Semester VI

CC 13 (MEDICAL MICROBIOLOGY)

UNIT : 1 – NORMAL MICROFLORA OF HUMAN BODY AND OST PATHOGEN INTERACTION

Types of normal flora:

1. Commensal microflora:
2. Mutualistic microflora:
3. Opportunistic microflora:

Commensal microflora:

- These microorganisms gets benefits from human host and give neither benefits nor harmful effects to host.
- Majority of normal flora are commensal types

Mutualistic microflora:

- These microorganisms gets benefits from host and at the same time give benefits to the host.

Opportunistic microflora:

- These microorganisms, under normal condition live as commensal on host body but they causes diseases if opportunity is available.
- For example; Normal flora of GI tract ie. E.coli causes urinary tract infection (UTI), if the site of habitat is changed.

Role of normal flora:

Beneficial role of normal flora;

1. **Prevent attachment and penetration of pathogenic microorganisms:** Normal flora prevent attachment and penetration of

pathogenic microorganism through skin and other tissue as they occupy the area. Some normal flora produces mucin and make the surface slippery so that pathogenic microorganism cannot attach to cause disease.

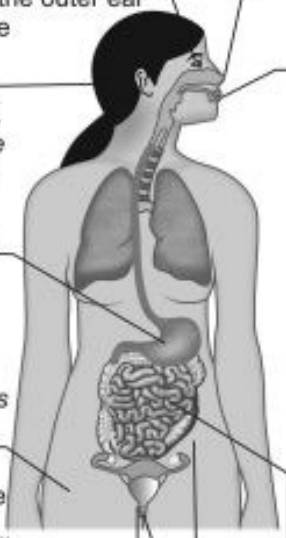
2. **Compete with pathogenic microorganisms:** Microflora compete with pathogenic bacteria for habitat and nutrition.
3. **Produces antibiotics:** Some normal flora produces antimicrobial chemicals (antibiotics) that kills pathogenic microorganism. For eg, *E. coli* produce Cloicine in intestine of human that kills many pathogenic bacteria.
4. **Immunity:** Antibodies produces against normal flora can neutralize pathogenic microorganism and prevent infection.
5. **Produces enzymes and vitamins:** Some intestinal normal flora produces useful substance for host such as vitamins and digestive enzymes. Eg. *E. coli* produces Vitamin B12 and Vitamin K.
6. **Helps in metabolism:** Intestinal normal flora produces enzymes such as cellulose, galactosidase, glucosidase etc and helps in digestion of food.
7. **Oxidation and hydrolysis of steroids:** Intestinal microflora also helps in metabolism of steroids. For eg. Bacteria in intestine carry out oxidation and hydrolysis of steroid ring of bile salt.

Harmful effects of normal flora:

1. **opportunistic infection:** Normal flora may causes opportunistic infection when immunity of host become weak or if normal flora of one tissue migrates to other habitat. For eg. If E. coli of GI tract migrates to Urinary tract it causes UTI.

Table 1. List of opportunistic flora activated in immunocompromised patients (combined of different studies)

Predominant location sites in immunocompetent subjects				
Oral cavity/ oropharynx	Skin/upper airways	Gut-associated	Urogenital	Lymph nodes, blood cells
Streptococcus haemolyticus *Staphylococcispp.* *M.avium* *M.bovis*	*Klebsiella pneumoniae;* *Pseudomonas aeruginosa* *Acinetobacter baumanni* *Staph. Epidermidis* *Staph. hominis* *Coagulase-negative staphylococci,* *Staphylococcus aureus* *Streptococcus pneumoniae,* *Streptococcus pyogenes*	*E.Coli* *Salmonella* *Enterobacter Enterococcus faecalis* *Enterococcus faecium* *Stenotrophomonas maltophilia* *Clostridium difficile* *Lactobacillus spp.* *Toxoplasma gondii*	*Proteus mirabilis* *Cor. urealytica* *Mycoplasma hominis* *Toxoplasma gondii*	*Bartonella spp.*
	Candida spp *Malassezia* *Aspergillus spp* *Mucor spp* *Pneumocystis jiroveci*		*Candida spp*	
	Herpes simplex virus Varicella zoster virus	Cytomegalovirus Adenovirus	Polyomavirus: BK; JC	Cytomegalovirus Epstein-Barr virus Adenovirus Human T lymphocyte virus type 1



Normal microbiota of the conjunctiva
1. Coagulase-nagative *Staphylococci*
2. *Haemophilus* spp.
3. *Staphylococcus aureus*
4. *Streptococci* (various species)

Normal microbiota of the outer ear
1. Coagulase-nagative *Staphylococci*
2. *Diphtheroids*
3. *Pseudomonas* spp.
4. *Enterobacteriaceae (Peptostreptococcus)*

Normal microbiota of the stomach
1. *Streptococcus*
2. *Staphylococcus*
3. *Lactobacillus*
4. *Peptostreptococcus*

Normal microbiota of the skin
1. Coagulase-nagative *Staphylococci*
2. *Diphtheroids* (including *Propionibacterium acnes*)
3. *Staphylococcus aureus*
4. *Streptococci* (various species)
5. *Bacillus* spp.
6. *Malassezia furfur*
7. *Candida* spp.
8. *Mycobacterium* spp. (occasionally)

Normal microbiota of the urethra
1. Coagulase-nagative *Staphylococci*
2. *Diphtheroids*
3. *Streptococci* (various species)
4. *Mycobacterium* spp.
5. *Bacteroides* spp. and *Fusobacterium* spp.
6. *Peptostreptococcus* spp.

