1. Normal Bacterial Flora in Human
2. Classification of Bacteria
3. Gram-Positive Cocci: Staphylococci

Normal Flora (Normal Microbiota) in Human

The term “Normal Flora” denotes the mixture of microorganisms (bacteria and fungi) that inhabit many sites (usually skin and mucous membranes) of healthy human body, but bacteria are the most numerous. Under normal conditions, normal flora does not cause disease, but if conditions be changed, it become opportunistic pathogen and it can cause disease.

In general, Bacterial-human relationships may be normal flora or opportunistic infection or Pathogenic infection. Normal Bacterial Flora can be arranged into two groups:

1. **Resident Normal Flora** consists of relatively fixed types of bacteria regularly found in a given area at a given age. It is acquired rapidly during & after birth and always present (permanent), if disturbed, it promptly reestablishes itself.

2. **Transient Normal Flora** consists of nonpathogenic or potentially pathogenic bacteria that is derived from the environment and inhabit many sites in human body for a period of time (hours, days, weeks, months) then move on or die off (not fixed). It is a temporary and it can't reestablish itself.

Normal flora inhabits at many anatomical sites of human body like: Skin; Eye (i.e. Conjunctiva); Nose (i.e. Respiratory tract); Mouth (i.e Oral Cavity); Ear; Urogenital tract; Elementry (Digestive)tract. On the other hand, in a healthy human, the internal tissues such as:
Blood, Brain, Muscle, Lungs, Cerbro-Spinal Fluid (CSF) are normally sterile tissues and free of microorganisms which mean it has no normal flora.

The normal flora that is constantly (continuously) present on body surfaces are commensals. Their flourishing in a given area depends on physiologic factors of temperature, moisture, and the presence of certain nutrients and inhibitory substances.

The presence of NormL Flora is not essential to life, yet, the resident flora of certain areas plays a definite role in maintaining health and normal function. For examples:

First: Members of the resident normal flora in the intestinal tract synthesize vitamin K & B complex and aid in the absorption of nutrients and Food metabolism by producing proteolytic enzymes.

Second: Normal flora contribute as a part of the first line of defense in innate Immune system. on mucous membranes and skin, the resident normal flora may prevent colonization by pathogens and possible disease through “Bacterial Interference.” The mechanism of bacterial interference may involve:

1. Competition for receptors or binding sites on host cells,
2. competition for nutrients,
3. mutual inhibition by metabolic or toxic products.
4. mutual inhibition by antibiotic materials or bacteriocins (An antibacterial substance produced by a strain of bacteria and harmful to another strain within the same family).
Suppression of the normal flora clearly creates a partial local void that tends to be filled by bacteria from the environment or from other parts of the body. Such bacteria behave as opportunists and may become pathogens.

On the other hand, members of the normal flora may themselves produce disease under certain circumstances (conditions). These organisms are adapted to a non-invasive mode of life defined by the limitations of the environment, if:

First: forcefully removed from the normally conditions of that environment and introduced into the bloodstream or tissues:
- Perforated mucous membranes
- or damage in the skin like burns and wounds) these organisms may become pathogenic.

For example:
1. viridans streptococci are the most common resident organisms in the mouth. If large numbers of them are introduced into the bloodstream (eg, after tooth extraction or oral surgery), they may settle on prosthetic heart valves and produce infective endocarditis.
2. E. coli normally found in the digestive tract where it causes no problems, but if it gets into the urinary tract it can become pathogenic and causes UTI(Urinary Tract Infection).
3. Staphylococcus aureus normally found in the upper respiratory tract, but if it gets into wounds or burns it can become pathogenic.

There are many other examples, but the important point is that the normal resident flora is harmless and may be beneficial in their normal location in the host and in the absence of coincident abnormalities. They
may produce disease if introduced into foreign locations in large numbers.

