Normal flora

Kusum Shrestha
Assistant Professor
School of Health and Allied Sciences
Pokhara University

Introduction

- The term **normal flora** is used to describe microorganisms that are frequently found in various body sites in normal, healthy individuals
- Also called as **indigenous microbiota**
- The constituents and numbers of the flora vary in different areas and sometimes at different ages and physiologic states
- They comprise microorganisms whose morphologic, physiologic, and genetic properties allow them to colonize and multiply under the conditions that exist in particular sites, to coexist with other colonizing organisms, and to inhibit competing intruders.

contd

- Each accessible area of the body presents a particular ecologic niche, colonization of which requires a particular set of properties of the invading microbe
- In humans, normal flora is located in various sites such as:

➢Gastrointestinal tract (GIT)

➢Respiratory tract

➢Genitourinary tract

➢Skin

contd

- Total population of normal flora in humans is roughly about 10^14 bacteria; which is more than *total* number of cells (10^13), present in human body.
- Organisms of the normal flora may have a **symbiotic relationship** that benefits the host or may simply live as **commensals** with a neutral relationship to the host.
- **Microbiome** is defined as the collection of microbes or microorganisms that inhabit environment , creating a sort of mini-ecosystem.
- **Human microbiome** is made of communities of **symbiotic , commensal and pathogenic microbiota** that share our body space.

Classification of normal flora

- Although there are many species of normal flora, these microbes typically fall into one of the *two* categories:

1) Resident flora
2) Transient flora

Resident flora

- Life long members of the body's normal microbial community
- They are very closely associated with a particular area and when disturbed, they again re-establish themselves
- For example *E. coli* is resident flora of gut.
- They do not cause harm; rather they have beneficiary effect on the host

Transient flora

- Microorganism that inhabit the body surface or mucous membrane temporarily for short interval
- Acquired from the environment and establish themselves briefly but tend to be excluded by competition from residents or by the host's innate or immune defense mechanisms.
- Many of the transient flora are potential pathogens which may cause disease under certain conditions, e.g. pneumococcus and meningococcus in nasopharynx

Contd

- In hospitals, patients may acquire many resistant organisms as transient flora from healthcare workers and hospital environment.
- For examples:

➢MRSA(Methicilin-resistant Staphylococcus aureus) in nose and skin

➢Multidrug resistant gram-negative organisms such as *Klebsiella, Escherichia coli, Pseudomonas, Acinetobacter* in respiratory tract

- They can be easily eliminated from the body surface by following proper hand hygiene and other infection control practices.

Factors affecting normal flora on body sites

- Local temperature and moisture
- pH
- Presence of nutrients
- Presence of inhibitory substances: example, bile and lysozyme
- Environmental factors(community or hospital)
- Immune status of individual
- Anatomical site
- Inhibition by the metabolic products of other organisms (eg, by hydrogen peroxide or volatile fatty acids)

Origin of normal flora

- The healthy fetus is sterile until the birth membranes rupture
- During and after birth, the infant is exposed to the flora of the mother's genital tract, to the skin and respiratory flora of those handling it, and to organisms in the environment.
- During the infant's first few days of life, the flora reflects chance exposure to organisms that can colonize particular sites in the absence of competitors.
- Subsequently, as the infant is exposed to a broader range of organisms, those best adapted to colonize particular sites become predominant