Normal microbiota of the vagina
1. *Lactobacillus* spp.
2. *Peptostreptococcus* spp.
3. *Diphtheroids*
4. *Streptococci* (various)
5. *Clostridium* spp.
6. *Bacteroides* spp.
7. *Candida* spp.
8. *Gardnerella vaginalis*

Normal microbiota of the nose
1. Coagulase-negative *Staphylococci*
2. *Viridans streptococci*
3. *Staphylococcus aureus*
4. *Neisseria* spp.
5. *Haemophilus* spp.
6. *Streptococcus pneumoniae*

Normal microbiota of the mouth and oropharynx
1. *Viridians streptococci*
2. Coagulase-negative *Staphylococci*
3. *Veillonella* spp.
4. *Fusobacterium* spp.
5. *Treponema* spp.
6. *Porphyromonas* spp. and *Prevotella* spp.
7. *Neisseria* spp. and *Branhamella catarrhalis*
8. *Streptococcus pneumoniae*
9. Beta-hemolytic *Streptococci* (not group A)
10. *Candida* spp.
11. *Haemophilus* spp.
12. *Diphtheroids*
13. *Actinomyces* spp.
14. *Ekenella corrodens*
15. *Staphylococcus aureus*

Normal microbiota of the small intestine
1. *Lactobacillus* spp.
2. *Bacteroides* spp.
3. *Clostridium* spp.
4. *Mycobacterium* spp.
5. *Enterococci*
6. *Enterobacteriaceae*

Normal microbiota of the large intestine
1. *Bacteroides* spp.
2. *Fusobacterium* spp.
3. *Clostridium* spp.
4. *Peptostreptococcus* spp.
5. *Escherichia coli*
6. *Klebsiella* spp.
7. *Proteus* spp.
8. *Lactobacillus* spp.
9. *Enterococci*
10. *Streptococci* (various species)
11. *Pseudomonas* spp.
12. *Acinetobacter* spp.
13. Coagulase-negative *Staphylococci*
14. *Staphylococcus aureus*
15. *Mycobacterium* spp.
16. *Actinomyces* spp.

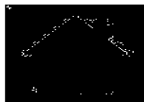
Normal flora of the skin

- Staphylococcus epidermidis
- Staphylococcus aureus (in small numbers)
- Alpha-hemolytic and nonhemolytic Streptococcus
- Micrococcus species
- Peptostreptococcus species
- Neisseriae species (nonpathogenic)
- Propionibacterium species
- Diphtheroids
- Candida species (small numbers)
- Acinetobacter species (small numbers)

Occurence	Type	Bacteria	Dominant species
Moist and dry areas of skin	staphylococci micrococci	aerobic gram-positive cocci	staphylococcus epidermidis et hominis
Moist and dry areas of skin	coryne bacteria brevi bacteria	aerobe coryneforme rods	
Hair follicles with many sebaceous glands	proprioni bacteria	anaerobic coryneforme rods	proprionibacterium acnes
Hair follicles with many sebaceous glands	malassezia furfur	yeast fungi	pityrosporum ovale

Normal respiratory microflora

- **Nasal cavity** has no specific microflora, there is skin microflora (frontal part) and pharyngeal microflora (back part)
- **In pharynx** (and also oral cavity) we can find oral streptococci, Neisserias, non-virulent strains of haemophili etc. Many other strains are also present, but we cannot culture them.
- **Lungs and lower respiratory ways** use to be nearly microbes-free in a healthy person
- **Other sites** (larynx) have transient microbes (larynx – like pharynx, but less microbes)



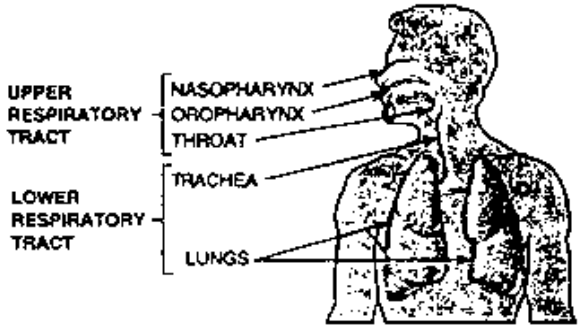
Normal microflora – Nose and nasopharynx

The nasopharynx of the infant is **sterile at birth** but in 2-3 days time it acquires the flora.

The nasopharynx is a ***natural habitat of the common pathogenic bacteria*** causing infection of the nose, throat, bronchi and lungs.

The flora of nose harbours

- *Diptheroids*
- *Straphylococcus*
- *Streptococcus*
- *Haemophilus*, and
- *Moraxella lacunata*



OROPHARYNX AND TONSILS

- ANAEROBES > AEROBES (100:1)
- PEPTOSTREPTOCOCCUS SPP
- VEILLONELLA
- ACTINOMYCES
- FUSOBACTERIUM
- STREPTOCOCCUS
- NEISSERIA
- STOMATOCOCCUS MUCILAGINOSUS
- GEMELLA SPECIES

Lower respiratory tract is usually sterile mainly because of the efficient cleansing action of the ciliated epitheliaum.

Normal flora of Gastrointestinal tract

- GI tracts consists of stomach, small intestine and large intestine. Various parts of GI tract differ in their environmental characteristics, chemical compositions and physiological properties. Therefore types and number of microflora vary in different parts.
- In general number of microorganisms increases from stomach to small intestine to large intestine.

Normal flora of stomach:

- Stomach receive large number of microorganism from mouth along with food and water but antimicrobial activity of HCl kills most of them. Few microorganisms that can tolerate acidity of stomach can form resident normal flora of stomach.
- Examples: *lactobacillus*, *Candida albicans*, *Helicobacter pylori*, *lactobacillus*, *Enterococcus* etc
- Number of microorganisms in stomach increases immediately after ingestion of food but number soon decreases after gastric juice is secreted.