**Second:** if abnormal health conditions are present which usually occur in people with underlying special conditions, such as: Immuno-suppression (AIDS); Radiation therapy & Chemotherapy; Rheumatic heart disease…etc

**Third:** On the other hand, some normal flora may produce carcinogens by modifying some chemicals into carcinogens through their enzymes, e.g., Artificial sweeteners may be enzymatically modified into bladder carcinogens.

**Normal Flora in Neonate**

The amniotic sac in which a fetus grows is a sterile environment to keep the fetus safe from infection. But at just one week old, neonate has a complex collection of microbes. In direct contrast to the highly differentiated communities of their mothers, neonates harbored bacterial communities that were undifferentiated across multiple body habitats, regardless of delivery mode. Thus, at its earliest stage of community development (<5 minutes post delivery), the human flora is homogeneously distributed across the body. Vaginally delivered neonates harbor bacterial communities (in all body habitats) that are most similar in composition to the vaginal communities of the mothers (Lactobacillus spp. Gardnerella vaginalis); caesarean section (C-section) neonates harbor bacterial communities (across all body habitats) that are most similar to the skin communities of the mothers (e.g., Staphylococcus, Corynebacterium, or Propionibacterium spp.).
The Role of the Normal Mouth Microbiota in Dental Plaque and Caries

Dental plaque, which has come to be viewed and managed as a complex biofilm, that forms on the tooth surface composed almost entirely of bacteria derived from the normal flora of the Mouth. Dental plaque is the most prevalent and densest of human biofilms. Metabolism of carbohydrates by organisms in dental plaque such as *Streptococcus mutans* is responsible for the initiation of caries or tooth decay.

Pathogenesis of Bacteria

The pathogenesis of bacteria is the ability of an infectious bacteria to cause disease. The pathogenesis includes initiation of the infectious process and the mechanisms that lead to the development of signs and symptoms of disease.

Definitions of Important terms in Pathogenesis

- **Adherence (Adhesion, Attachment):** The process by which bacteria stick to the surfaces of host cells. After bacteria have entered the body, adherence is a major initial step in the infection process. The terms adherence, adhesion, and attachment are often used interchangeably.

- **Carrier:** A person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.

- **Infection:** Multiplication of an infectious agent within the body. Multiplication of the bacteria that are part of the normal flora is generally not considered an infection; on the other hand, multiplication of pathogenic bacteria (eg, *Salmonella spp.*) even if the person is asymptomatic is deemed an infection.
Invasion: The process where by bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body.

Nonpathogen: A microorganism that does not cause disease; may be part of the normal microbiota.

Opportunistic pathogen: An agent capable of causing disease only when the host’s resistance is impaired (ie, when the patient is “immunocompromised”).

Pathogen: A microorganism capable of causing disease.

Toxigenicity: The ability of bacteria to produce a toxin that contributes to the development of disease.

Virulence: The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers. Virulence Involves adherence, persistence, invasion, and toxigenicity.

Classification of Bacteria

It has been estimated that we currently have the capacity to identify fewer than 10% of the pathogens responsible for causing human disease because of our inability to culture or target these organisms using molecular probes. Yet the diversity of even these identifiable pathogens is so great that it is important to understand the subtle differences associated with each infectious agent. The reason for understanding these subtleties is significant because each infectious agent has specific adapted to a particular mode(s) of transmission, a mechanism(s) to grow in human hosts (colonization), and a mechanism(s) to cause disease (pathology). Bacterial Taxonomy is the classification of bacteria in an
ordered system that indicates a natural relationship. The taxonomy of any group of organisms is based on three sequential stages: Classification, Nomenclature, and Identification, which are separate but interrelated areas of bacterial taxonomy.

Bacterial Classification is the categorization of bacteria into taxonomic groups which requires experimental and observational techniques; this is because biochemical, physiologic, genetic, and morphologic properties are often necessary for an adequate description of a taxon. Nomenclature refers to the naming of an organism by international rules (Scientific Name) according to it's characteristics,

Identification is practical use of a classification scheme. For example of Bacterial Classification see table(1)

Table(1): Taxonomic ranks of *Escherichia coli*

<table>
<thead>
<tr>
<th>Domain</th>
<th>Prokaryotae</th>
</tr>
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<tbody>
<tr>
<td>Kingdom</td>
<td>Eubacteria</td>
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<tr>
<td>Phylum</td>
<td>Proteo-bacteria</td>
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<tr>
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<td>Escherichia</td>
</tr>
<tr>
<td>Species</td>
<td><em>E. coli</em></td>
</tr>
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</table>

Table 1: Taxonomic ranks of *Escherichia coli*
Criteria for Classification of Bacteria

1. Morphological Characteristics
   - Useful for identifying prokaryotes and eukaryotes

2. Microscopical Examination
   - Gram staining, acid-fast staining
     - Cell wall structure

3. Biochemical tests
   - Determine presence of bacterial enzymes, metabolic activities

4. Immunological tests
   - Determine antigens
     - toxins

5. Genetic tests
   - Determine presence and structure of genes

Criteria for Classification of Bacteria
1. Morphological Characteristics (Growth on media)
2. Microscopical examination
3. Biochemical Tests
4. Immunological Tests
5. Genetic features

Depend on Gram staining, bacteria are classified into two groups: Gram-positive bacteria and gram-negative bacteria. Gram-positive bacteria have a thick cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin.
Gram stain

Gram − (pink)

Cocci

Neisseria spp.

Haemophilus influenzae (requires factors V and X)

Pasteurella — animal bites

Brucella — brucellosis

Bordetella pertussis

Maltose fermenter

N. meningitidis

Maltose nonfermenter

N. gonorrhoeae

Rods

Lactose

Lactose fermenter

Klebsiella

E. coli

Enterobacter

Citrobacter

Serratia

Others

Oxidase −

Shigella

Salmonella

Proteus

Oxidase +

Pseudomonas
Gram positive rod shaped bacteria

- **Gram positive**
  - **rods**
    - **Aerobic**
    - **Anaerobic**
      - Spore Forming: Bacillus sp., B. anthracis, B. cereus
      - No spores
      - Spores/Large: Clostridium sp.
      - Branching: Actinomyces
    - Motile: Listeria
    - Corynebacterium
Gram - Positive Cocci

Catalase Positive - Gram Positive Cocci

Genus: Staphylococcus

Spherical cells, characteristically dividing in more than one plane to form irregular grape-like clusters, Gram-stain-positive, Non-motile. Cell wall contains peptidoglycan and teichoic acid, Facultative anaerobes. Growth is more rapid and abundant under aerobic conditions. Staphylococcus is catalase-positive and oxidase-negative. Most strains grow in the presence of 10% NaCl and between 18-40°C. Susceptible to lysis by lysostaphin, but resistant to lysis by lysozyme. Natural populations are mainly associated with skin, skin glands, and mucous membranes. Some species may be isolated from a variety of animal products (e.g., meat, milk, cheese) and environmental sources (e.g., fomites, soil, sand, dust, air, or natural waters). Some species are opportunistic pathogens of humans and/or animals. Depend on coagulase test, Staphylococci classify into two groups:

1. **Coagulase-Positive Staphylococci**
2. **Coagulase-Negative Staphylococci** (CoNS)
1. **Coagulase-Positive Staphylococci: *Staphylococcus aureus***

*S. aureus* is a Gram-positive, catalase-positive cocci, that occurs in irregular “grape-like” clusters. It is facultative anaerobes, non-spore forming, non-motile and non gas forming from carbohydrates. It has long been recognized as one of the major human pathogens and is by far one of the most common nosocomial bacteria with it's ability to cause large spectrum of human diseases, ranging from skin lesions (abscesses, impetigo) to invasive and more serious infections, e.g., osteomyelitis, septic arthritis, pneumonia, endocarditis.

