INTRODUCTION

The first simple forms of life appeared on earth more than three billion years ago. Their descendants have changed and developed into the several million types of animals, plants and microorganisms recognized today. Of course, thousands more remain to be discovered and officially described.

Microscopic forms of life are present in vast numbers in nearly every environment known, i.e. soil, water, food, air, etc. Since the conditions that favor the survival and growth of many are the same as those under which people normally live.

Many microscopic microorganisms or microbes occur as single cell, others are multicellular, and still others such as viruses, do not have a true cellular appearance. Some organisms called anaerobes are capable of carrying out their vital functions in the absence of free oxygen; whereas other organisms can manufacture the essential compounds for their physiological needs from atmospheric sources of nitrogen and carbon dioxide. Other microorganisms such as viruses and certain bacteria are totally dependent for their existence on the cells of higher forms of life. The branch of science known as microbiology embraces all of these properties of microorganisms and many more.

One of the attractive features of microbiology is a number of investigations and work remaining to be done. Many decisions affecting the future of the world may depend upon and involve the activities of microorganisms in areas like ***food production***, ***pollution control***, ***energy production*** and the ***control*** and ***treatment of diseases***. In short, ***microbiology*** has assumed a position of great importance in modern society.

Medical microbiology (Gr. ***mikros***-small, ***bios***-life, ***logos***-science) is the study of causative agents of infectious disease of human beings and their reactions to such infections. In other words it deals with etiology, pathogenesis, laboratory diagnosis, treatment, epidemiology and control of infection.

Obviously, medical microbiology has close link with other disciplines, i.e. pathology, clinical medicine, surgery, pharmacology cum therapeutics and preventive medicine.

Microbiologists are enthusiastic to confirm the diagnosis and cause of macroscopic changes by taking smears and preparing cultures from the lesions to demonstrate microorganisms, e.g. circumscribed boil of staphylococcus, the spreading cellulitis of streptococcus, the red-liver-like appearance of lung in pneumococcal pneumonia, the tubercles followed by syphilis and intestinal ulceration of salmonella organism, etc. The pathology of infection is quite fascinating as it includes affinity of pathogens for certain tissue and initiation of infection and characteristic tissue reaction. Each investigation is regarded as research project in miniature.

Historical Events

Long before the discovery of microorganisms certain processes caused by their life activities, such as fermentation of wine juice, milk, yeast, etc. were known to mankind. In ancient times at the beginning of civilization, man by using these processes learned to prepare koumurs, sour milk and other products.

- ***Antoni van Leeuwenhoek (1683)***

He was the first scientist who observed bacteria and other microorganisms, using a single-lens microscope constructed by him and he named those small organisms as 'Little animalcules'.

- ***Robert Hook***, a contemporary of Leeuwenhoek developed compound microscope in ***1678*** and confirmed Leeuwenhoek's observation.

- ***Edward Jenner (1796)*** developed the first vaccine of the world, the smallpox vaccine. He used the cowpox virus (Variolae vaccinae) to immunize children against smallpox from which the term ' vaccine ' has been derived. The same principles are still used today for developing vaccines.

♦. ***Louis Pasteur.*** Microbiology developed as a scientific discipline from the era of ***Louis Pasteur*** (**1822- 1895**). He is also known as ***father of microbiology***. He was a professor of chemistry France. His studies on fermentation led him to take interest to work in microbiology. His contributions to microbiology are as follows:

1- He had proposed the **principles of fermentation** for preservation of food.

2- He introduced the **sterilization techniques** and developed steam sterilizer, hot air oven and autoclave.

3- He described the method of **pasteurization of milk**.

4- He had also contributed for the vaccine development against several diseases, such as anthrax, fowl cholera and rabies.

5- He disproved the theory of spontaneous generation of disease and postulated the **'germ theory of disease'**. He stated that disease cannot be caused by bad air or vapor but it is produced by the microorganisms present in air.

6- Liquid media concept: He used nutrient broth to grow microorganisms.

7- He was the founder of the Pasteur Institute, Paris.

♦. ***Joseph Lister***

Joseph Lister (**1867**) is considered to be the **father of antiseptic surgery**. He had observed that postoperative infections were greatly reduced by using disinfectants such as diluted carbolic acid during surgery to sterilize the instruments and to clean the wounds.

♦. ***Robert Koch (father of bacteriology)***. ***Robert Koch*** provided remarkable contributions to the field of microbiology. He was a German general practitioner (**1843- 1910**). His contributions are as follows:

1- He introduced solid media for culture of bacteria, Eilshemius Hesse, the wife of, one of Koch's assistants had suggested the use of agar as solidifying agents.

2- He also introduced methods for isolation of bacteria in pure culture.

3- He described hanging drop method for testing motility.

4- He discovered bacteria such as the anthrax bacilli, tubercle bacilli and cholera bacilli.

5- He introduced staining techniques by using aniline dye.

6- Koch's phenomenon: Robert Koch observed that guinea pigs already infected with tubercle bacillus developed a hypersensitivity reaction when injected with tubercle bacilli or its protein. Since then, this observation was called as Koch's phenomenon.

7- He also suggested criteria before blaming the organism responsible for disease. It goes by the name of **Koch's postulate**, according to which:

1-The specific micro-organism should be isolated from all cases of a specific disease, and should not be found in healthy individuals.

2-The specific micro-organism should be isolated from the diseased individual and grown in pure culture on an artificial medium.

3-The isolated micro-organism should reproduce the specific disease when inoculated into a healthy individual.

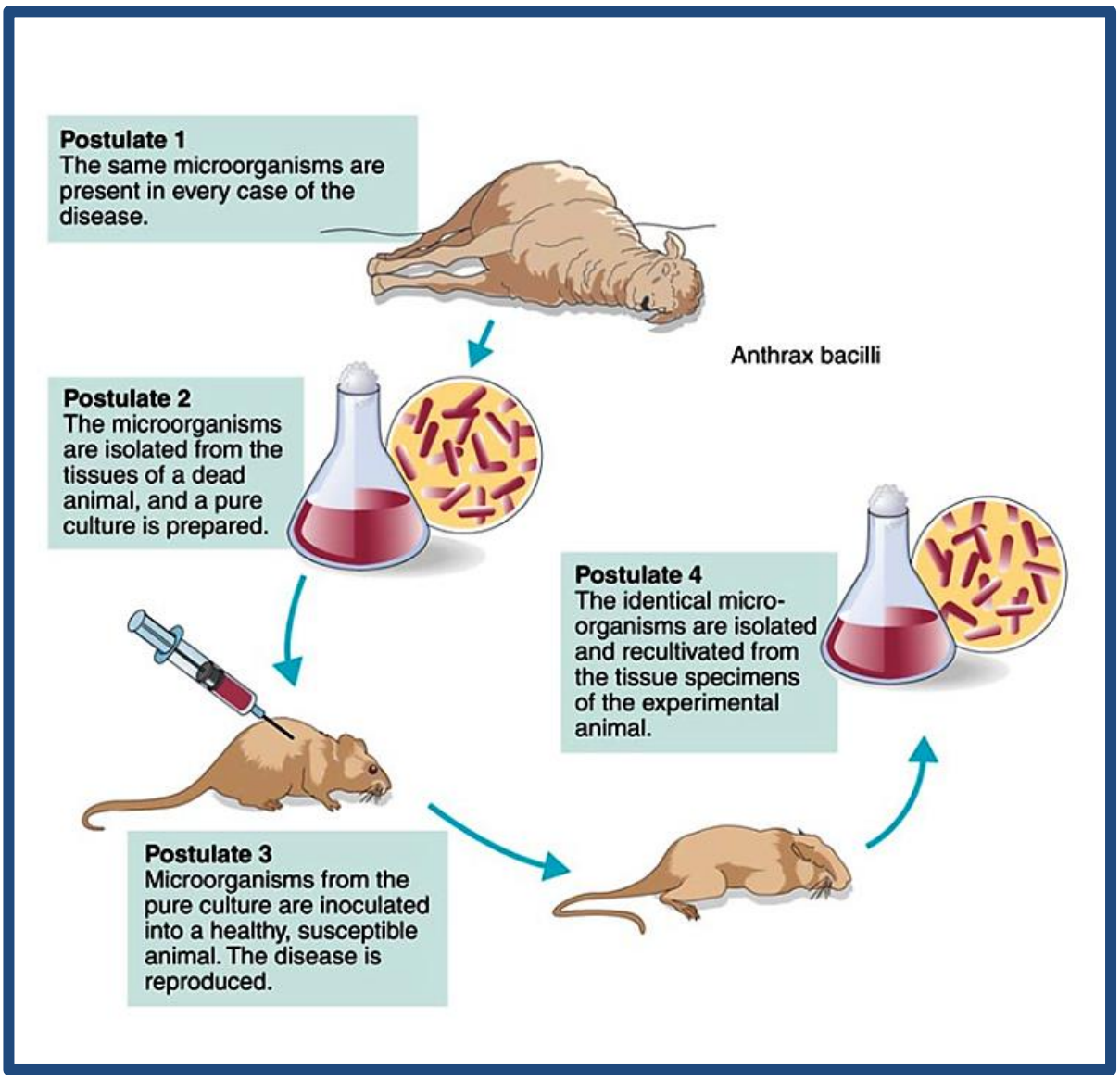
4-The specific micro-organism should be re-isolated in pure culture from the experimental infection.

An additional **fifth** criterion was introduced subsequently which states that antibody to the causative organism should be demonstrable in the patient's serum.

Exceptions to Koch's postulates: It is observed that it is not always possible to apply these postulates to study all the human diseases. There are some bacteria that do not satisfy all the four criteria of Koch's postulates. Those organisms are:

(i)- *Mycobacterium leprae* and *Treponema pallidum*: They cannot be grown ***in vitro***; however, they can be maintained in experimental animals.

(ii)- *Neisseria gonorrhoeae:* There is no animal model; however, it can be grown ***in vitro***.



. ***Neisser (1879)*** described gonococcus

. ***Ogston (1881)*** discovered staphylococcus

. ***Loeffler (1884)*** isolated diphtheria bacillus.

. ***Loeffler and Frosch (1898)*** observed that foot and mouth disease of cattle was caused by a microbe, i.e. filter passing virus.

. ***Walter Reed (1902)*** observed that yellow fever was caused by filterable virus and that it was transmitted through the bite of mosquitoes.

. ***Landsteiner*** and ***Popper (1909)*** showed poliomyelitis was caused by filterable virus.

. ***Towert (1951)*** and ***Herelle (1917)*** discovered lytic phenomenon in bacterial culture. The agent responsible was termed as bacteriophage (viruses that attack bacteria).

. ***Alexander Fleming*** discovered penicillin in **1928** (He made the accidental discovery that the fungus penicillium produces a substance which destroys staphylococci).