Normal flora of small intestine:

- Duodenum is adjacent to stomach and hence it is slightly acidic in nature. Therefore microorganisms in duodenum is similar to that of stomach. Mainly *Lactobacillus* and *Enterococcus* are found in deuodenum.
- From duodenum ileum, intestine become less acidic and hence number of microorganism increases.
- In jejunum *Enterococci*, *lactobacillus*, Diphtheroid and *Candida albican*s are found.
- In Ileum microorganism begins to resemble to that of large intestine. Mainly obligate anaerobes such as *Clostridium perfinges*, Bacteroides and anaerobic E. coli are found.

Normal flora of large intestine:

- Large intestine is anaerobic in nature. It contains obligate anaerobes and facultative anaerobes.
- C*lostridium perfingens, Bifidobacterium, Bacteroides, Streptococcus fecalis, E. coli*

Role of Intestinal normal flora:

1. Synthesize vitamin B12 and vitamin K
2. Produces various carbohydrate metabolizing enzymesand helps in food digestion. Eg. Cellulase, glucosidase, galactosidase
3. Helps in steroid metabolism
4. Produces gases such as CH4 and CO2
5. Produces other substances such as Indole, Skatole, butyric acid etc.

Normal flora of urogenital tract

	Men	Women
Urogenital microbial normal flora	*Aerococcus, Anaerococcus, Atopobium, Corynebacterium, Enterococcus, Escherichia, Finegoldia, Gardnerella, Gemella, Klebsiella, Lactobacillus, Mycoplasma, Prevotella, Sneathia, Staphylococcus, Streptococcus, Ureaplasma, Veillonella*	*Actinobacteria, Actinobaculum, Actinomyces, Aerococcus, Allisonella, Alloscardovia, Anaerococcus, Anoxybacillus, Arthrobacter, Atopobium, Bacteroidetes, Bifidobacterium, Burkholderia, Corynebacterium, Dialister, Enterobacteriaceae, Enterococcus, Escherichia, Finegoldia, Gardnerella, Klebsiella, Lactobacillus, Peptoniphilus, Prevotella, Ralstonia, Rhodanobacter, Shigella, Sneathia, Staphylococcus, Streptococcus, Trueperella, Veillonella*

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.

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

ENTRY:

Sites of Entry / Cause / S/S

Ingestion into gastrointestinal tract;

- microorganisms contaminating food or water
  - Salmonella, Vibrio cholera
- S/S abdominal pain, nausea, vomiting, diarrhea

Inhalation into respiratory tract;

- microorganisms in air
- S/S cough, chest pain, shortness of breath, coughing blood

Ascension into urinary tract;

- microorganisms that enter bladder through urethra or catheter
- S/S painful urination, blood in urine, pelvic pain, flank pain

Ascension into biliary tree

- microorganisms entering common bile duct from GI tract Black (2008)
- S/S abdominal pain, jaundice

Sites of Entry / Cause / S/S

Crossing of mucosal surfaces

- microorganisms that penetrate oral, anal, genital, or conjunctival linings
  - S/S Human papillomavirus, HIV, herpes simplex virus, Neisseria gonorrhea
- Experience local irritation, ulceration, pain, redness

Entrance through wound sites

- Direct inoculation of micro-organisms leads to direct spread

COLONIZATION

Colonization is the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

Mechanisms of Adherence to Cell or Tissue Surfaces

The mechanisms for adherence may involve two steps:

1. **nonspecific adherence: reversible attachment** of the bacterium to the eucaryotic surface (sometimes called "docking")

2. **specific adherence: irreversible permanent attachment** of the microorganism to the surface (sometimes called "anchoring").

The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur.

Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eucaryotic cell surface. Possible interactions and forces involved are:

1. hydrophobic interactions

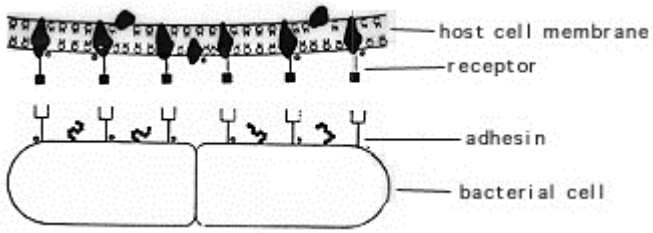
2. electrostatic attractions

3. atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies

4. Brownian movement

5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.



Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

Several types of experiments provide direct evidence that receptor and/or adhesin molecules mediate specificity of adherence of bacteria to host cells or tissues. These include:

1. The bacteria will bind isolated receptors or receptor analogs.

2. The isolated adhesins or adhesin analogs will bind to the eucaryotic cell surface.

3. Adhesion (of the bacterium to the eucaryotic cell surface) is inhibited by:

    a. isolated adhesin or receptor molecules

    b. adhesin or receptor analogs

    c. enzymes and chemicals that specifically destroy adhesins or receptors

    d. antibodies specific to surface components (i.e., adhesins or receptors)

EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexos-amine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	Type IV pili (N-methylphenyl-alanine pili)	Glucosamine-galactose carbohydrate	Urethral/ cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-I fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type I fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenyl-alanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera

Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

Pathogens (Pathogenic microorganisms) cause diseases and they may be:
(a) Opportunistic

(b) True pathogens.

Opportunistic Pathogens:
Many commensal or non-pathogenic microorganisms may be transmissible from person to person or derived from the environment and are present, in large numbers, on the skin, in the upper respiratory tract, in the intestine and lower urinogenital tract hence they are normal micro flora of the body and sometimes they may act against invading pathogenic microorganisms and are unable to invade the tissues as they cannot overcome the healthy body defences.