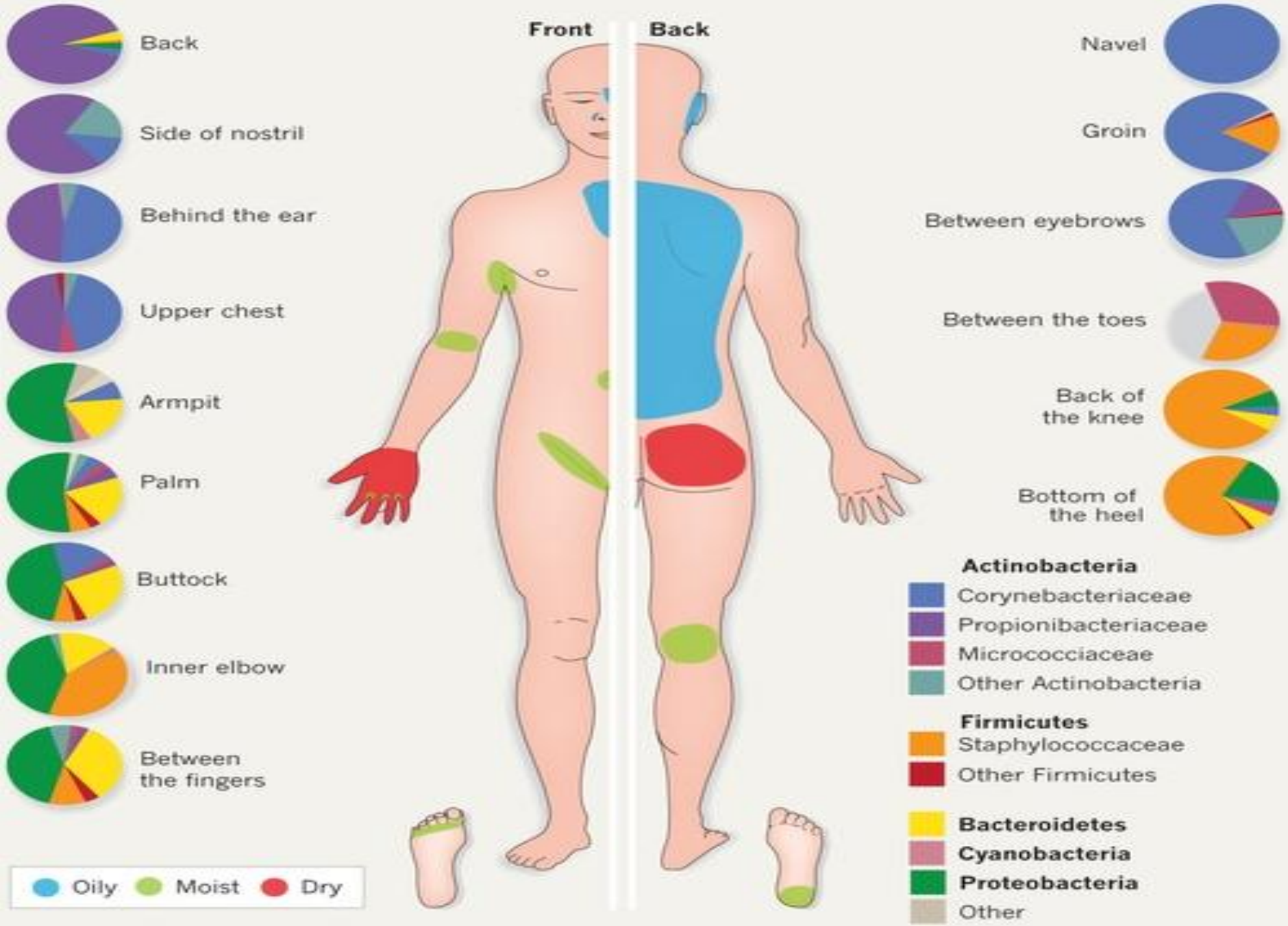
contd

- Thereafter, the flora generally resembles that of other individuals in the same age group and cultural milieu
- Once established onto or into a particular body site of the host, microbes develop particular relationship with that host that may be symbiosis or commensalism

Skin normal flora

- Skin and its appendages - physical barrier, protecting our bodies from pathogenic microbes/ toxic substances.
- Skin exposed to external environment colonized with diverse collection of microbes
- Flora is most abundant on moist skin areas (axillae, perineum, and between toes)
- Organisms of the skin flora are resistant to the bactericidal effects of skin lipids and fatty acids, which inhibit or kill many extraneous bacteria
- Normal flora of skin: *Staphylococcus epidermidis,* Propionibacteria, Micrococci, diptheroids

The human skin is rich with bacteria. The population and ratios vary by region, and depend on the whether the skin site is oily, moist or dry.