The major human infections caused by this species are furuncle, carbuncle, impetigo, toxic epidermal necrolysis (scalded skin syndrome), pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cervicitis, cerebritis, meningitis, enterocolitis, mastitis, cystitis, prostatitis bacteremia, toxic shock, syndrome, and abscesses of the muscle, skin, central nervous system, urogenital tract.

In addition, staphylococcal enterotoxin is involved in food poisoning.
Methicillin-Resistant S. aureus (MRSA):

Introduction of methicillin into medical practice in the early 1960s quickly resulted in methicillin-resistant S. aureus (MRSA). Some MRSA are resistant to all but one or two antibiotics. Methicillin(Meticillin) is a narrow-spectrum β-lactam, semisynthetic derivative of penicillin. Methicillin acts by inhibiting the synthesis of bacterial cell wall. It inhibits cross-linkage between the linear peptidoglycan polymer chains by binding to penicillin-binding proteins (PBPs). Methicillin is actually a penicillinase-resistant β-lactam antibiotic even if this enzyme is present. Previously, it was used to treat infections.
caused by susceptible Gram-positive bacteria, in particular, penicillinase-producing organisms such as *S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Today, it isn't as effective against these organisms due to resistance. MRSA is any strain of *S. aureus* that has developed resistance to β-lactam antibiotics, which include the Penicillins (Methicillin, Dicloxacillin, Nafcillin, Oxacillin, etc.) and the Cephalosporins. Strains unable to resist these antibiotics are classified as Methicillin-Sensitive *S. aureus* (MSSA). The evolution of such resistance make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous. MRSA is treated by using vancomycin. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have emerged in the 1980s as a major clinical and epidemiological problem in hospitals. These strains are beginning to spread out of the hospitals and into communities.

**Virulence Factors of *S. aureus***

The ability of *S. aureus* to cause disease has been attributed to an impressive spectrum of cell-wall-associated factors and Extracellular toxins as virulence determinants.

First: Capsule
Second: Teichoic Acid
Third: Staphylococcal Enzymes:

1) Coagulase
2) Hyaluronidase
3) Catalase  
4) Fibrinolycin  
5) Lipase  
6) Nuclease  
7) Penicillinase 

Fourth: Staphylococcal toxins  
1) Enterotoxins  
2) Cytotoxins  
3) TSST-1 

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First: the Capsule protects bacteria by inhibiting the chemotaxis and phagocytosis. In addition, Capsule facilitates the adherence of bacteria to catheters and other synthetic materials.

Second: Teichoic acid mediates the attachment of staphylococci to mucosal surfaces through its specific binding to fibronectin.

Third: Staphylococcal Enzymes:  
1) Coagulase: converts the fibrinogen (in plasma) to insoluble fibrin, causing the staphylococci to clump or aggregate…. Detection of this enzyme is the primary test for identifying _S. aureus_. 
What is the role of coagulase in the pathogenesis of _S. aureus_? the formation of fibrin layer (clot) around a staphylococcal abscess contributes in localizing the infection and protecting the organisms from phagocytosis.
S. aureus possess two forms of coagulase: bound coagulase and free coagulase.

2) **Hyaluronidase** hydrolyzes hyaluronic acid, (hyaluronic acid is the acidic muco-poly-saccharides present in the acellular matrix of connective tissues), thus, this enzyme facilitates the spreading of S. aureus through tissues… more than 90% of S. aureus strains produce this enzyme.

3) **Catalase**: all staphylococci produce catalase, which catalyzes the conversion of toxic hydrogen peroxide to water and oxygen…. Hydrogen peroxide can accumulate during bacterial metabolism or after phagocytosis.

4) **Fibrinolycin (Staphylokinase)**: dissolves fibrin threads in the plasma clot, allowing S. aureus to free it self from clots, and spread.

5) **Lipase** digests the lipids, allowing S. aureus to grow on the skin surface and in cutaneous oil glands.