. ***Ruska*** **(1934)** introduced electron microscope and hence detailed study of morphology of virus was possible.

New agents of infectious diseases continue to emerge, e.g. HIV (identified in **1980**). The outbreaks of plague in **1994**, cholera in **1995**, and dengue hemorrhagic fever in **1996**. As such many workers in medicine have been awarded **Nobel Prizes** for their outstanding contributions in microbiology (Table -1).

The methods of many infectious diseases and vaccine production have been revolutionized, e.g. **recombine DNA technology**, **PCR**, **nuclear anaprobes**, radioimmunoassay, **ELISA**, etc.

One of the best ways of learning microbial nomenclature is to look up the characteristics of each genus and species of organism that is encountered in the classroom, in the laboratory, or in practice. With this practice, a lot of information will quickly become a part of working knowledge, on which one can build the sound practice of the profession.

TABLE 1: Noble prize winners in microbiology

1901	Behring	Antitoxins
1902	Ross	Malaria
1905	Koch	Bacteriology
1907	Laveran	Malaria
1908	Ehrlich and Metchnikoff	Theories of immunology
1913	Richet	Anaphylaxis
1919	Bordet	Immunology
1928	Nicolle	Typhus fever
1930	Landsteiner	Blood groups
1939	Domagk	Sulfonamide
1945	Fleming, Florey and Chain	Penicillin
1951	Theiler	Yellow fever vaccine
1952	Waksman	Streptomycin
1954	Ender	Cellular culture of polio virus
1958	Lederberg, Taitum and Beadle	Genetics
1959	Ochoa Kornberg	Genetics RNA
1960	Burnet and Medawar	Theories of immunity
1961	Watson and Crick	Genetic code, structure of DNA
1965	Jacob, Mond and Lwoff	Genetic episome and prephage
1966	Rous	Viral etiology of cancer
1968	Nirenberg, Holley and Khuranna	Synthesis of DNA
1969	Dulbeco, Luria and Delbruck	Genetics, mutations
1972	Porter and Edelman	Structure of immunoglobulin
1974	Christian	Lysosomes
1975	Dulbeco, Baltimore and Teonin	Genetics and mutations
1976	Gajdusck and Blumberg	Slow virus and Australia antigen
1977	Rosalyn Yalow	Radioimmunoassay
1978	Arber, Nathans and Smith	Restriction enzyme
1980	Snell, Dausset and Benacerrof	Major histocompatibility complex (MHC) and genetic control of immune response
1983	Barbara McClintoch	Mobile genetic element
1984	Georges Koehler and Danish Niele Jerne	Monoclonal antibodies
1987	Tonegawa Susuma	Generation of immunoglobulin diversity
1988	Gertrude Elion, George Hitchings and James Black	Discoveries of important principles that have resulted in the development of a series of new drugs including Acyclovir for herpes and AZT for treating AIDS
1989	Michael Bishop and Harold Varmus	Discovery of cellular origin of viral oncogenes
1993	Richard J Robert	Split genes
1997	Stanley B Prusiner	Prion
2005	Barry J Marshal and Roben Warren	*Helicobacter pylori* as causative agent of gastritis and peptic ulcer
2006	Andrew Fire and Craig Mello	RNA interference—gene silencing by DS RNA

Branches of Microbiology

Microbiology is one of the largest and most complex of the biological sciences because it integrates subject matter from many diverse disciplines. Microbiologists study every aspect of microbes their genetics, their physiology, characteristics that may be harmful or beneficial, the ways they interact with the environment, the ways they interact with hosts, and their uses in industry and agriculture.

Each major discipline in microbiology contains numerous subdivisions or specialties that deal with a specific subject area or field (Branches of Microbiology).

✶. *Branches of Microbiology*

1-Medical microbiology

2-Industrial microbiology

3-Food microbiology

4- Microbial Genetics &Molecular Biology deals with the function of genetic material and biochemical reactions of cells involved in metabolism and growth.

5- Microbial Ecology deals with Interrelationships between microbes and the environment; the roles of microorganisms in the nutrient cycles of soil, water, and other natural ecosystems.

Here we are concerned with ***medical microbiology***. It is studied under following headings:

A- ***Parasitology*** deals with the study of parasites causing diseases in human being.

B- ***Mycology*** deals with the study of fungi causing diseases in human beings.

C- ***Immunology*** is concerned with mechanism involved in the development of resistance by body to infectious diseases.

D- ***Bacteriology*** deals with the study of bacteria.

E- ***Genetics*** is the study of heredity and variations.

F- ***Virology*** is the study of viruses.

SCOPE OF MICROBIOLOGY

1- Diagnostic, e.g. isolation and identification of causative organism from the pathological lesions. We can also diagnose typhoid fever by doing Widal's test.

2- Prognosis of disease, e.g. in Widal's test rising titer signifies active disease and ineffective treatment. Falling titer means effective treatment and curing of disease.

3- Guidance in treatment, e.g. by culturing the organism in pure form and then performing drug sensitivity test we can suggest the effective drug for the treatment of that particular infection.

4- Source of infection, e.g. in sudden outbreak of infectious disease we can find out the source of infection.

5- Detection of new pathogens and then development of vaccines.

BACTERIAL TAXONOMY

Bacterial taxonomy comprises of three separate but interrelated important areas.

1-Classificalion: It is the arrangement of bacteria into taxonomic groups or taxa (in singular, taxon) on the basis of similarities or differences in their biochemical, physiological, genetic, and morphological properties.

2-Nomenclature: It refers to the naming of taxa according to their characteristics, by following the international rules.

3-Identification: It is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon.

CHAPTER CHECKPOINTS

- Taxonomy is the formal filing system scientists use to classify living organisms. It puts every organism in its place and makes a place for every living organism.
- The taxonomic system has three primary functions: classification, nomenclature, and identification of species.
- The eight major taxa, or groups, in the taxonomic system are (in descending order): domain, kingdom, phylum or division, class, order, family, genus, and species.
- The binomial system of nomenclature describes each living organism by two names: genus and species.
- Taxonomy groups organisms by phylogenetic similarity, which in turn is based on evolutionary similarities in morphology, physiology, and genetics.
- Evolutionary patterns show a treelike branching from simple, primitive life forms to complex, advanced life forms.
- The Woese-Fox classification system places all eucaryotes in the Domain (Superkingdom) Eukarya and subdivides the procaryotes into the two Domains Archaea and Bacteria.
- The Whittaker five-kingdom classification system places all bacteria in the Kingdom Procaryotae and subdivides the eucaryotes into Kingdoms Protista, Myceteae, Animalia, and Plantae.

BIOLOGICAL WEAPONS

Now it is quite clear and understandable that biological warfare is not new at all. Biological warfare was reported by early Romans who polluted water sources of their enemies by dumping animal carcasses. Then British distributed blankets to Indians in 1763. These blankets had been used by smallpox patients. Hence Indian users contracted smallpox. British had detonated an experimental anthrax bomb in Gruinard Island in Second World War.

In 1984, 750 people fell ill to food poisoning in Oregaon because of spread of salmonella that had been cultured in the laboratory.

Biological warfare may be defined as intentional use of doses to harm or kill an adversary military forces, population, food or livestock and includes any living, or nonliving organisms or its bioactive substance (toxin). Hence, germ warfare can be spearheaded by bacteria, viruses, fungi, toxins, etc.

Organisms which can be used for biological war are ***Bacillus anthracis*** (causing pneumonia with a mortality rate 95%, if untreated), **smallpox** (contagious with high mortality rate), ***Yersinia pestis*** (plague causing bacteria), ***Francisella tularensis*** (tularemia), Ebola and Marburg viruses (hemorrhage fever), ***Clostridium botulinum*** (botulinism), etc.

Molecular Epidemiology

Molecular epidemiology is defined as a science that focuses on the contribution of potential genetic and environmental risk factors, identified at molecular level, to the etiology, distribution and prevention of diseases within families, countries and continents. Genetic variability within and between infectious agents/disease pathogens forms the foundation of molecular microbiology.

Table-2 Epidemic Prone Diseases

Ancient	Middle Era (killer diseases)	Middle Era (emerging/ reemerging)	Current Era	Threat of Rare Diseases
Smallpox	Cholera	O_{139}V. cholerae	Viral hepatitis	Ebola virus
Plague	Malaria	Dengue	HIV.	Yellow fever
Anthrax	Typhoid	Leptospira		Rift valley
	Tuberculosis			

-Application of Molecular Epidemiology

1- Tracing the source and origin of infection.

2- Tracking the routes of pathogen transmission.

3- Identifying reservoirs sustaining transmission.

4- Identifying new, emerging and reemerging pathogens.

5- DNA finger printing in actual diagnosis of pathogens.

6- Characterizing drug resistant strains.

7- Monitoring the progress of disease and central activities.

8- Identifying links between cases and infections.

9- Linking pathogen variants to endemicity and epidemicity.

10- Monitoring impact of immunization program, e.g. polio eradication.

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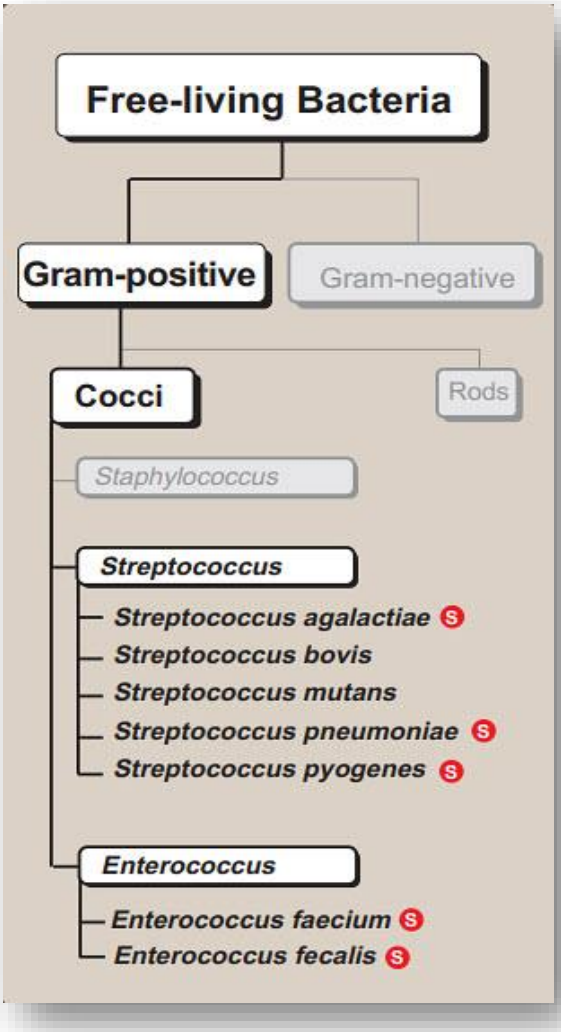
Streptococci, Enterococci, and Related Genes

The streptococci are Gram- positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human flora; others are associated with important human diseases attributable in part to infection by streptococci.