Sometimes, when the body defence mechanism is lowered and when these commensals leave their natural habitat and reach other parts of the body, e.g., coliform bacilli (Escherichia coli) are mostly harmless commensals in the intestine, but they may cause infection in the urinary tract; similarly Clostridium welchii, an intestinal commensal, can cause gangrene in locally damaged tissues; Streptococcus viridians is the commensal of the mouth; after tooth extraction they may invade

the blood stream and settle on previously damaged heart valves as opportunistic pathogens.

True Pathogens:
They are those microorganisms which are able to overcome the normal body defence mechanism and initiate the infection.

Several properties are essential for pathogenicity of microorganisms:
(a) Transmissibility:
The ability of a pathogen to grow profusely in the body and to be shed in large numbers in body fluids or secretions which are capable of dissemination and reach new host after surviving in the adverse conditions, e.g., desiccation in the dry dust.

(b) Infectivity:
Pathogenic microbes are able to initiate the infection by penetrating the healthy body's first line of defence, that is, skin, mucous membranes to which they readily gain access. To infect a person, only a few of the pathogens can cross the protective barriers in the respiratory and alimentary tracts. The pathogen may initiate a localised lesion at the site of infection, e.g., staphylococcal boil on the skin or streptococcal pharyngitis in the throat.

The capacity of the microbes to initiate the infection is mostly related to the dosage of the pathogen, its phase of growth and its virulence factors. In salmonella family, the infecting dose of Salmonella typhi is very small; whereas large number of S. typhimurium (food poisoning salmonellae) must be ingested to produce acute vomiting and diarrhoea.

Microorganisms which are in the logarithmic stage of growth are more likely to overcome host resistance than those in the latent phase: Streptococcus pyogenes is more infective when transferred directly from a person with a sore throat than when it is inhaled after drying in dust particles, because Strepto pyogenes has the capsular M protein

(anti-phagocytic component) during the active phase of sore throat infection.

Virulence:

The virulence of a pathogen is the ability to kill susceptible animals (mouse, guinea pig etc.).

Virulence factors refer to the properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease. Virulence factors include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium, and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium.

Function	Virulence factors
Attachment	Adhesins (fimbriae, afimbrial surface proteins) Exopolysaccharides Lipoteichoic acid Outer membrane proteins Outer membrane vesicles
Invasion	Flagella Enzymes (collagenase, hyaluronidase, chondroitin sulfatase, fibrinolysin, acid phosphatase, and Dnase)
Survival (evasion of host defenses or acquisition of nutrients)	Exopolysaccharides (capsule) IgA, IgG, IgM, C3, and C5 proteinases Lipopolysaccharide (antigen-O portion) Flagella Exotoxins Heat-shock proteins Metabolic end-products
Direct damage	Exotoxins Enzymes (collagenase, hyaluronidase, chondroitin sulfatase, gingipains, aminopeptidases, phospholipase, neuraminidase, and acid phosphatase) Metabolic end-products (short-chain fatty acids, polyamines, volatile sulfur compounds, indole, ammonia)
Indirect damage	Lipopolysaccharide (mainly lipid A portion) Peptidoglycan Lipoteichoic acid Fimbriae Exopolysaccharides Outer membrane proteins (porins) Lipoproteins DNA Heat-shock proteins

Exoenzymes

Some pathogens produce extracellular enzymes, or **exoenzymes**, that enable themto invade host cells and deeper tissue

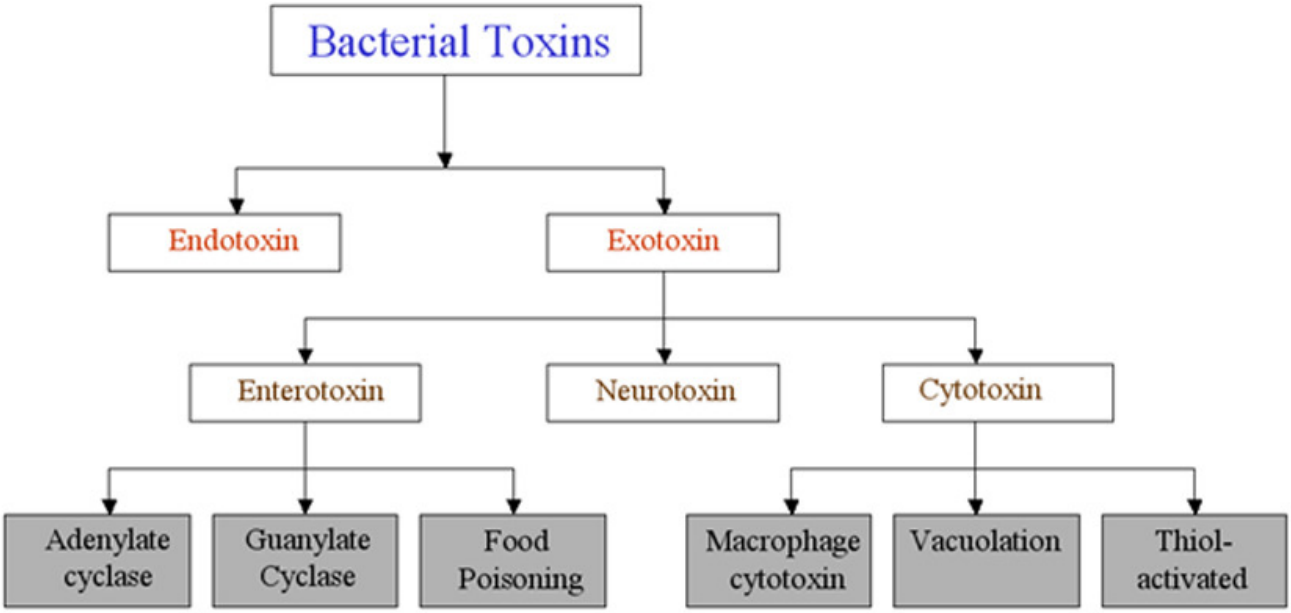
Some Classes of Exoenzymes and Their Targets		
Class	**Example**	**Function**
Glycohydrolases	Hyaluronidase S in *Staphylococcus aureus*	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by *S. aureus*	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread
Phospholipases	Phospholipase C of *Bacillus anthracis*	Degrades phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm
Proteases	Collagenase in *Clostridium perfringens*	Degrades collagen in connective tissue to promote spread