Oral flora

- The mouth consists several distinct habitats which include teeth, ginival sulcus , gingiva , lips, palate, tongue.
- Oral habitats from highly heterogeneous ecological system and support the growth of significantly different microbial communities.
- The mother transmit microbes to child and delivery mode also influences the diversity of oral flora
- Feeding, eruption of teeth, replacement of teeth with an adult dentition alters the oral flora.

Anatomical Site	Total bacteria/ gm or mL	Anaerobic/ Aerobic Ratio	Anaerobic Normal flora (common)	
Mouth				**Predominant**
Saliva	10^8–10^9	1:1	• Anaerobic cocci • *Actinomyces* • *Fusobacterium* • *Bifidobacterium* • *Prevotella* • Spirochetes	Viridans streptococci
Tooth surface	10^{10}–10^{11}	1:1		
Gingiva	10^{11}–10^{12}	10^3:1		

Gut flora

- Among various habitats, human GIT possess most abundant and diverse number of microbes containing at least 10^{14} bacteria with a total weight of approx 1kg and with total genome in excess of 100 times as that of human host.
- Mostly human gut belongs to phyla *Firmicutes* and *Bacteroidetes*(70-75%), other phyla comprises *Actinobacteria* ,*Proteobacteria* and *Verrucomicrobia*
- Most predominant genera are *Bacteroides*, *Clostridium*, *Ruminococcus*,*Petidococcus*,*Peptidostreptococcus* and *Bifidobacterium.*

Stomach 10^2

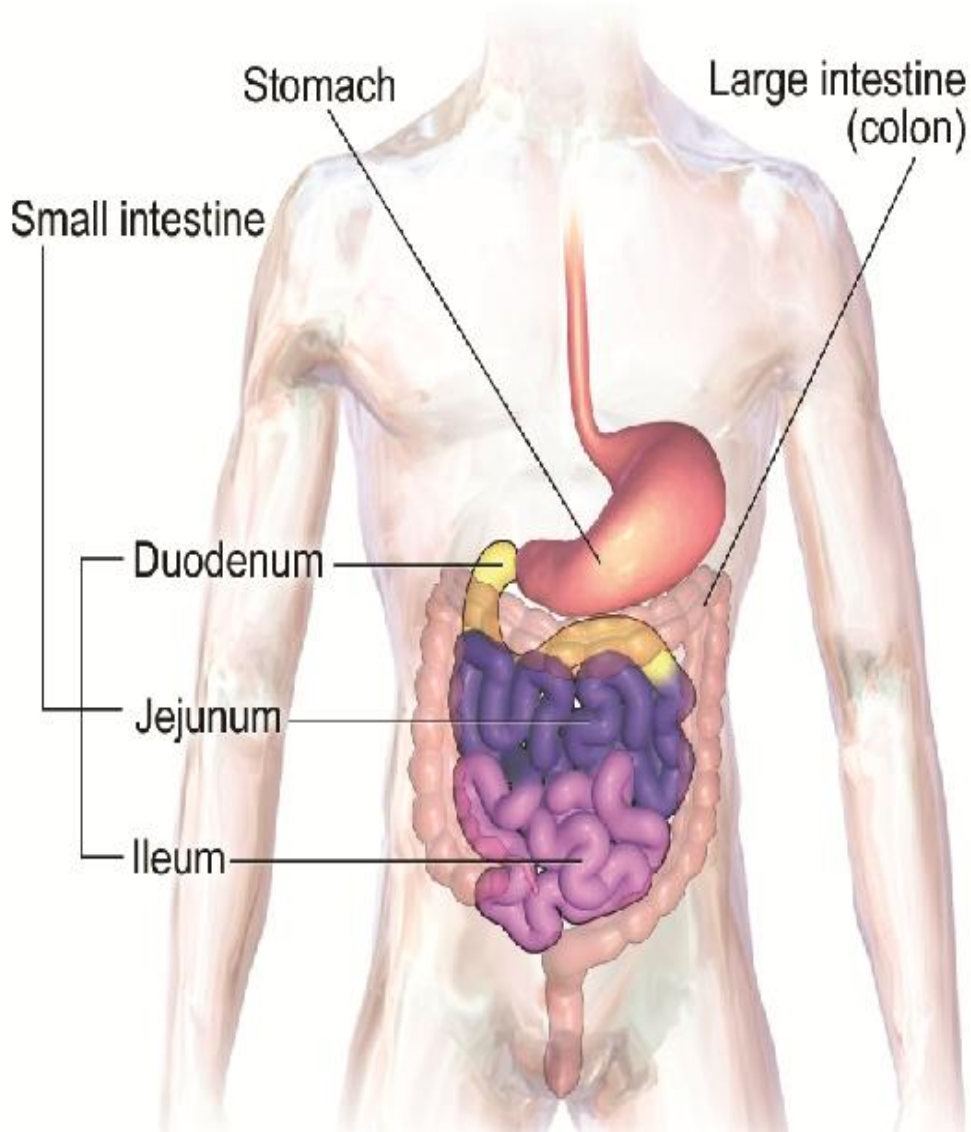
Lactobacillus
Candida
Streptococcus
Heliobacter pylori
Peptostreptococcus

Duodenum 10^2

Streptococcus
Lactobacillus

Jejunm 10^2

Streptococcus
Lactobacillus



Proximal ileum 10

Streptococcus
Lactobacillus

Distal ileum 10^8

Clostridium
Streptococcus
Bacteroides
Actinomycinae
Corneybacteria

Colon 10^{12}

Bacteroides
Clostridium
Bifidobacterium
Enterobacteriacae

Genitourinary tract flora

- The urinary tract is sterile in health above the distal 1 cm of the urethra, which has a scanty flora derived from the perineum
- The vagina has a flora that varies according to hormonal influences at different ages.
- Members of the genus *Lactobacillus* are commonly identified as the hallmark of normal or healthy vagina

Female genital tract				Predominant
Vagina	10^7–10^9	10:1	▪ Anaerobic cocci ▪ *Lactobacillus* ▪ *Prevotella* ▪ *Bifidobacterium* ▪ *Clostridium*	▪ *Corynebacterium* species ▪ *Lactobacillus* species ▪ Streptococci *(α*, non hemolytic and *S.agalactiae)* ▪ *Neisseria* (non-pathogenic species)

Respiratory tract flora

- The nares have a flora similar to that of the skin except that it is the primary site of carriage of a pathogen, *Staphylococcus aureus*
- The nasopharynx has a flora similar to that of the mouth; however, it is often the site of carriage of potentially pathogenic organisms such as pneumococci, meningococci, and *Haemophilus* species
- The respiratory tract below the level of the larynx is protected by the action of the epithelial cilia and by the movement of the mucociliary blanket; thus, only transient inhaled organisms are encountered in the trachea and larger bronchi.

contd

Nasopharynx				**Predominant**
			• *Prevotella* species • Anaerobic cocci • *Fusobacterium*	• Streptococci (α and non-hemolytic) • *Neisseria* (non-pathogenic species) • Diphtheroids • *Staphylococcus epidermidis*

Role of normal flora

❖Beneficial effects:

- **Prevent colonization of pathogen:** The normal flora prevent colonization of pathogens by competing for attachment sites or for essential nutrients.
- **Synthesize vitamin:** Human enteric bacteria secrete several vitamins such as Vitamin K and B complex (e.g. Vitamin B12) in excess; which can be absorbed by the host as nutrient.
- **Waste produced antagonize other bacteria:** Normal flora may inhibit or kill other nonindigenous organisms by producing a variety of waste substances such as:
  - Fatty acids and peroxides
  - Lactic acid: Lactobacilli present as normal flora in vagina of adult females maintain the acidic pH by producing lactic acid, thereby prevent the growth of pathogenic bacteria.
  - Bacteriocin: Some bacterial flora may produce bacteriocin or colicins which are antibiotic-like substances that can inhibit or kill other bacteria.

Beneficial effects contd

Immune stimulation: Normal microbial flora being foreign to the host stimulates the host's immune system.

- **Development of lymphatic tissues:** Immune stimulation helps in development of lymphatic tissues in the local sites (e.g. Peyer's patches in intestine).
- **Stimulate antibody production:** The antigens of the normal microbial flora stimulate the host immune system to produce antibodies that cross-react with pathogens having related or shared antigens and prevent their entry.

Beneficial effects contd

Prevent allergic diseases (Hygiene hypothesis): It states that a lack of early childhood exposure to symbiotic microorganisms (e.g. gut flora or probiotics), infectious agents and parasites increases susceptibility of the individual to allergic diseases by suppressing the natural development of the immune system.

Complement activation: The endotoxins liberated by the Gram-negative population of normal flora may help the defense mechanisms of the body by triggering the alternative complement pathway.

Harmful effects of normal flora

May be agents of disease: Members of the normal flora may cause endogenous disease (Table 53.2).

- When the host immunity is lowered, the transient flora may invade and produce disease, e.g. Gram-negative organisms (*E. coli*) colonizing the respiratory tract can cause pneumonia.
- If they enter a wrong site or tissue (e.g. blood, sterile body cavities)—then even the resident flora can produce disease. For example *E. coli* which is a resident flora of intestine may cause urinary tract infection if enters into urinary tract.

Harmful effects contd

- **Bacterial synergism:** Bacterial vitamins and growth factors produced by members of the normal flora may promote the growth of the potential pathogens.
- **Contribute to the drug resistance of pathogens:** Some members of normal flora produce enzymes such as beta lactamases which destroy the beta lactam antibiotics; thus indirectly contribute to the drug resistance of pathogens that are otherwise susceptible to the drug.
- **Competition for host nutrients:** Bacteria in GIT absorb some of the host's nutrients for their survival. Germ-free animals are known to grow more rapidly and efficiently than conventional animals.

TABLE 53.2: Diseases produced by normal flora

Diseases produced by normal flora	Anatomical site from which the flora is transferred
Urogenital infections including UTI	Intestinal flora such as *Escherichia coli, Klebsiella, Proteus*
Endocarditis	Oral flora (Viridans streptococci)
Dental caries and periodontal disease	Oral flora (*Streptococcus mutans*)
Peritonitis, abdominal infection	Intestinal flora
Pneumonia	Transient respiratory flora
Septicemia	From any site

Probiotics

The term "Probiotics" is defined as the live microorganisms (part of normal flora) which, when administered in adequate amounts, confer a health benefit to the host.

- They are extremely useful in the conditions where the normal intestinal flora is suppressed.
- Probiotics are commercially available in the form of capsule or sachet, consisting of mixture of some important beneficiary bacteria and yeast of human intestinal flora such as *Bacillus coagulans, Bifidobacterium longum, Lactobacillus acidophilus, Saccharomyces boulardii,* etc.

Role of probiotics

To treat various forms of GIT conditions like:

- Gastroenteritis due to any cause
- Antibiotic-associated diarrhea
- Lactose intolerance
- Irritable bowel syndrome and colitis
- Necrotizing enterocolitis
- *Helicobacter pylori* infection

Reducing serum cholesterol level by breaking down bile in the gut, thus inhibiting its reabsorption.

Reducing blood pressure (by producing ACE inhibitor-like peptides during fermentation).

Modulate inflammatory and hypersensitivity responses, hence can be given in allergic disorders, eczema and atopic dermatitis.

Prebiotics

Prebiotics

In contrast to probiotics, prebiotics are the dietary non digestible fibers which when administered, stimulate the growth and activity of commensal microorganisms and thereby exert beneficiary effect to the host indirectly.

Examples: fructans, galactans, pectin

Pathogenic microorganisms

- A pathogenic organism is an organism which is capable of causing diseases in a host
- It can be either bacteria, virus, fungi or parasites
- Pathogens fall into two basic types:

1) **primary pathogens** that cause disease among at least a portion of normal individuals

2)opportunistic pathogens that cause disease only in individuals who are compromised in either their innate or humoral immune defences.

Primary pathogens

- Refers to microorganisms causing diseases in a proportion of susceptible individuals with apparently intact specific and non-specific defense systems
- Also called as principal pathogens
- Their pathogenicity depends on various virulence factors

Virulence factors

1)Adhesins:

- helps in adhesion to host cell
- Fimbriae, teichoic acid, M protein of Streptococcus pyogenes,

2)Capsule:

- Adhesion
- Antiphagocytic
- Inhibits complement

3)Endotoxin: lipid A of LPS

- Inhibits complement activation
- Coagulation pathway activation
- Platelet activation

contd

4)Exotoxin:

- neurotoxin(botulinum toxin, tetanus toxin) , enterotoxin(cholera toxin, staphylococcal enterotoxins), cytotoxin(shiga toxin, Panton Valentine leukocicin toxin)
- Different exotoxins have different effect
- Toxin of Bacillus anthracis can cause edema
- Toxin of Clostridium perfringens can cause myonecrosis
- Tetanus toxin and botulinum toxin can cause spastic paralysis

contd

- Enzymes:

➢Various enzymes help to invade host cell and damages host cell

- Examples: hyaluronidase (cleaves proteoglycans in connective tissue), streptokinase and staphylokinase (breaks down fibrin clots)
- Virulence marker antigen or invasion plasmid antigens in *Shigella*

Pathogenicity Islands

Pathogenicity islands (PAIs) are large genomic islands located in the chromosomal regions of some bacteria containing sets of genes encoding numerous virulence factors, such as adhesins, secretion systems, toxins, invasins, iron uptake system, etc.

- Genes encoded in a pathogenicity island are expressed in a coordinated way to initiate the virulence process.

These genes may be turned on by a single stimulus (e.g. the temperature of the gut) and can be transferred as a unit by horizontal gene transfer mechanisms to different sites within a chromosome or to other bacteria.

Pathogenicity islands have been detected in some bacteria, such as *Shigella*, *Salmonella*, *Vibrio cholerae*, *Yersinia pestis*, *Staphylococcus aureus*, uropathogenic *E. coli*, *Helicobacter*.

Classification of infective microorganisms by risk group (WHO)

- **Risk Group 1** (no or low individual and community risk) A microorganism that is unlikely to cause human or animal disease. Example: Escherichia coli K-12, Saccharomyces cerevisae
- **Risk Group 2** (moderate individual risk, low community risk):

➢A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.

➢Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

➢ Example: *Staphylococcus, Streptococcus,* Herpes virus

contd

- **Risk Group 3** (high individual risk, low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment is available. Example: *Bacillus anthracis, Brucella* spp
- **Risk Group 4** (high individual and community risk): A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Example: Ebola virus, Marburg virus

Oppurtunistic microorganisms

- Refers to those microorganisms which do not usually cause disease in people with intact host defence systems; yet they can clearly cause devastating disease in many hospitalised and immunocompromised patients
- Commensal bacteria are among the most prevalent opportunistic pathogens.
- The use of antibiotics, which usually kill commensals besides pathogens has increased the incidence of infections due to environmental microorganisms presenting reduced susceptibility to antibiotics

contd

- Infections caused by them are called opportunistic infection
- There are mainly two aspects to study opportunistic pathogens. They are as follows:

1)Oppurtunistic organism in reference to site change:

➢Example: Escherichia coli is normal flora of colon but change in anatomic site to urinary tract, it causes urinary tract infection

2)Oppurtunistic organism in reference to immunocompromised conditions:

➢Example: Oppurtunistic infections related HIV/AIDS like Cryptococcus neoformans, Pneumocystic jiroveci, Cytomegalo virus

contd

- Immunocompromised conditions are:

➢HIV/AIDS

➢Chemotherapy

➢Steroid therapy

➢Organ transplantation

➢Ageing

➢Immunodeficiency diseases like Bruton disease, DiGeorge syndrome