6) **Nuclease (Nucleo-de-polymerase or Poly-nucleo-tidase)** is an enzyme capable of cleaving the phosphodiester bonds between monomers of nucleic acids.

7) **Penicillinase (β-Lactamase)** breaks down penicillin, and allowing S. aureus to survive treatment with β-Lactam Antibiotics.

**Fourth: Staphylococcal Toxins**

Depending on the strain, S. aureus is capable of secreting several exotoxins. Many of these toxins are associated with specific diseases.

1) **Toxic Shock Syndrome Toxin (TSST-1)**
2) **Exfoliative toxins (Exfoliatin**
3) **Cytotoxins**
4) **Enterotoxins**
1) **Toxic Shock Syndrome Toxin (TSST-1)** is a heat and proteolytic resistant. The ability of TSST-1 to penetrate mucosal barriers, even though the infection remains localized in the vagina or at the site of a wound, is responsible for the systemic effects of TSS. Death in patients with TSS is due to hypovolemic shock leading to multiorgan failure. Hypovolemic shock is an emergency condition in which severe blood and fluid loss make the heart unable to pump enough blood to the body. This type of shock can cause many organs to stop working.

2) **Exfoliative Toxins (Exfoliatin)** causes a blistering of the skin known as staphylococcal scalded skin syndrome usually in infants.

3) **Cytotoxins Or Cytolytic Toxins (Heamolysins)**

   Heamolysins cause lysis of red blood cells by destroying their cell membrane. In *S. aureus* heamolysins include α-hemolysin, β-hemolysin, and γ-Hemolysin.

   α-hemolysin also known as α-toxin, is the major cytotoxic agent released by *S. aureus* and the first identified member of the pore forming toxins. It's major function is development of pores in the cellular membrane which are able to cause the lysis of erythrocytes, leukocytes, and platelets.

   On Blood Agar plates, colonies of *S. aureus* are frequently surrounded by zones of clear beta-heamolysis (complete heamolysis).
4) **Enterotoxins:** once a food product has been contaminated with producing staphylococci and it's enterotoxins, neither reheating the food nor the digestive process will be protective. Enterotoxin A is the most commonly associated with disease. Enterotoxin C and D are found in contaminated milk products, and Enterotoxin B causes staphylococcal pseudomembranous enterocolitis.

**Pathogenesis of *S. aureus***

The pathogenic capacity of *S. aureus* is the combined effect of extracellular factors and toxins together with the invasive properties. *S. aureus* expresses many potential virulence factors.

1. Surface proteins that promote colonization of host tissues.
2. Factors that probably inhibit phagocytosis.
3. Toxins that damage host tissues and cause disease symptoms.
Toxins produced by bacteria are generally classified into two groups: **Exotoxins and Endotoxins.** Exotoxins are proteins that are most often excreted from the cell. However some exotoxins accumulate inside the cell and are either injected directly into the host or are released by cell lysis. Endotoxins are lipid molecules that are components of the bacterial cell membrane.

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extrachromosomal control. There are multiple (A–E, G–J, K–R and U, V) enterotoxins. Approximately %50 of *S. aureus* strains can produce one or more of them. The Enterotoxins are heat stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods and results in vomiting and diarrhea.

*S. aureus* is able to cause many superficial pyogenic (pus forming) infections of the dermis and underlying tissues as well as serious systemic infections.

**Most Common Infections by *S. aureus***

- Purulent Infections of the skin: Folliculitis, Furunculus, Carbunculus, Wound infections, Otitis Media, Mastitis.
- Invasive Infections: pneumonia, Bacteraemia, Sepsis, Meningitis, Ostitis, Osteomyelitis, Endocarditis.
- Toxin Mediated Infections: Gastroenteritis, Toxic Shock Syndrom(TSS), Staphylococcal Scalded Skin Syndrome(SSSS)
- Abscesse formation.