Virulence factors of fungi:

VIRULENCE FACTORS	FUNCTIONS	FUNGAL PATHOGENS
A. SURFACE COMPONENTS		
a. Cell wall glycoproteins	Adherence to epithelial surfaces	Candida species
b. Melanin pigment	Shield against immunologically active cells, hydrolyses	Cr. Neoformans W. dermatitidis
c. Capsules, glucans	Anti - phagocytic	Cr. neoformans
B. Thermotolerance	Survive and replicate at 37°C	Human pathogens
C. Resistance to microbiocidal products of neutorophils e.g. H_2O_2, by dimorphic primary pathogen	Evasion of host defence mechanisms by tissue phase (yeast, spherule) of virulent dimorphic fungi	Primary pathogens Blastomyces, Coccidiodes, Histoplasma, Paracoccidiodes, Sporothrix schenkii
D. Epithelial cell and monocyte cytocidal activity	Evasion of host defences	Candida albicans Candida tropicalis

VIRULENCE FACTORS	FUNCTIONS	FUNGAL PATHOGENS
E. Exoenzymes		
a. Elastase	Degrades elastin, scleroproteins, enhances invasion of elastin containing tissue (lung, skin, blood vessels)	Aspergillus flavus, A. Fumigatus Dermatophytes
b. Alkaline protease	Degrades collagen, elastin, enhances invasion of lung tissue	Aspergillus flavus, A. Fumigatus Rhizopus spp.
c. Keratinase, collagenases	Degrades scleroproteins in skin	Dermatophytes
d. Acid protease	Cleavage of IgA_2	Candida spp., A. Fumigatus
F. Toxins		
a. Aflatoxin	Hepatotoxicity	Aspergillus flavus
b. Endotoxin	Tissue necrosis	A. Flavus, A. Fumigatus
G. Dimorphism	Evasion of host defences Environmental and tissue forms present different and surface features, requiring different host response of mechanisms to contain each form	True pathogens Opportunistic pathogens

Bacterial toxins may be (a) exotoxins or (b) endotoxins



Exotoxins are usually secreted by bacteria and act at a site removed from bacterial growth. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides, that act enzymatically or through direct action with host cells and stimulate a variety of host responses. Most exotoxins act at tissue sites remote from the original point of bacterial invasion or growth. However, some bacterial exotoxins act at the site of pathogen colonization and may play a role in invasion.

Most bacterial exotoxins are proteins, and they may be subdivided into three groups based on their site of action:

(a) Type I toxins do not enter the target cell. They trigger a harmful response by binding to a receptor on the cell surface. For example, heat-stable toxin a (STa) is made by some pathogenic strains of *E. coli* that cause diarrhea. STa is a hormone analog that binds to guanylate cyclase in the animal cell membrane, causing overproduction of cyclic GMP.

(b) Type II toxins act on the cell membrane of the target cell. Some degrade membrane lipids, and others create pores in the membrane. For example, hemolysin A is made by some pathogenic strains of *E. coli* that cause kidney damage. Hemolysin A was named for its ability to lyse red blood cells, but it disrupts the membranes of many other types of animal cells also.

(c) Type III toxins enter the target cell. They consist of a toxic factor (A-protein) together with a delivery system (B-protein). The A ("active") and B ("binding") proteins may be separate molecules or may be different domains of a single protein. Sometimes the delivery system consists of multiple B-subunits and, conversely, cases are known where multiple A-proteins share the same delivery system

Exotoxins are often given the name of the disease they produce (e.g., tetanus toxin, diphtheria toxin, botulinum toxin) and can be classified into four types based on their structure and physiologjcal activities.

These types are:

(i) AB exotoxins affecting general tissues,

(ii) AB exotoxins affecting specific host sites,

(iii) Membrane-disrupting exotoxins

(iv) Superantigen exotoxins.

AB exotoxins affecting general tissues	AB exotoxins affecting specific host sites	Membrane-disrupting exotoxins	Superantigen exotoxins
Exotoxins that affect general tissues of the host are composed of two covalently bonded subunits, A and B. The B subunit binds to a cell surface receptor, allowing the transfer of the A subunit across the targeted cell memberane, where it functions to damage the cell. Isolated A subunits are enzymatically active but lack binding and cell entry capability, whereas isolated B subunits possess binding	There are various exotoxins, mostly AB exotoxins, that affect the specific sites in the body of the host by acting extracellularly or intracellularly. **These exotoxins can be categorized as:** (i) Neurotoxins (e.g., tetanus toxin, botulinum toxin), (ii) Enterotoxins (e.g., cholera toxin, E. coli heat-labile toxins), and	Some exotoxins lyze host cells by disrupting the integrity of the plasma membrane. **There are two subtypes of membrane-disrupting exotoxins:** (i) Protein exotoxins and (ii) Enzyme exotoxins.	Superantigens are the antigens that provoke drastic immune response. Certain exotoxins function as superantigen and hence called superantigen exotoxins. Superantigen exotoxins act indirectly on host cells, using a novel immune mechanism to cause extensive host tissue damage. They directly stimulate large number of immune response cells resulting in extensive inflammatory

capability but are nontoxic and biologically inactive.	(iii) Cytotoxins (e.g., nephrotoxin. hepatotoxin, cardiotoxin).		reactions.
Example : Diphtheria toxin			

Types of AB toxins affecting specific host site :

Cytotoxin	**Neurotoxins**	**Enterotoxins**
Cytotoxins are the AB toxins that act upon cells/tissues of specific organs in victim's body and are designated as per the cell/tissue or organ for which they are specific.	Neurotoxins target the cells of central nervous system and usually are ingested as performed toxins.	Enterotoxins (G. enter = intestine) are the exotoxins whose activity directly affects the mucosa of small intestine generally causing profuse secretion of fluid into the intestinal luman.
Examples of cytotoxins are nephrotoxin (kidney), hepatotoxin (liver), and cardiotoxin	e.g., tetanus toxin, botulinum toxin	e.g cholera toxin

(heart).		

Two subtypes of membrane-disrupting exotoxins:

Protein exotoxins	Enzyme exotoxins.
Various pathogenic bacteria produce proteins that disrupt the host plasma membrane causing cell lysis and death. Some such proteins are leucocidins and haemolysins. Leucocidins kill phagocytic leucocytes and most of them are produced by pneumonococci, streptococci, and staphylococci bacteria.	Some membrane-disrupting exotoxins are the phospholipasc enzymes. These enzymes attack the phospholipid of the cell plasma membrane by removing the charged head group from the lipid portion of the phospholipids An example of phospholipase enzyme is the α-toxin secreted by Clostridium perfringens to cause gas gangrene disease.

Examples of AB type of bacterial toxins:

Toxin and Organism	Structure and Mode of Action
Cholera toxin *Vibrio cholerae*	AB_5 ADP-ribosylation of G-protein that controls adenylate cyclase
Pertussis toxin *Bordetella pertussis*	$A'B'_5$ (five B-subunits are nonidentical) ADP-ribosylation of G-protein that controls adenylate cyclase
Shiga toxin *Shigella*	AB_5 Removal of adenine in 28S rRNA; inhibition of protein synthesis
Diphtheria toxin *Corynebacterium diphtheriae*	A-B (A = N-terminal domain) ADP-ribosylation of translation factor EF2; inhibition of protein synthesis
Exotoxin A *Pseudomonas aeruginosa*	A-B (A = C-terminal domain) ADP-ribosylation of translation factor EF2; inhibition of protein synthesis
Botulinum toxin *Clostridium botulinum*	A-B Zn-dependent protease that cleaves proteins in nerve synapses so blocking transmission in peripheral nerves
Edema toxin *Bacillus anthracis*	$A\text{-}B_7$ (edema factor plus protective antigen) Adenylate cyclase activity
Lethal toxin *Bacillus anthracis*	$A\text{-}B_7$ (lethal factor plus protective antigen) Protease that cleaves mitogen-activated protein kinase kinases (MAPKKs)

Endotoxins are cell-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. In the context of this article, endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) located in the outer membrane of Gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics. Endotoxins generally act in the vicinity of bacterial growth or presence.

Cholera toxin

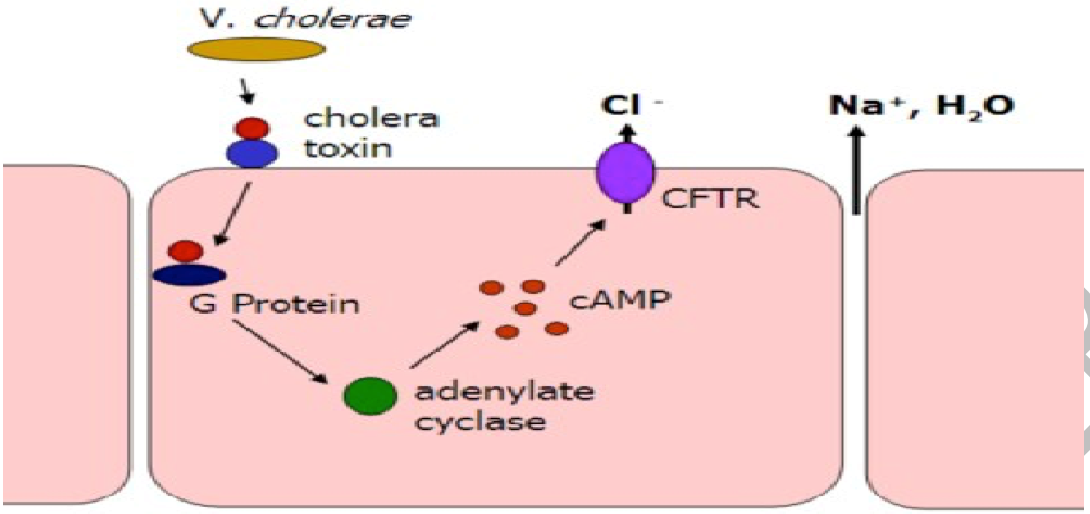
Cholera toxin **activates the adenylate cyclase enzyme** in cells of the intestinal mucosa leading to increased levels of intracellular cAMP, and the secretion of H_20, Na^+, K^+, Cl^-, and HCO_3^- into the lumen of the small intestine. The effect is dependent on a specific receptor, monosialosyl ganglioside (GM1 ganglioside) present on the surface of intestinal mucosal cells. The bacterium produces an invasin, neuraminidase, during the colonization stage which has the interesting property of degrading gangliosides to the monosialosyl form, which is the specific receptor for the toxin.

The toxin has been characterized and contains **5 binding (B) subunits** of 11,500 daltons, an active **(A1) subunit** of 23,500 daltons, and a **bridging piece (A2)** of 5,500 daltons that links A1 to the 5B subunits. Once it has entered the cell, the A1 subunit enzymatically transfers ADP ribose from NAD to a protein (called Gs or Ns), that regulates the adenylate cyclase system which is located on the inside of the plasma membrane of mammalian cells.

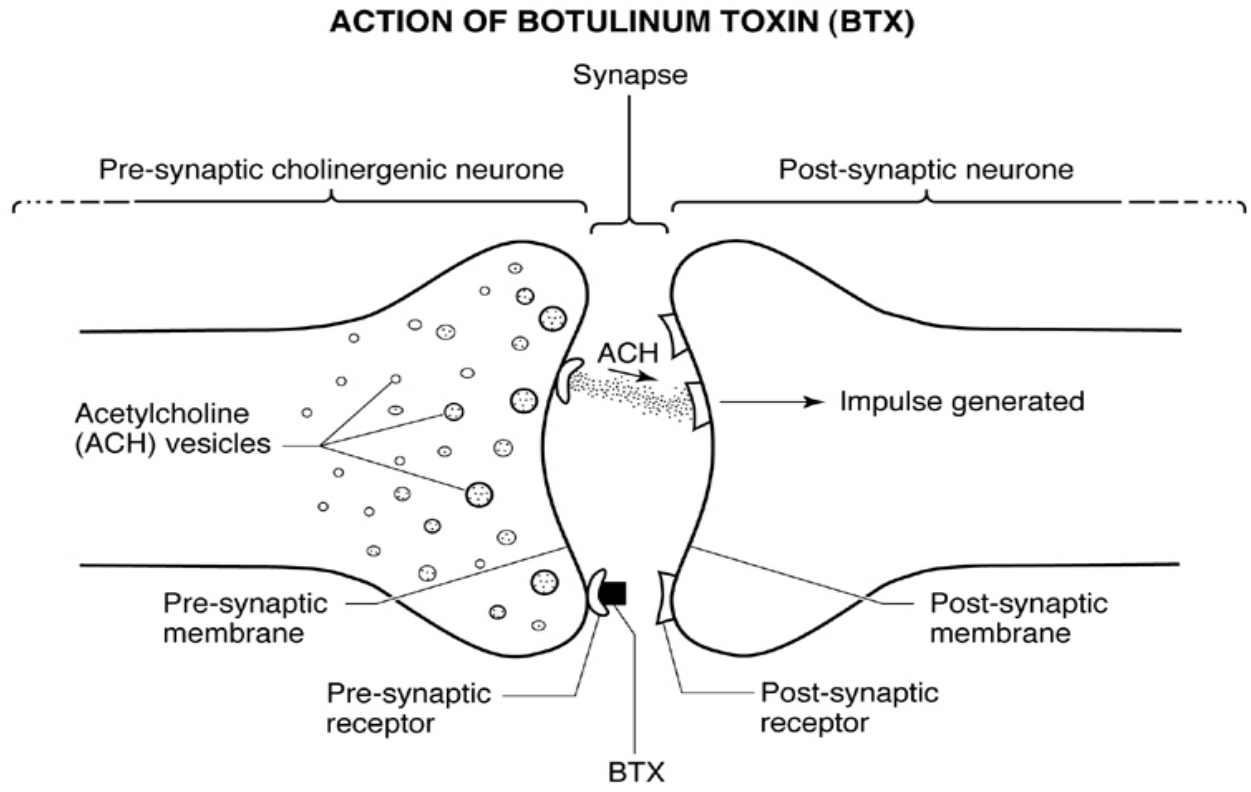
Enzymatically, fragment A1 catalyzes the transfer of the ADP-ribosyl moiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP; however activation is normally brief because another regulatory protein (Gi), hydrolyzes GTP. The normal situation is described as follows.

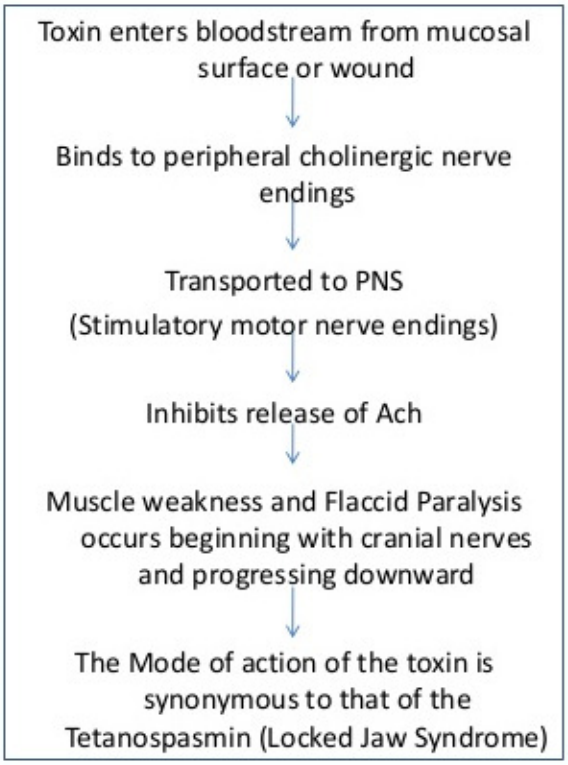
The A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates the adenylate cyclase, the enzyme remains continually activated.

Thus, the net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, Na^+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-. The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.