Streptococci and ***staphylococci*** constitute the main groups of medically important **gram-positive cocci**. Streptococci are ***gram-positive***, ***nonmotile,*** and ***catalase-negative***. Clinically important genera include ***Streptococcus*** and ***Enterococcus***. They are ovoid to spherical in shape, and occur as pairs or chains.

Most are ***facultative anaerobes*** (capable of growing in the absence or presence of oxygen), but grow fermentatively even in the presence of oxygen. Because of their complex nutritional requirements, blood-enriched medium is generally used for their isolation.

Diseases caused by this group of organisms include ***acute infections of the throat and skin caused by Group A streptococci***(***Streptococcus pyogenes***); ***female genital tract colonization***, resulting in **neonatal sepsis** caused by **Group B streptococci** (***Streptococcus agalactiae***); ***pneumonia, otitis media, and meningitis*** caused by ***Streptococcus pneumoniae***; and ***endocarditis*** caused by the ***viridans group of streptococci***.



♦. Morphology and Identification

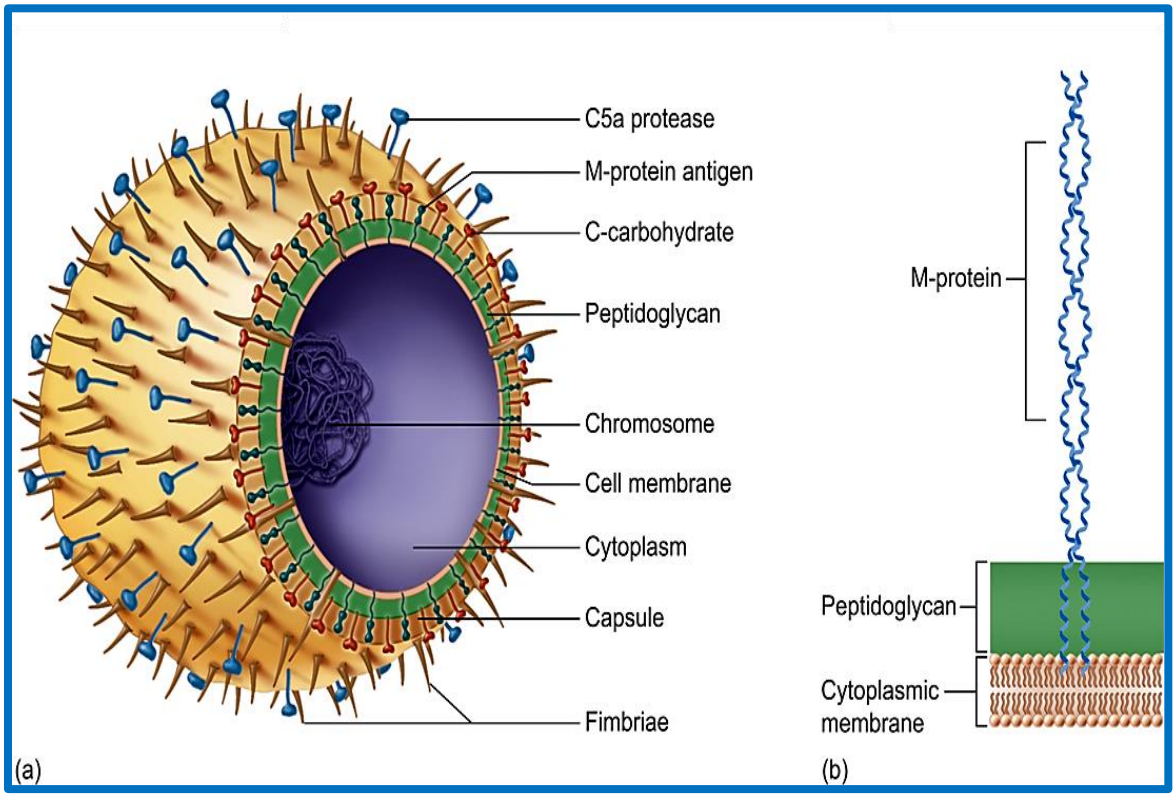
A- Typical organisms

Individual cocci are spherical ore ovoid and are arranged in chains. The cocci divide in a plane perpendicular to the long axis of the chain.The members of the chain often have a striking diplicoccal appearance, and rod-like forms are occasionally seen. The lengths of the chains vary widely and are conditioned by environmental factors.

Most **group A** strains produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede **phagocytosis**. The hyaluronic acid capsule likely plays a greater role in virulence than is

generally appreciated and together with M protein was believed to be an important factor in the resurgence of rheumatic fever (RF) in the United States in the 1980s and 1990s.The capsule binds to hyaluronic-acid-binding protein, CD44, present on human epithelial cells. Binding induces disruption of intercellular junctions allowing microorganisms to remain extracellular as they penetrate the epithelium. Capsules of other streptococci (eg, ***S. agalactiae and S. pneumoniae***) are different.

The ***S. pyogenes*** **cell wall** contain proteins (**M,T,R** antigens), carbohydrates (**group- specific**), and peptidoglycans . **Hair – like pili** project through the capsule of group **A streptococci**. The pili consist partly of **M ptotein** and are covered with **lipotechoic acid**. The latter is important in the attachment of streptococci to epithelial cells.



B- Culture

Most streptococci grow in solid media as discoid colonies, usually 1–2 mm in diameter. *S pyogenes* is β-hemolytic; other species have variable hemolytic characteristics.

C- Growth Characteristics

Energy is obtained principally from the utilization of sugars .Growth of streptococci tends to be poor on solid media or in broth unless enriched with blood or tissue fluids. Nutritive requirements vary widely among different species. The human pathogens are most exacting, requiring a variety of growth factors. Growth and hemolysis are aided by incubation in **10**% CO_2.

Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

.Classification of streptococci

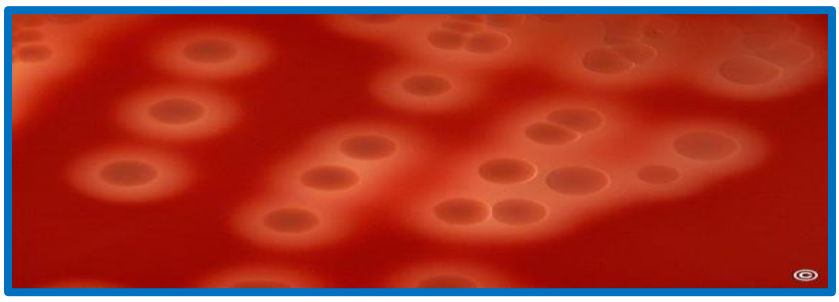
Four different classification systems exist for this important microorganism:

A - Hemolysis

Brown on the basis of red blood cell lysis on blood agar plates, divides the streptococci into:

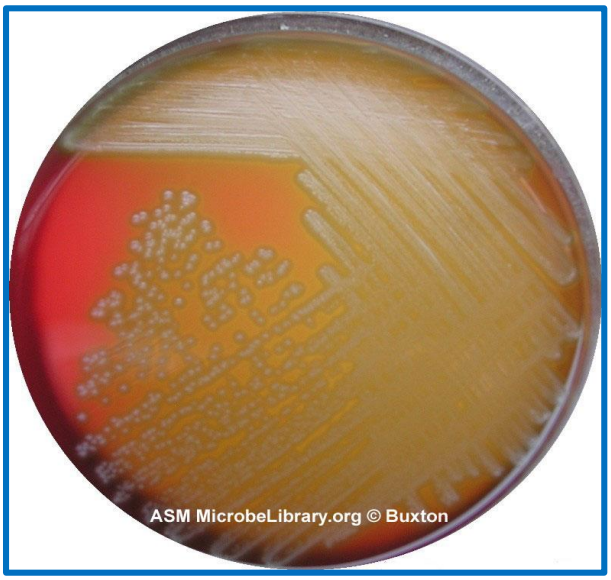
1- Beta hemolytic streptococci (β- Hemolysis)

This type produce wide, clear, translucent zone of complete hemolysis around the colony (complete disruption of erythrocytes with release of hemoglobin),e.g ***Streptococcus pyogenes***.



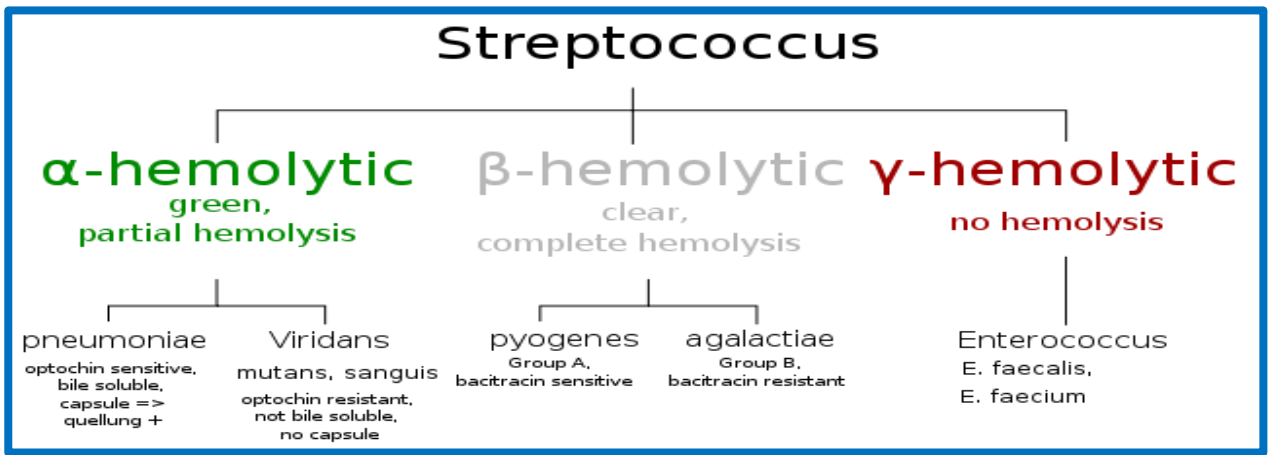
2 - Alpha hemolytic streptococci (α- Hemolysis)

They produce green zone around the colony due to incomplete lysis of erythrocytes with the formation of green pigment, eg. ***Streptococcus pneumoniae***.



3- Non hemolytic streptococci (ϒ- Hemolysis)

No change on the blood agar, e.g. ***Streptococcus bovis***.



B- Serologic Group-Specific Substance (Lancefield Classification)

The most precise and useful classification, has developed from the work of **Rebecca Lancefield**. She extracted serologically reactive material (the carbohydrate group – specific substances) with hot dilute hydrochloric acid and using precipitin techniques (with specific antisera). The serologic specificity of the group specific carbohydrate is determined by an amino sugar.

Streptococci can be placed into groups, referred to as **Lancefield groups**, based on the major cell wall carbohydrates they possess. Commercial agglutination kits are available for streptococcal grouping. At least **18** groups are recognized, designated groups **A** through **H** and **K** through **U**, but not all are equally important as human pathogens.

-The following are worthy of note:

1-Group A includes the important human pathogen ***Streptococcus pyogenes***.

2- Group B contains one species, ***Streptococcus agalactiae***, an inhabitant of the female tract; causes infection in neonates.

3- Group C mainly causes diseases in animals.

4- Group D includes the enterococci (***Streptococcus faecalis***, etc.)

C - Capsular Polysaccharides

The antigenic specificity of the capsular polysaccharides is used to classify ***Strep. pneumoniae*** into **90** types and to type the **group BStreptococci** (***Strep. agalactiae***).

D-Biochemical Reactions

Biochemical tests include **sugar fermentation reactions**, tests for the **presence of enzymes**, and tests for **susceptibility** or **resistance to certain chemical agents**. **Biochemical test** are most often used to classify **streptococci** after the colony growth and hemolytic characteristics have been observed.

Biochemical tests are used for species that typically do not react with the commonly used antibody preparations for the group-specific substances, groups A, B, C, F, and G.

Streptococci of particular medical interest

♦. Streptococcus pyogenes (Group A)

Habitat and transmission

Normal habitat is the human upper respiratory tract and skin; may survive in dust for some time. Spread by airborne droplets and by contact.

Characteristics

A commensal in the nasopharynx of a minority of healthy adults but more commonly (about 10%) in children. Grows well on blood agar ,with a characteristic halo of **β- haemolysis** . Some strains produce mucoid colonies as result of having a hyaluronic acid capsule. This may contribute to virulence by offering resistance to phagocytosis.

.Antigenic structure

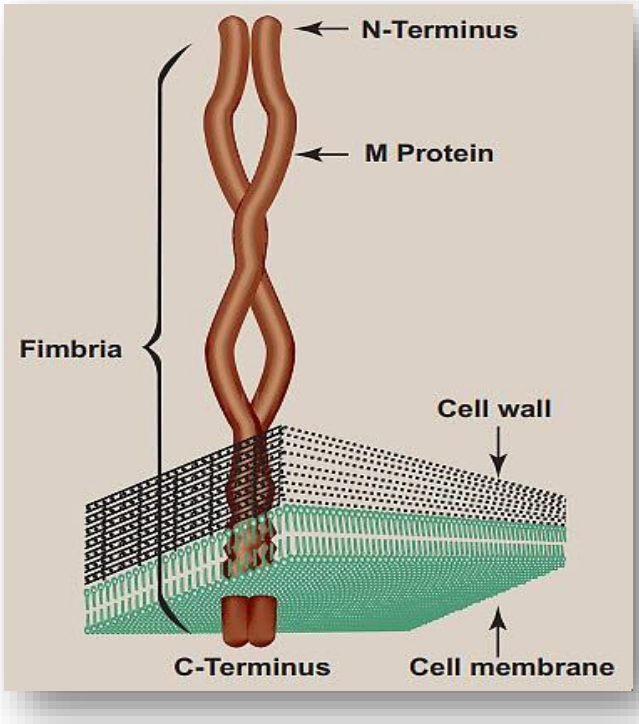
1- Group- specific cell wall antigen

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping (**Lancefield group** '**C**-substance' **A-H, K-U**).

2- M protein

This substance is a major virulence factor of **group A *S. pyogenes***. **M protein** is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall.

When **M protein** is present, the streptococci are virulent, and the absence of **M type-specific antibodies**, they are able to resist phagocytosis by polymorphonuclear leukocytes. **Group A streptococci** that lack **M protein** are not virulent. Because there are more than 150 types of M protein, a person can have repeated infections with ***S. pyogenes*** of different M types.



M protein is thought to serve as a virulence factor in two ways:

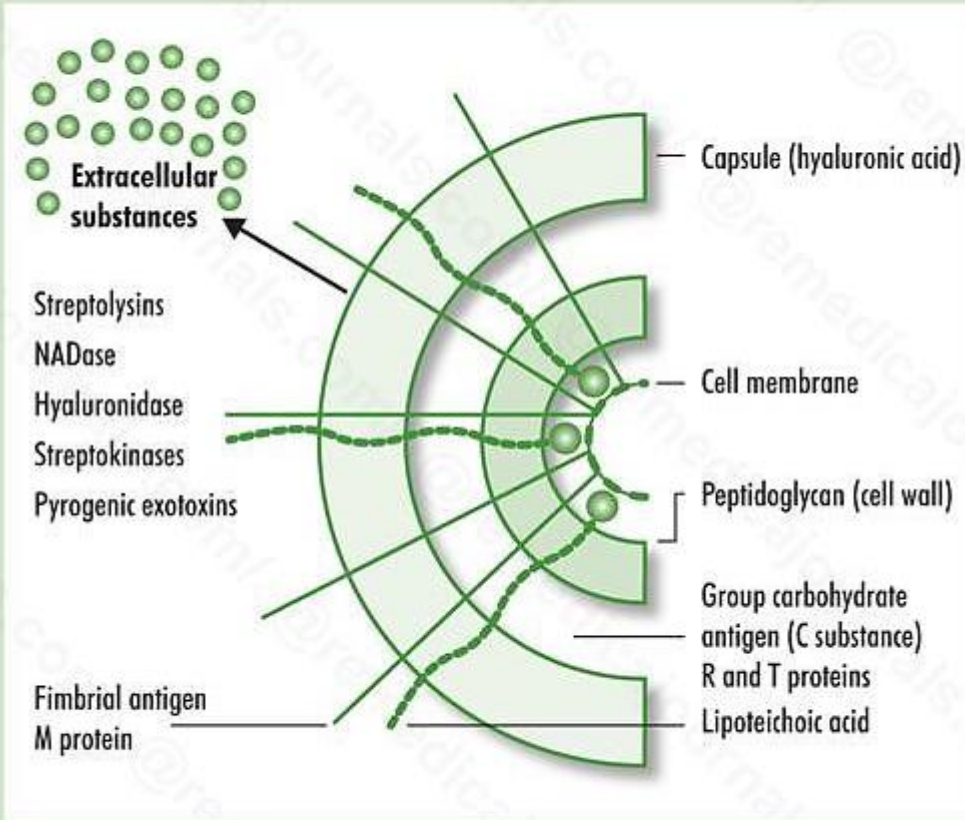
(1) by enabling *S. pyogenes* cells to adhere to surfaces and

(2) by functioning as an antiphagocytic.

3- T substance

This antigen has no relationship to virulence of streptococci. Unlike **M protein**, **T substance** is acid-labile and heat-labile. **T substance** permits differentiation of certain types streptococci by agglutination with specific antisera, while other types share the same **T substance**. Yet another surface antigen has been called **R protein**.

Figure 1. Cell surface structure of *Streptococcus pyogenes* and secreted products that are involved in virulence.



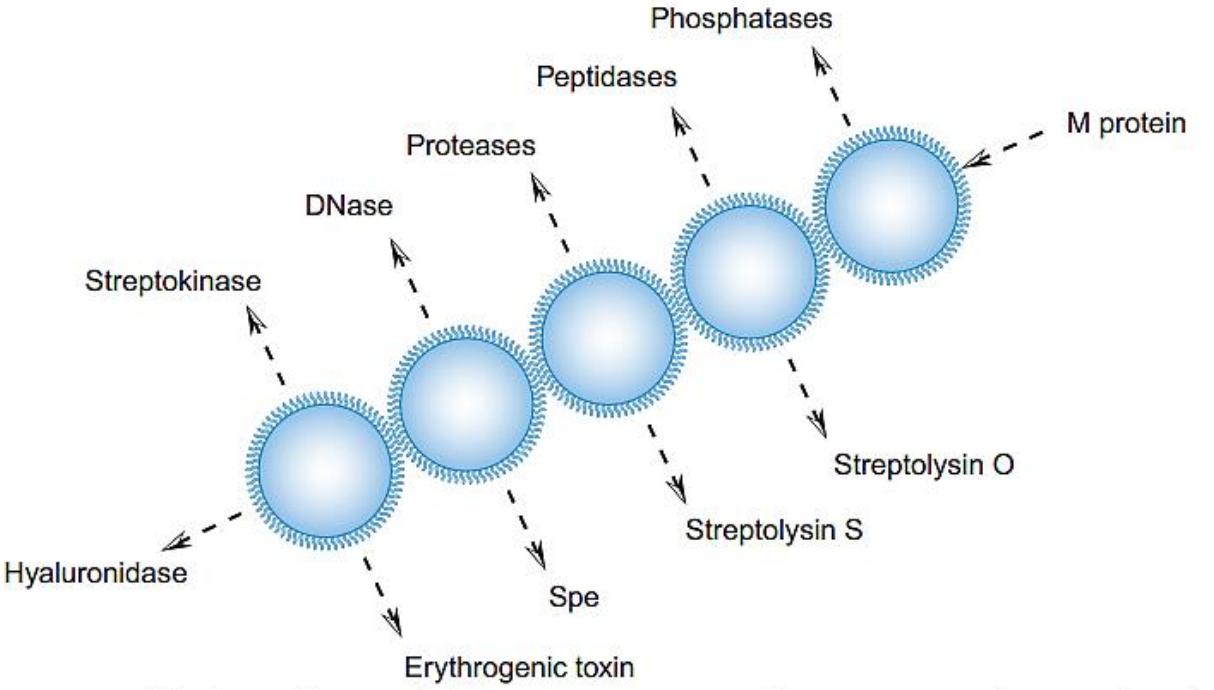
Reproduced with permission from Todar's Online Textbook of Bacteriology [69].

4- Protein F (fibronectin-binding protein)

Protein F mediates attachment to fibronectin in the pharyngeal epithelium. **M proteins** and lipoteichoic acids also bind to fibronectin.

♦.Toxins and Enzymes

More than 20 extracellular products that are antigenic are elaborated by ***S. pyogenes***, including the following:

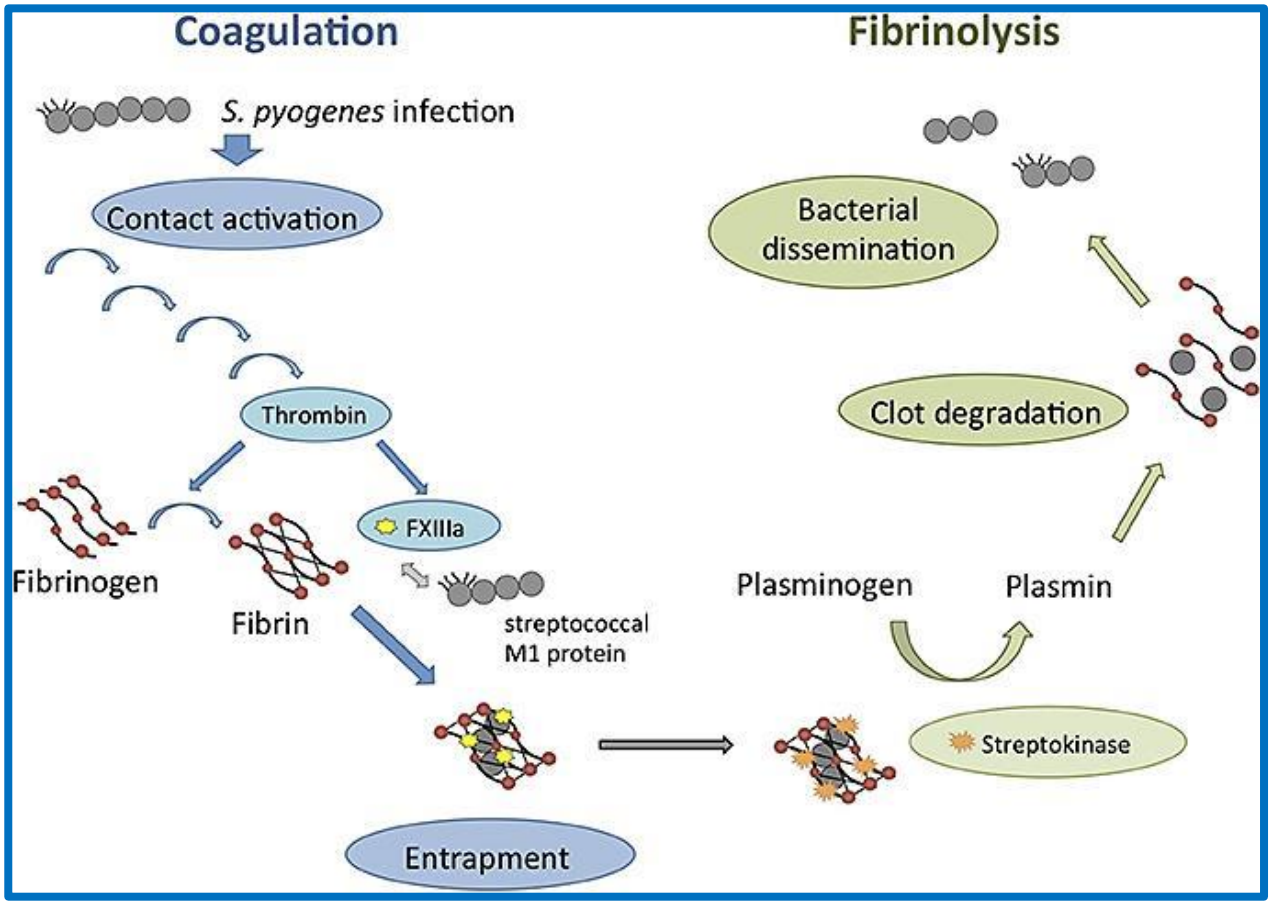


Virulence factors of *Streptococcus pyogenes*. Spe, streptoccocal pyrogenic toxin.

A- Sreptokinase (Fibrinolysin)

Streptokinase is produced by many strains of **group A** β-hemolytic streptococci.It transforms the **plasminogen** of human plasma into **plasmin**, an active proteolytic enzyme that digests **fibrin** and other proteins. This process of digestion may be interfered with by nonspecific serum inhibitors and by a specific antibody, antistreptokinase.

Streptokinase has been given intravenously for treatment of pulmonary emboli and of coronary artery and venous thrombosis.



B-Streptodornase

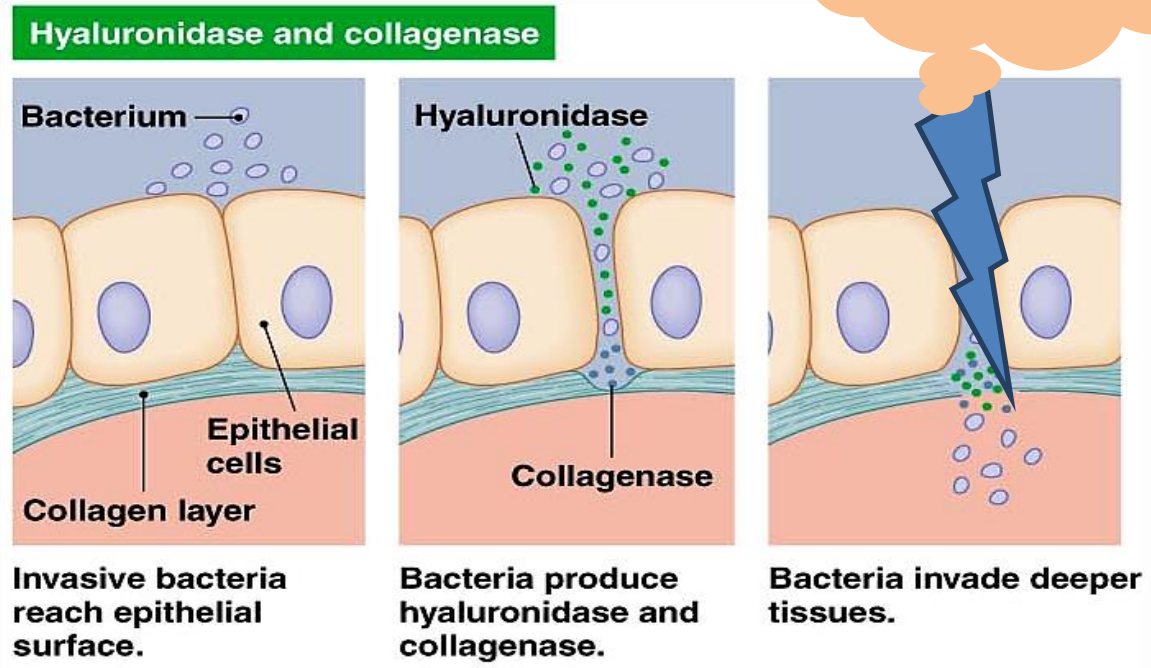
Streptodornase depolymerizes DNA. The enzymatic activity can measured by the decrease in viscosity of known DNA solutions.

Mixture of **streptodornase** and **streptokinase** are used in "enzymatic debridement". They help to liquefy exudates and facilitate removal of pus and necrotic tissue; antimicrobial drugs thus gain better access, and infected surfaces recover more quickly.

C- Hyaluronidase

Hyaluronidase cleaves hyaluronic acid (an important component of the ground substance of connective tissue) into numerous small fragments, thus converting the gel state of the extracellular matrix to a sol state . The consequence of this reaction is to permit the rapid spread of the infecting microorganisms through the connective tissue spaces (so called **spreading factor**).

Digestion of connective tissues and Invasion of tissues



D- Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by **group A streptococci**. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever.

E- Diphosphyridin Nucleotidase

This enzyme is elaborated into the environment by some streptococci. This substance may be related to the organism's ability to kill leukocytes. Proteinases and amylase are produced by some strains.

F-Hemolysins

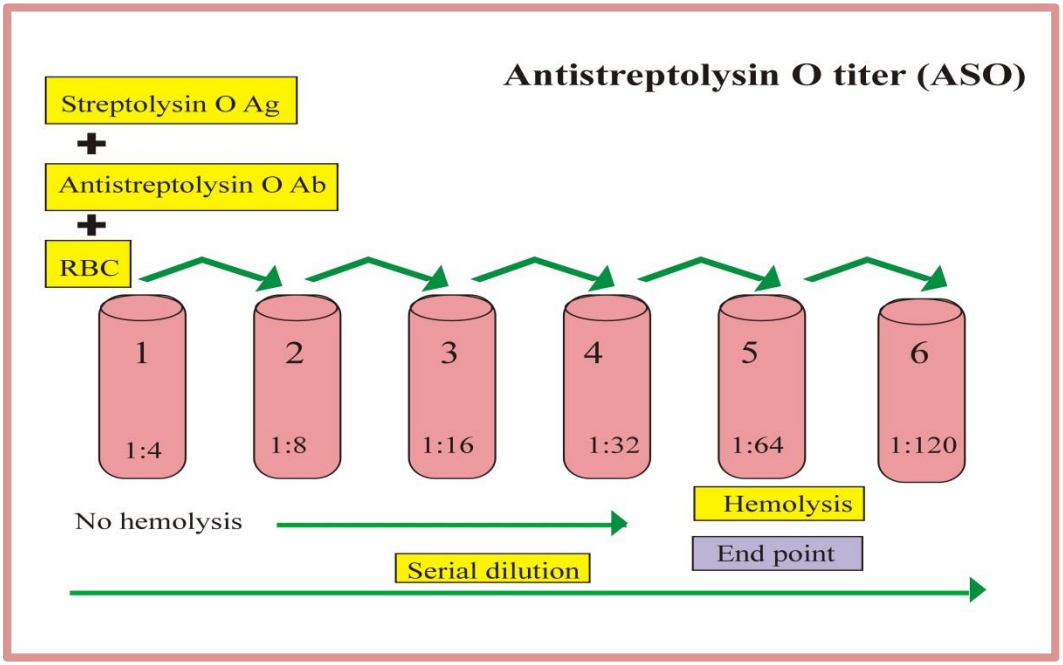
Many streptococci are able to hemolyze red blood cells in vitro in varying degrees.**β-Hemolytic group A *Strep. pyogenes*** elaborates two hemolysins (**streptolysins**) :

(i)- Streptolysin O

Streptolysin O is a protein that is hemolytically active in the reduced state but rapidly inactivated in the presence of oxygen. **Streptolysin O** is responsible for some of the hemolysis seen when growth is in cuts deep into the medium in blood agar plates.

Streptolysin O combines quantitatively with **antistreptolysin O** (**ASO**), an antibody that appears in humans following infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O.

This phenomenon forms the basis of a quantitative test for the antibody. An **antistreptolysin O (ASO)** serum titer in excess of **160-200 units** is considered abnormally high and suggests either recent infection with streptococci or persistently high antibody levels due to an exaggerated immune response to an earlier exposure in a hypersensitive person.



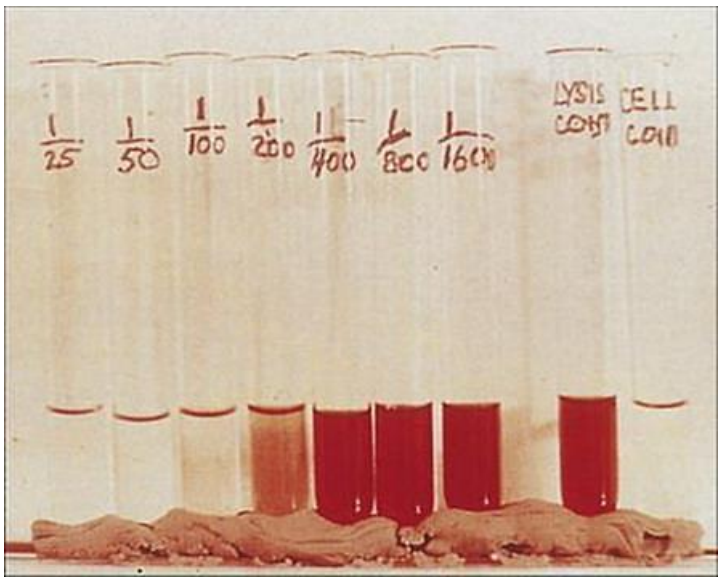


Figure-Illustration of the **anti-streptolysin O (ASO) test** which is now performed primarily by automated instrumentation**. The O-toxin lyses red cells.** Test serum is diluted until the antibodies it contains it no longer inhibit lysis by a standard concentration of toxin**. Positive and negative controls** are included in the test **(right).**

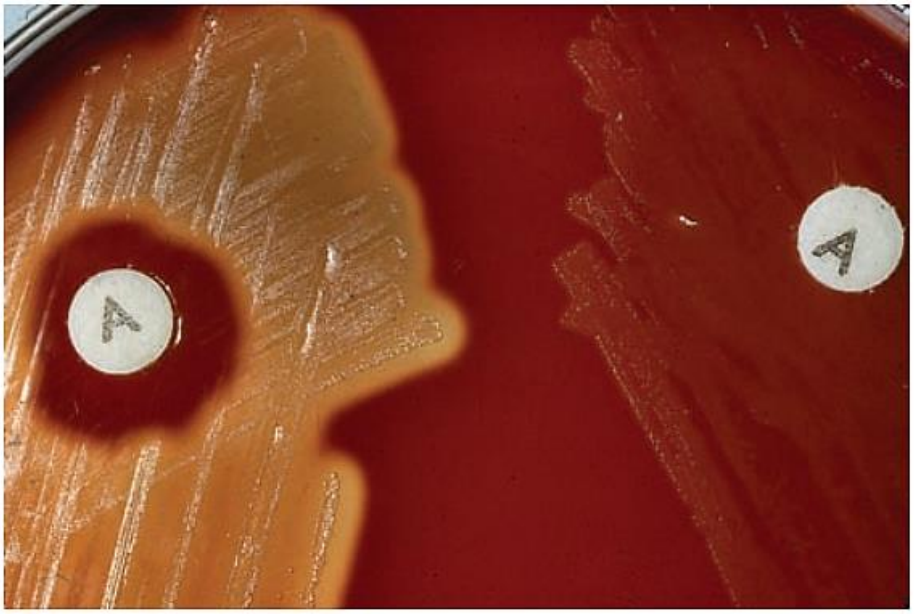
(ii)- Streptolysin S

Streptolysin is the agent responsible for the hemolytic zones around streptococcal colonizes growing on the surface of blood agar plates. It is elaborated in the presence of serum, hence the name **streptolysin S**.

Note : Not all these products are produced by every strain ; the combined action of enzymes and toxins contribute to the pathogenicity.

Culture and identification

Culture on blood agar yields characteristic **β-haemolytic** colonies (lysis of blood due to **streptolysins O** and **S**) . A Gram – Strained smear shows Gram positive cocci in chains ; which are well developed in liquid rather than in solid media . The isolate can be presumptively identified as ***Steptococcus pyogenes*** if it is sensitive to **bacitracin**.



Bacitracin sensitivity test (also known as the A-disk test). *Streptococcus pyogenes*, to the left, is susceptible to bacitracin, whereas *Streptococcus agalactiae*, to the right, is resistant. (From Winn WC Jr, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Ed. Philadelphia: Lippincott Williams & Wilkins, 2006.)

If rheumatic fever is suspected , then testing the patient`s **antistreptolysin O** (**ASO**) antibody titer will demonstrate previous exposure to ***Steptococcus pyogenes***.

Pathogenicity

Steptococcus pyogenes cause a number of infections ; the most notable are :

- Tonsillitis and pharyngitis
- Peritonsillar abscess (now rare)
- Scarlet fever
- Mastoiditis and sinusitis
- Otitis media (middle – ear infection)
- Wound infections leading to cellulitis and lymphangitis.
- Impetigo (a skin infection).

- **Invasive group A streptococcal disease**: Common during the first half of the century, invasive group A streptococcal (GAS) disease became rare until its resurgence during the past decade. Patients may have a deep local invasion either without necrosis (cellulitis) or with it (necrotizing fasciitis/myositis) [Note: The latter disease led to the term "flesh-eating bacteria].Invasive GAS disease often spreads rapidly, even in otherwise healthy individuals, leading to bacteremia and sepsis. Symptoms may include a toxic shock–like syndrome, fever, hypotension, multiorgan involvement, a sunburn-like rash, or a combination of these symptoms.
- **Streptococcal toxic shock syndrome (Toxic Shock–Like Syndrome [TSLS]):** This syndrome is defined as isolation of group A β-hemolytic streptococci from blood or another normally sterile body site in the presence of shock and multiorgan failure. The syndrome is mediated by the production of streptococcal pyrogenic exotoxins that function as superantigens causing massive, nonspecific T-cell activation and cytokine release. Patients may initially present with flulike symptoms, followed shortly by necrotizing soft tissue infection, shock, acute respiratory distress syndrome, and renal failure. Treatment must be prompt and includes antistreptococcal antibiotics, usually consisting of high-dose penicillin G plus clindamycin.

Complications (Sequelae)

After an episode of infection some patients develop complications, such as rheumatic fever , glomerulonephritis and erythema , which may have long – lasting effects . Note that :

- **In cellulitis** , hyaluronidase (spreading factor) mediates the subcutaneous spresd of infection.
- Erythrogenic toxin causes the **rash** of scarlet fever.
- Post –Streptococcal infection, manifesting as:

A- rheumatic fever : This autoimmune disease occurs 2 to 3 weeks after the initiation of pharyngitis. It is caused by cross-reactions between antigens of the heart and joint tissues, and the streptococcal antigen (especially the M protein epitopes). It is characterized by fever, rash, carditis, and arthritis.

B- A cute glomerulonephritis is caused by immune complexes bound to glomeruli.

Treatment and prevention

S. pyogenes has not developed resistance to **penicillin**, so **penicillin** remains the drug of choice to treat strep throat and most other *S. pyogenes* infections. Some strains have developed resistance to **erythromycin** and many strains are resistant to **tetracycline**. No vaccine available.

In a penicillin allergic patient, a macrolide such as **clarithromycin** or **azithromycin** is the preferred drug. **Penicillin G** plus **clindamycin** are used in treating necrotizing fasciitis and in streptococcal toxic shock syndrome. **Clindamycin** is added to penicillin to inhibit protein (i.e., toxin) synthesis so that a huge amount of toxin is not released abruptly from rapidly dying bacteria.

♦. *Streptococcus agalactiae* (Group β)

Increasingly recognized as a human pathogen, especially as a cause of neonatal meningitis and sepsis.

Habitat and Transmission

The human vagina , and sometimes anorectal carriage. Babies acquire infection from colonized mother during nursing .Common in cattle and cause bovine mastitis .

Characteristics

Gram – positive cocci in chains. Catalase-negative organisms.

Culture and identification

Gram – stained smear and culture which yield β- haemolytic colonies on blood agar ; colonies on blood agar are generally larger colonies and less hemolysis than group A *Steptococcus pyogenes*. Lancefield group determined by antiserum against cell wall polysaccharide.

Virulence factors

Encapsulated strains of GBS are resistant to phagocytosis by white blood cells. Other potential virulence factors include hemolysin, CAMP factor, peptidase, and hyaluronidase.

Pathogenicity

Cause neonatal meningitis and septicemia; also associated with septic abortion and gynecological sepsis .

Treatment

Penicillin and ampicillin are the antibiotics of choice ; **Erythromycin** in patients hypersensitive to penicillin.

In life-threatening infections, an aminoglycoside can be added to the regimen. [Note: Pregnant carriers should be treated with ampicillin during labor if risk factors such as premature rupture of membranes or prolonged labor are present]. Intrapartum prophylaxis of group B streptococcal carriers and administration of antibiotics to their newborns reduce neonatal group B streptococcal sepsis by as much as 90 percent.

♦. Enterococci

These belong to lancefield **group D** and include ***Enterococcus faecalis*** and other less important species.

Habitat and Transmission

Normal intestinal inhabitants.

Culture and identification

Most strains non-haemolytic, grow readily on bile-containing media, e.g. MacConkey medium. Their ability to grow in 6.5% sodium chloride, which is seven times the concentration found in normal tissue fluids.

Pathogenicity

Most commonly cause acute infections of the urinary tract, and sometimes wound infections, especially after intestinal operation.

Treatment

Antimicrobial susceptibility testing is very important for enterococci.

♦. Viridans Streptococci

This is mixed group of streptococci with variable characteristics. Hence the nomenclature of this group is in a constant state of flux. Typically they are α-hemolytic, but they may be nonhemolytic. These streptococci principally live in the oropharynx. Oral streptococci can be divided into four main 'species group' as follows:

1- ***Steptococcus mutans*** **group**

2- ***Steptococcus salivarius*** **group**

3- ***Steptococcus milleri*** **group**

4- ***Steptococcus oralis*** **group**

Each of the above groups comprise a number of species.

Habitat and Transmission

The viridans group of streptococci are common inhabitants of the oral cavity and comprise roughly one-quarter of the total cultivable flora from supragingival and gingival plaque and one-half of the isolates from the tongue and saliva. They are vertically transmitted from mother to child.

Infection endocarditis caused by the viridans group is generally a result of their entry into the bloodstream during intraoral surgical procedures (e.g. tooth extraction), and sometimes even during tooth brushing.

Culture and identification

Gram-positive cocci in chains; α-hemolytic; catalase negative. Growth not inhibited by bile or optochine (ethylhydrocupreinhydrochloride), in contrast to pneumococci. Commercially available kits are highly useful in laboratory identification of these organisms (e.g. API kit system).

Pathogenicity

The major agent of dental caries is *Steptococcus mutans* . They have a characteristic ability to produce voluminous amounts of sticky, extracellular polysaccharides in the presence of dietary carbohydrates, these help tenacious binding of the organisms to enamel and to each other.

Steptococcus mutans may act as an opportunistic pathogen under a number of circumstances. Breakdown of the dental enamel from the acidic fermentation products in the development of the carious lesion results in invasion of dentin by the microorganism and eventually in pulpal infection. *Steptococcus mutans* has been isolated from the root canals of such teeth but at a low incidence. It has been demonstrated experimentally that *Steptococcus mutans* can destroy periapical bone when it is inoculated into the dental pulp and that the same organism is isolated from the blood as late as 21 days after the inoculation.

They are also important agents of infective endocarditis. Usually bacteria released during dental procedures settle on damaged heart valves, causing infective endocarditis.

A not on *Steptococcus mutans*

Steptococcus mutans gained notoriety in the 1960s when it was demonstrated that caries could be experimentally induced and transmitted in animals which were orally inoculated with the organism. The name 'mutans' results from its frequent transition from coccal phase to coccobacillary phase.

They are currently seven distinct species of human and animal mutans streptococci (***Steptococcus cricetus, Steptococcus downei, Steptococcus ferus, Steptococcus macacae, Steptococcus mutans***, ***Steptococcus rattus,*** and ***Steptococcus sobrinus***) the term ***Steptococcus mutans*** is limited to human isolates belonging to three serotypes (***c,e*** and ***f***).

Treatment

Prophylactic therapy against **endocarditis** resulting from *Steptococcus mutans* probably is most effective with the use of **Ampicillin** combined with **Gentamicin**. The organism is also sensitive to **Tetracycline**, **Vancomycin**, **Erythromycin**, and **Chloramphenicol**, but it is resistant to **Cephalosporins** and **Streptomycin**.

♦. Streptococcus Pneumoniae (Pneumococcus)

The pneumococci (strep. Pneumoniae) are gram-positive diplococci often lancet-shape or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are differentiated from many species of oral streptococci by being sensitive to optochin and soluble in bile.

Pneumococci are normal inhabitants of the upper respiratory tract of 5-40% humans. S. pneumoniae is the most common cause of community acquired pneumonia and adult bacterial meningitis and is an important cause of otitis media, sinusitis and mastoiditis. The risk of disease is highest among young children (Figure), older adults, smokers, and persons with certain chronic illnesses.

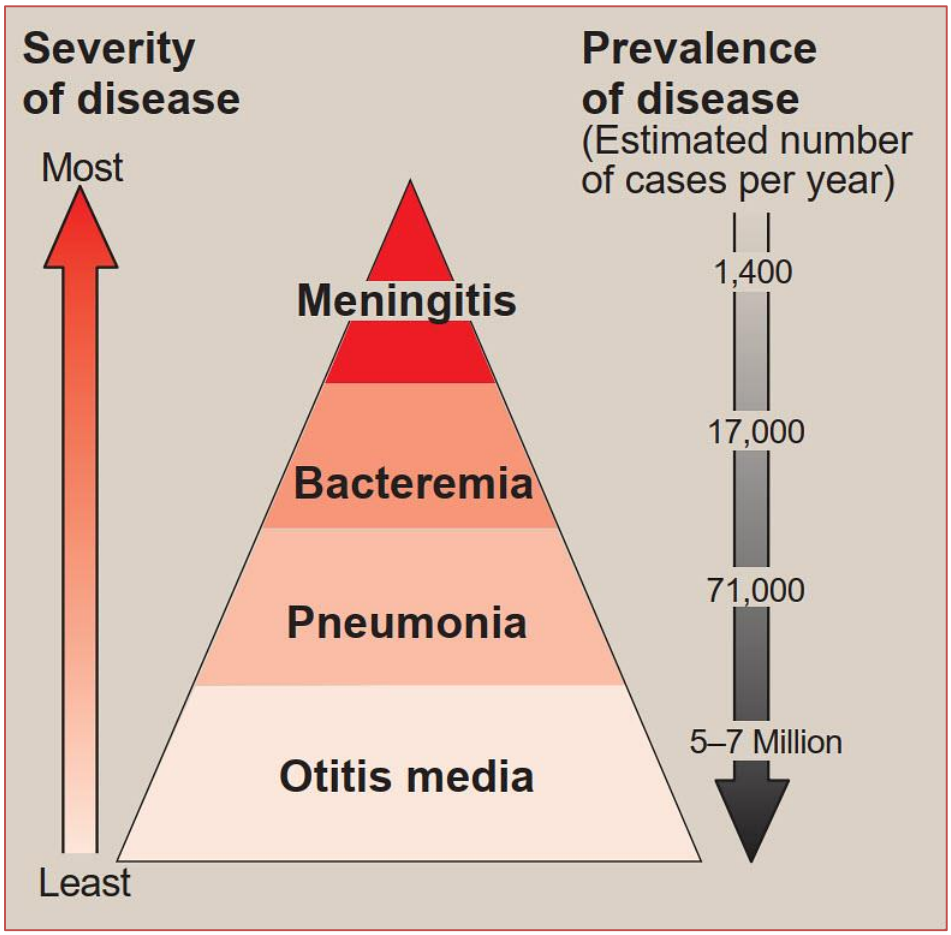


Figure Comparison of severity and prevalence of some pneumococcal infections in children in the United States.

Culture

Pneumococci form a small round colony, at central plateau with an elevated rim. Pneumococci are alph –hemolytic on blood agar.Growth is enhanced by 5-10% CO_2 .

Quellung Reaction

When pneumococci of a certain type are mixed with specific antipolysaccharide serum of the same type-or with polyvalent antiserum –on a microscope slide, the capsule swells markedly. This reaction is useful for rapid identification and for typing of the organisms, either in sputum or in cultures.

The polyvalent antiserum, which contains antibody to the 84 type (omni serum), is a good reagent for rapid microscopic determination of whether pneumo-cocci are present in fresh sputum.

Pathogenesis

A-Production of Disease

Pneumococci produce disease through their ability to multiply in the tissues. They produce no toxin of significance. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes.

A serum that contains antibodies against the type specific polysaccharide protects against infection.

B- Loss of Natural Resistance

Since 40-70% of humans are at some time carries of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus. Among the factors that probably lower this resistance and thus predispose to pneumococcal infection are the Following:

1. Abnormalities of the respiratory tract

Viral and other infections that damage surface cells; abnormal accumulations of mucus (e.g. allergy), which protect pneumococci from phagocytosis.

2. Alcohol or Drug intoxication

Which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.

3. Abnormal circulatory dynamics (e.g. pulmonary congestion, heart failure).

4. Other mechanisms

Malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency.

Treatment

S. pneumoniae isolates were highly sensitive to penicillin G, the initial agent of choice, until the late 1980s. Since then, the incidence of penicillin resistance has been increasing worldwide. Most resistant strains remain sensitive to third generation cephalosporins (such as cefotaxime or ceftriaxone), and all are still sensitive to vancomycin. These antibiotics are therefore the agents of choice for invasive infections by penicillin-resistant strains of *S. pneumonia*.

♦. Peptostreptococcus and related Genera

These streptococci grow only under anaerobic or microaerophilic conditions and variably produce hemolysins. They are part of the normal microbiota of the mouth, upper respiratory tract, bowel, and female genital tract.

They often participate with many other bacterial species in mixed anaerobic infections. Such infections may occur in wounds in the breast, in postpartum endometritis, after rupture of an abdominal viscus, in the brain, or in chronic suppuration of the lung. The pus usually has a foul odor.

Table. Characteristics of Medically Important Streptococci.

Name	Group-Specific Substance[1]	Hemolysis[2]	Habitat	Important Laboratory Criteria	Common and Important Diseases
Streptococcus pyogenes	A	Beta	Throat, skin	Large colonies (> 0.5 mm), PYR[3] test positive, inhibited by bacitracin	Pharyngitis, impetigo, rheumatic fever, glomerulonephritis
Streptococcus agalactiae	B	Beta	Female genital tract	Hippurate hydrolysis, CAMP-positive[4]	Neonatal sepsis and meningitis
Streptococcus dysgalactiae subspecies *equisimilis;* others	C, G	Beta (human) infections), alpha, none	Throat	Large (> 0.5 mm) colonies	Pharyngitis, pyogenic infections similar to group A streptococci
Enterococcus faecalis (and other enterococci)	D	None, alpha	Colon	Growth in presence of bile, hydrolyze esculin, growth in 6.5% NaCl, PYR-positive	Abdominal abscess, urinary tract infection, endocarditis
Streptococcus bovis (non-enterococcus)	D	None	Colon	Growth in presence of bile, hydrolyze esculin, no growth in 6.5% NaCl, degrades starch	Endocarditis, common blood isolate in colon cancer
Streptococcus anginosus group (*S anginosus, S intermedius, S constellatus, S milleri* group)	F (A, C, G) and untypable	Alpha, beta, none	Throat, colon, female genital tract	Small (< 0.5 mm) colony variants of beta-hemolytic species. Group A are bacitracin-resistant and PYR-negative. Carbohydrate fermentation patterns	Pyogenic infections, including brain abscesses
Viridans streptococci (many species)	Usually not typed or untypable	Alpha, none	Mouth, throat, colon, female genital tract	Optochin-resistant. Colonies not soluble in bile. Carbohydrate fermentation patterns	Dental caries *(S mutans),* endocarditis, abscesses (with many other bacterial species)
Streptococcus pneumoniae	None	Alpha	Throat	Susceptible to optochin. Colonies soluble in bile, quellung reaction-positive	Pneumonia, meningitis, endocarditis
Peptostreptococcus (many species)	None	None, alpha	Mouth, colon, female genital tract	Obligate anaerobes	Abscesses (with multiple other bacterial species)

[1]Lancefield classification.

[2]Hemolysis observed on 5% sheep blood agar after overnight incubation.

[3]Hydrolysis of L-pyrrolidonyl-2-naphthylamide ("PYR").

[4]Christie, Atkins, Munch-Peterson test.

Streptococcus pyogenes
(group A, β-hemolytic)

- Acute pharyngitis or pharyngotonsillitis
- Acute rheumatic fever
- Erysipelas
- Puerperal sepsis
- Invasive group A streptococcal disease

1 Penicillin G[1,2]

2 Clarithromycin[3]
2 Azithromycin[3]

[1] *S. pyogenes* has not acquired resistance to penicillin G.
[2] Clindamycin may be added to pencillin G for soft tissue infection such as necrotizing fasciitis.
[3] For penicillin-allergic patient.

Streptococcus agalactiae
(group B, β-hemolytic)

- Meningitis and septicemia in neonates
- Endometritis
- Septicemia or pneumonia in individuals with impaired immune systems
- Diabetic foot infections

1 Penicillin G[4]
2 An aminoglycoside[5]

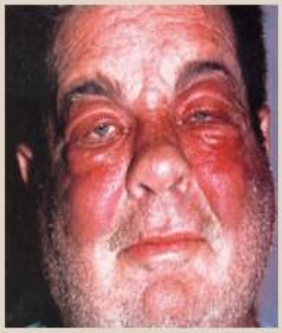
[4] All isolates remain sensitive to penicillin G and ampicillin.
[5] In life-threatening infections, an amino-glycoside can be added to the regimen.

Streptococcus pneumoniae
(α-hemolytic)

- Acute bacterial pneumonia
- Otitis media
- Meningitis

1 Penicillin G[6]
1 Cefotaxime
1 Ceftriaxone
2 Vancomycin[7]

[6] Penicillin G has been the drug of choice, but resistant strains are regularly seen.
[7] Most resistant strains remain sensitive to vancomycin.



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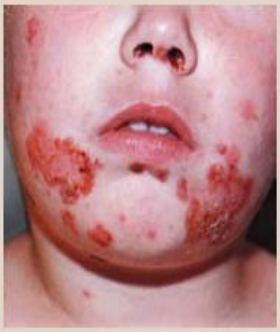
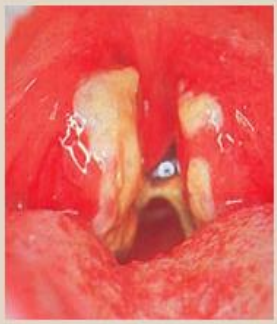


Impetigo



Streptococcal pharyngitis

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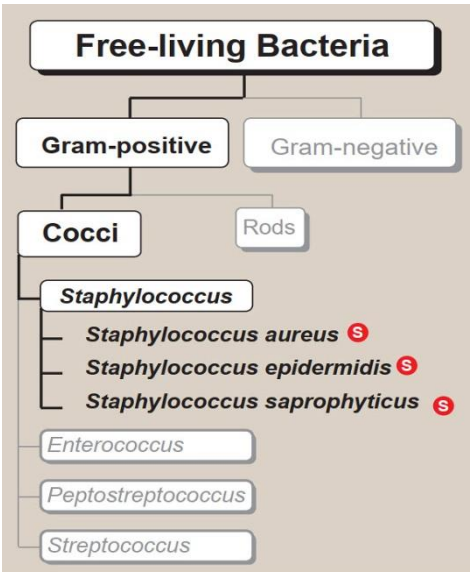
The Staphylococci

Staphylococci and **streptococci** constitute the main groups of medically important gram-positive cocci. Staphylococcal infections range from the trivial to the rapidly fatal. They can be very difficult to treat, especially those contracted in hospitals, because of the remarkable ability of staphylococci to become resistant to antibiotics. Staphylococci are ubiquitous in nature, with about a dozen species occurring as part of human flora. The most virulent of the genus, ***Staphylococcus aureus***, is one of the most common causes of bacterial infections, and is also an important cause of food poisoning and toxic shock syndrome. Among less virulent staphylococcal species, ***Staphylococcus epidermidis*** is an important cause of prosthetic implant infections, whereas ***Staphylococcus saprophyticus*** causes urinary tract infections, especially cystitis in women. Figure summarizes the staphylococci described in this chapter.



General Features

Staphylococci generally stain darkly gram positive. They are round rather than oval and tend to occur in bunches like grapes. Because growth of staphylococci requires supplementation with various amino acids and other growth factors, they are routinely cultured on enriched media containing nutrient broth and/or blood. Staphylococci are facultatively anaerobic organisms.

They produce catalase, which is one feature that distinguishes them from the **streptococci**[The catalase test can be used to differentiate between ***Staphylococcus* spp**. and ***Streptococcus* spp.** It is important for you to remember that virtually all ***Staphylococcus* spp.** are catalase positive (i.e., they produce catalase), whereas all ***Streptococcus* spp.** are catalase negative (i.e., they do not produce catalase)].

The most virulent species of ***staphylococcus*** is ***S. aureus***, almost all isolates of which secrete coagulase, an enzyme that causes citrated plasma to clot. Other species that occasionally cause disease and lack coagulase are often referred to as **coagulasenegative staphylococci**. Staphylococci are hardy, being resistant to heat and drying, and thus can persist for long periods on fomites (inanimate objects), which can then serve as sources of infection.

♦.Classification of Staphylococci

A- Based on pigment production

1- *Staphylococcus aureus* :- Golden-yellow pigmented colonies

2- *Staphylococcus albus* :- White colonies

3- *Staphylococcus citrus* :- Lemon yellow colonies

B-Based on coagulase production

Coagulase is an enzyme that causes the formation of clots. Specifically, coagulase catalyzes the conversion of a plasma protein called fibrinogen into a sticky substance called fibrin. ***Staphylococcus aureus*** is **coagulase positive**, which differentiates it from the other species. ***S aureus*** is a major pathogen for humans. Almost every person will have some type of ***S aureus*** infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections.

The **coagulase-negative staphylococci (CoNS)** are normal human microbiota and sometimes cause infection, often associated with implanted devices, such as joint prostheses, shunts, and intravascular catheters, especially in very young, old, and immunocompromised patients.

Approximately 75% of these infections caused by coagulase-negative staphylococci are caused by ***S epidermidis***; infections caused by ***S lugdunensis***, ***Staphylococcus warneri***, ***Staphylococcus hominis***, and other species are less common. ***S saprophyticus*** is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients. Other species are important in veterinary medicine.

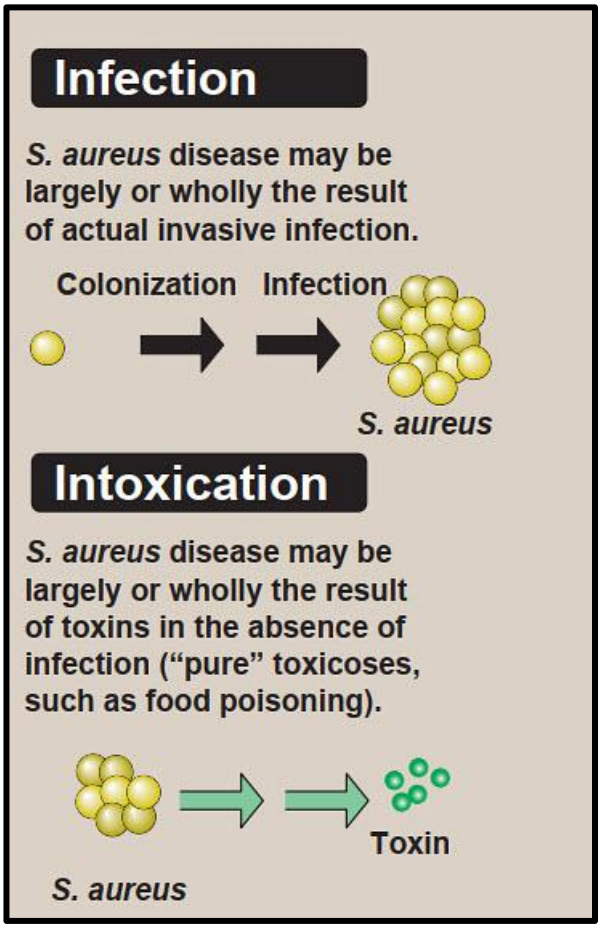
I. *Staphylococcus aureus*

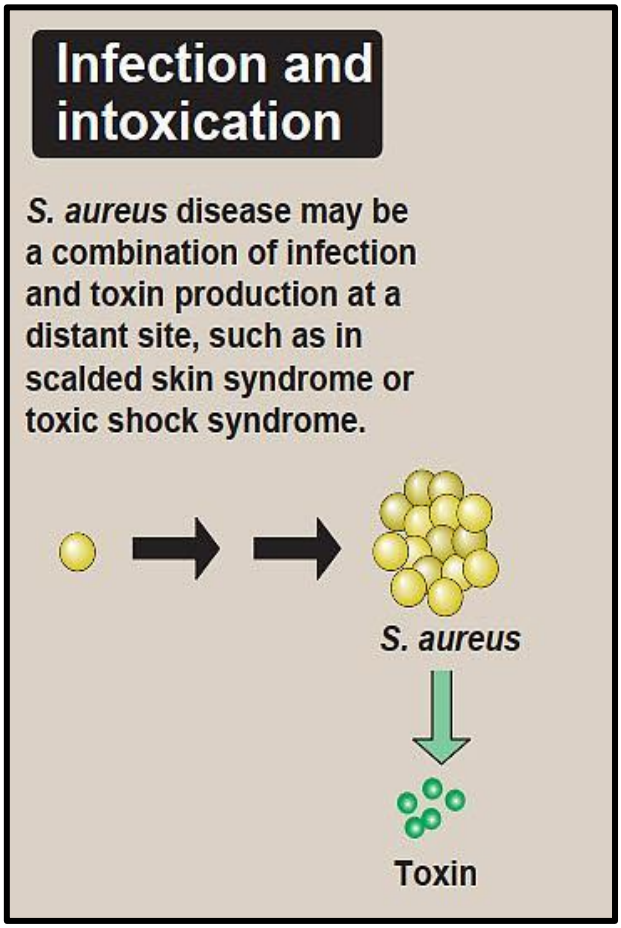
Generally, significant host compromise is required for *S. aureus* infection, such as a break in the skin or insertion of a foreign body (for example, wounds, surgical infections, or central venous catheters), an obstructed hair follicle (folliculitis), or a compromised immune system. *S. aureus* disease may be:

1. largely or wholly the result of actual invasive infection, overcoming host defense mechanisms, and the production of extracellular substances which facilitate invasion;

2. a result of toxins in the absence of invasive infection ("pure" toxinoses); or

3. a combination of invasive infection and intoxication.





A- Epidemiology

S. aureus is frequently carried by healthy individuals on the skin and mucous membranes. Carriers serve as a source of infection to themselves and others; for example, by direct contact, by contamination of fomites (objects such as a doorknob, which in turn can be a source of infection) or contamination of food, which can then result in food poisoning.

B. Pathogenesis

Virulence factors are the genetic, biochemical, or structural features that enable an organism to produce disease. The clinical outcome of an infection depends on the virulence of the pathogen and the opposing effectiveness of the host defense mechanisms. *S. aureus* expresses many potential virulence factors (Figure). For the majority of diseases caused by *S. aureus*, pathogenesis depends on the combined actions of several virulence factors, so it is difficult to determine precisely the role of any given factor.

Cellwall associated structures	Extracellular toxins	Enzymes
• Peptidoglycan • Capsule • