterial cytoplasm directly into thecytosol of target host cells without the generation of an extracellular intermediate is the hallmark feature of the type Ill secretion system . Effector proteins that are translocated into host cells harbor enzymatic activities that manipulate cellular processes of the eukaryotic host, resulting in a variety of processes that culminate in perpetuation of the bacterium at the infection site . The type Ill secretion mechanism was first characterized in pathogenic Bacterial Invasins Yersinia species, but has subsequently been identified and extensively studied in vanus-pzthogefifincluding enteropathogenic E. coli (EPEC), P aeruginosa, Salmonella enterica and *Shigella flexneri* . Analysis of genetic information has evolved for type Ill systems in several other gram-negative bacteria, and thus may represent a highly conserved pathogenic strategy . Even though the process of injection of virulence factors by the type Ill pathway is a recently described phenomenon, the type Ill secretion apparatus appears both structurally and functionally similar to the basal body of the flagellar secretion system in gram-negative bacteria. In fact, the flagellar secretion system is now considered a type Ill pathway and recent observations suggest that the flagellar export system may also support the secretion of virulence factors . *Yersinia* species employ the type Ill pathway to maintain an extracellular lifestyle in the lymphoid tissues of their mammalian hosts and cause a variety of diseases ranging from bubonic plague in *Yersinia pestis* to acute enteritis in Y enterocolitica.

TYpe IV Secretion

The type IV secretion mechanism is employed for a wide range of functions in gram-negative bacteria, Several species utilize the type IV mechanism for interbacterial conjugative transfer of mobilized genetic elements. Pathogenic Agrobacterium tumefaciens employs the type IV system for the transfåumorigenic

DNA and protein into host plant cells, and several vertebrate pathogens such as *Brucella* spp., B. pertussis, Helicobacter pylori, and Legionella pneumophila use

modified type IV secretion system for the secretion of toxins or the delivery of effector proteins into the host cell . There is evidence to suggest that the secretion of substrates through the type IV apparatus requires a Sec-dependent translocation step, such as in the secretion of pertussis toxin; however, specialized systems such as those for H. pylori and L. *pneumophila* may bypass this requirement. All type IV systems represent a modification of the conjugative transfer system found in strains of E. coli. In general, the mechanism involves the assembly of a secretion apparatus with a pilus-like projection that will provide intimate contact between the donor and recipient cell . The transfer of DNA from bacterium to host in the pathogen A. tumefaciens represents the archetype for the type IV secretion pathway.

•

Type V secetion pathway autotransporter

The autotransporter pathway represents an alternate branch ofthe GSP that serves as a simplified mechanism for the translocation of substrates out«cell. Analysis of various genomes suggests that the autotransporter mechanismis widely conserved across gram-negative bacteria . Rather than a requirement for factors in the the autotransporter secretion substrate harbors infomqtion in its carboxy-terminus for insertion into the outer membrane, and for translocation of the amino- terminal out of bacteria

Lect 10 bBacterial exotoxin

Exotoxins .

Exotoxins (usually from Gram-positive bacteria) are secreted from viable bacteria and are potent toxins. Some act directly on cells to cause cytolysis; others act via the A-B toxin system and bind to cell membranes with a receptor (B subunit) and deliver a second toxic molecule (A subunit) into the cytoplasm. As examples, A-B toxin systems are used in botulism (*C. botulinum*), tetanus (*C. tetani*), and diseases caused by *Corynebacterium* spp. Vacuolating toxin of Helicobacter *pylori*, *E. coli* hemolysin, and superantigens belonging to *S. pyogenes* and *Staphylococcus aureus* are surface-acting exotoxins. Surface-acting exotoxins bind to cell membranes and form pores through which cell lysis occurs. *S. aureus* also has pore-forming cytotoxins called α-toxin.

Exotoxins, in contrast to endotoxin, are diffusible proteins secreted into the external medium by the pathogen. Most pathogens secrete various protein molecules that facilitate adhesion to, or invasion of, the host. Many others cause damage to host cells. The damage may be physiological, for example cholera toxin promotes electrolyte (and fluid) excretion from enterocytes without killing the cells, or pathological, where the toxin (e.g. diphtheria toxin) inhibits protein synthesis and induces cell death. Exotoxins vary in their molecular structure, biological function, mechanism of secretion and immunological properties. The list of bacterial exotoxins is vast and

increasing; however, they are often classified by their mode of action on animal cells:

- Type I (membrane acting) toxins bind surface receptors and stimulate transmembrane signals, and include the *super-antigenic* toxins.
- Type II (membrane damaging) toxins directly affect membranes, forming pores or disrupting lipid bilayers.
- Type III (intracellular effector) toxins translocate an active enzymatic component into the cell and modify an intracellular target molecule.

Examples of exotoxins and their effects on target cells are shown in Table 1.. Bacteria secrete toxins and other proteins using a number of distinct mechanisms that are understood to varying extents.

Table 1. Some effects of bacterial exotoxins

Toxic effect	**Examples**
Lethal action	
Effect on neuromuscular junction	*Clostridium botulinum* toxin A
Effect on voluntary muscle	Tetanus toxin
Damage to heart, lungs, kidneys, etc.	Diphtheria toxin
Pyrogenic effect	

Toxic effect	Examples
Increase in body temperature and polyclonal T cell activation	Super-antigenic exotoxins of *Staphylococcus aureus* and *Streptococcus pyogenes* (e.g. staphylococcal toxic shock syndrome toxin 1)
Action on gastrointestinal tract	
Secretion of water and electrolytes	Cholera and *Escherichia coli* enterotoxins
Pseudomembranous colitis	*Clostridium difficile* toxins A and B
Bacillary dysentery	Shigella toxin (Shiga toxin)
Vomiting	*Staphylococcus aureus* enterotoxins A–E
Action on skin	
Necrosis	Clostridial toxins; staphylococcal α-toxin
Erythema	Diphtheria toxin; streptococcal erythrogenic toxin

Toxic effect	Examples
Permeability of skin capillaries	Cholera enterotoxin; *E. coli* heat-labile toxin
Nikolsky sign[a]	*Staphylococcus aureus* epidermolytic toxin
Cytolytic effects	
Lysis of blood cells	*Staphylococcus aureus* α-, β- and δ-lysins; leucocidin Streptolysin O and S *Clostridium perfringens* α and perfringolysin (θ toxins)
Inhibition of metabolic activity	
Protein synthesis	Diphtheria toxin; shiga toxin

.

Enterotoxins cause symptoms of gastrointestinal disease, including diarrhoea, dysentery and vomiting. In some cases the disease is caused by ingestion of preformed

toxin in food, but in most cases colonization of the intestine is required before toxin is made.
Cholera toxin and heat-labile toxins of enterotoxigenic *E. coli* (ETEC) do not induce inflammatory changes in the intestinal mucosa, but perturb the processes that regulate ion and water exchange across the intestinal epithelium). In contrast, the enterotoxins of *Clostridium difficile*, C. perfringens type A and *Bacillus cereus* cause structural damage to epithelial cells, resulting in inflammation. Another gastrointestinal pathogen, enteropathogenic *E. coli* (EPEC), mediates damage by a process that involves secretion of a protein.
B. pertussis, the causative agent of whooping cough, produces various extracellular products, including a tracheal cytotoxin that inhibits the beating of cilia on tracheal epithelial cells, pertussis toxin, which exhibits several systemic effects, and an adenylate cyclase that interferes with phagocyte function.
Another group of toxins causes damage to subepithelial tissues following penetration and multiplication of the pathogen at the site of infection. Many of these toxins also inhibit or interfere with components of the host immune system. Membrane-damaging toxins such as staphylococcal α and β toxins, streptolysin O and streptolysin S, and *C. perfringens* α and θ toxins inhibit leucocyte chemotaxis at subcytolytic concentrations, but cause necrosis and tissue damage at higher concentrations.

Direct toxicity caused by exotoxins.

Different exotoxins cause disease by different means and in different locations, depending on the proclivities of the individual bacterial species
. For example, infection with *V. cholerae* results in the local release of an exotoxin that binds to gut epithelial cells. A massive release of

electrolytes and tissue fluids is induced that is manifested as the severe diarrhea that characterizes cholera.

Although they are derived from extracellular bacteria, many bacterial exotoxins have the ability to translocate into mammalian cells and wreak havoc on intracellular processes. The diphtheria exotoxin secreted by *Corynebacterium* diphtheriae travels the body systemically and is absorbed by cells of the heart and peripheral nervous system. The toxin inhibits protein synthesis in these cells, leading to myocarditis and neuritis. The diphtheria exotoxin also promotes colonization of the throat by the bacterium, which provokes an acute inflammatory response resulting in severe respiratory obstruction. As mentioned previously, *C. botulinum* produces a neurotoxin that blocks the transmission of nerve impulses to the muscles, resulting in paralysis. This toxin is much feared as a potential biological weapon because a dose of less than 1 µg is fatal to humans.

Another *Clostridium* species, *Clostridium tetani*, synthesizes a neurotoxin that causes uncontrollable muscle contractions. Other exotoxins trigger specific host cell necrosis, such as the leukocidin produced by *S. aureus* that is toxic to granulocytes. Another example is the exotoxin produced by *E. coli* O157:H7, which causes severe hemorrhaging because it blocks protein synthesis within vascular endothelial cells and kills them. *B. anthracis* produces two exotoxins called *lethal toxin* and *edema toxin* that damage phagocytes in an unknown way. Lethal toxin is composed of a zinc protease called *lethal factor* and a protein called *protective antigen*, while edema toxin is composed of an adenylate cyclase called *edema factor* plus protective

Diphtheria Toxoid

The exotoxin produced by C. *diphtheriae* is by far the most important pathogenic factor associated with the organism. The extensive study of the biology of diphtheria toxin has pioneered many biomedical developments over the past century. The basic biology of diphtheria toxin, including its production and actions, has become reasonably well understood, although some gaps remain.[41,42]
The ability of strains *C. diphtheriae* to produce toxin results from a nonlytic infection by one of a series of related bacteriophages that contain a genetic sequence encoding the toxin. The phage integrates into specific sites present in *C. diphtheriae* and other *Corynebacterium* species. The presence of the phage is thought to confer a survival advantage to the bacterium by increasing the probability of transmission in a susceptible population; transmission may be facilitated by local tissue damage resulting from the toxin.[43,44] The sequence of diphtheria toxin has been demonstrated to be highly conserved in *C. diphtheriae* strains, suggesting that immunologically important differences among the toxins produced by different strains are unlikely to occur.
Diphtheria toxin is a polypeptide with a molecular weight of approximately 58,000 Da. The toxin is secreted as a proenzyme, requiring enzymatic cleavage into two fragments (fragments A and B) to become active. Fragment B is responsible for attachment to and penetration of the host cell. Although nontoxic by itself, fragment B appears to be the antigen responsible for clinical immunity. The receptor domain of fragment B binds to a cell surface receptor, heparin-binding epidermal growth factor precursor,[48] with CD9 as a coreceptor.[49] After receptor-mediated endocytosis and penetration of the cell, fragments A and B are detached.[50] The released fragment A is the toxic moiety,

and it acts by inhibiting protein synthesis, resulting in cell death.

Group A Streptococcal and Staphylococcal Infections

Pyrogenic Exotoxins A, B, C, MF, and SSA

The pyrogenic exotoxins A, B, C, MF, and SSA, also called scarlatina toxins and erythrogenic toxins, induce lymphocyte blastogenesis, potentiate endotoxin-induced shock, induce fever, suppress antibody synthesis, and act as superantigens.

The gene for streptococcal pyrogenic exotoxin A (*speA*) is transmitted by bacteriophage,[39] and stable toxin production depends on lysogenic conversion in a manner analogous to that for diphtheria toxin production by *Corynebacterium diphtheriae*. Factors controlling SPEA production are not fully understood, though the quantity of SPEA produced can vary dramatically from decade to decade. Historically, SPEA-producing strains have been associated with severe cases of scarlet fever and, more recently, with Strep TSS.

Although all GAS possess the gene for SPEB (*speB*), the quantity of toxin produced varies greatly and its regulation is highly complex. Several different roles in pathogenesis have been postulated for SPEB, mostly based on its *in vitro* proteolytic and superantigenic activities. SPEB cleaves pre-IL-1β to release biologically active IL-1β, activates matrix metalloproteases that disrupt endothelial integrity and induce apoptosis, and cleaves fibronectin, vitronectin, immunoglobulin, and serum properdin such that bacterial survival and dissemination may be facilitated. SPEB also cleaves the terminal portion of M protein, rendering the organism more susceptible to phagocytosis by normal serum but

protecting it from opsonization by M-type-specific antibody. SPEB, like other superantigens, induces production of both monokines and lymphokines that mediate fever, shock, and tissue injury. However, proteolytic cleavage of some superantigens by SPEB reduces their proliferative and immunomodulatory activities. Despite this panoply of activities, SPEB's role in pathogenesis remains to be established with certainty. For instance, some studies have shown that insertional inactivation of *speB* decreases mouse lethality[41] while others using a similar approach failed to demonstrate a role for *speB* in virulence.[42] This discrepancy remains to be resolved.

Pyrogenic exotoxin C (SPEC), like SPEA, is bacteriophage-mediated and its expression is likewise highly variable. Some scientists was found mild cases of scarlet fever in England have been associated with strains of GAS producing SPEC.

Streptococcal superantigen (SSA) was described in an M type 3 GAS strain isolated from a patient with Strep TSS.SSA resembles the other pyrogenic toxins, though its distribution and its role in pathogenesis have not been defined.

Mitogenic factor (MF) has all the features of other pyrogenic exotoxins, is a potent inducer of cytokines and lymphokines, and has been found in most strains of GAS examined.

Many other GAS superantigens have been described, though their roles in pathogenesis remain unknown.[46]