BOTULINUM TOXIN:





Difference between endotoxin and exotoxin:

Property	Exotoxin	Endotoxin
Chemical properties	Proteins, excreted by certain gram-positive or gram-negative Bacteria; generally heat-labile	Lipopolysaccharide-lipoprotein complexes (see Figures 3.33 and 3.34); released on cell lysis as part of the outer membrane of gram-negative Bacteria; extremely heat-stable
Mode of action; symptoms	Specific: either cytotoxin, enterotoxin, or neurotoxin with defined specific action on cells or tissues	General; fever, diarrhea, vomiting
Toxicity	Highly toxic, often fatal	Weakly toxic, rarely fatal
Immunogenicity	Highly immunogenic: stimulate the production of neutralizing antibody (antitoxin)	Relatively poor immunogen; immune response not sufficient to neutralize toxin
Toxoid potential	Treatment of toxin with formaldehyde will destroy toxicity, but treated toxin (toxoid) remains immunogenic	None
Fever potential	Do not produce fever in host	Pyrogenic, often produce fever in host

Transmission of diseases:

Direct contact

Infectious diseases are often spread through direct contact. Types of direct contact include:

Person-to-person contact

Infectious diseases are commonly transmitted through direct person-to-person contact. Transmission occurs when an infected person touches or exchanges body fluids with someone else. This can happen before an infected person is aware of the illness. Sexually transmitted diseases (STDs) can be transmitted this way.

Pregnant women can also transmit infectious diseases to their unborn children via the placenta. Some STDs, including gonorrhea, can be passed from mother to baby during childbirth.

2. Droplet spread

The spray of droplets during coughing and sneezing can spread an infectious disease. You can even infect another person through droplets created when you speak. Since droplets fall to the ground within a few feet, this type of transmission requires close proximity.

Indirect contact

Infectious diseases can also be spread indirectly through the air and other mechanisms. For example:

1. Airborne transmission

Some infectious agents can travel long distances and remain suspended in the air for an extended period of time. You can catch a disease like measles by entering a room after someone with measles has departed.

2. Contaminated objects

Some organisms can live on objects for a short time. If you touch an object, such as a doorknob, soon after an infected person, you might be exposed to infection. Transmission occurs when you touch your mouth, nose, or eyes before thoroughly washing your hands.

Germs can also be spread through contaminated blood products and medical supplies.

3. Food and drinking water

Infectious diseases can be transmitted via contaminated food and water. *E. coli* is often transmitted through improperly handled produce or undercooked meat. Improperly canned foods can create an environment ripe for *Clostridium botulinum*, which can lead to botulism.

4. Animal-to-person contact

Some infectious diseases can be transmitted from an animal to a person. This can happen when an infected animal bites or scratches you or when you handle animal waste. The *Toxoplasma gondii* parasite can be found in cat feces. Pregnant women

and people with compromised immune systems should take extra care (disposable gloves and good hand washing) when changing cat litter, or avoid it altogether.

5. Animal reservoirs

Animal-to-animal disease transmission can sometimes transfer to humans. Zoonosis occurs when diseases are transferred from animals to people. Zoonotic diseases include:

- anthrax (from sheep)
- rabies (from rodents and other mammals)
- West Nile virus (from birds)
- plague (from rodents)

6. Insect bites (vector-borne disease)

Some zoonotic infectious agents are transmitted by insects, especially those that suck blood. These include mosquitos, fleas, and ticks. The insects become infected when they feed on infected hosts, such as birds, animals, and humans. The disease is then transmitted when the insect bites a new host. Malaria, West Nile virus, and Lyme disease are all spread this way.

7. Environmental reservoirs

Soil, water, and vegetation containing infectious organisms can also be transferred to people. Hookworm, for example, is transmitted through contaminated soil. Legionnaires' disease is an example of a disease that can be spread by water that supplies cooling towers and evaporative condensers.

Nosocomial infections are infections that have been caught in a hospital and **are** potentially caused by organisms that **are** resistant to antibiotics.

Mode of transmission	Nosocomial infection	Infectious reservoir	Source of infection
Air	Measles, pulmonary tuberculosis	Infected individuals	Air-borne particles
Direct contact	Staphylococcal neonatal infection	Colonized / infected individuals	Hands containing secretions from infected wounds
Indirect contact	Respiratory Syncytial Virus Antibiotic-resistant bacteria	Infected individuals Colonized/ infected individuals	Hands and fomites Hands and fomites
Droplets	Whooping cough, invasive meningococcal disease, Streptococcal infection - group A	Colonized / infected individuals	Large respiratory droplets
Endogenous	Bacteremia caused by coagulase-negative Staphylococcus Urinary tract infection caused by *Escherichia coli*	Skin at the insertion site of the vascular catheter Periurethral skin and mucous membranes	Intravascular catheter Urinary catheter
Common source	Bacteremia caused by gram-negative bacteria Posttransfusion infection with HIV, HBV, HCV, CMV	Liquid substances in the environment Infected individuals	Contaminated IV fluids Donor's contaminated blood products
Vectors	Salmonellosis Enteric infection	Infected / colonized individuals Infected individuals or infectious material	Contaminated food Flies, ants

Opportunistic pathogen:

An infectious microorganism that is normally a commensal or does not harm its host but can cause disease when the host's resistance is low.

Examples of opportunistic pathogens are:

- *Candida albicans* – a causal agent of opportunistic oral and genital infections in human.
- *Staphylococcus aureus* – occur as commensal on human skin but may cause staph infections.
- *Pseudomonas aeruginosa* – most common cause of burn and external ear infections, and is the most frequent colonizer of medical devices (e.g.catheters).

Toxigenicity :

The ability of a pathogenic organism to produce injurious substances that damage the host.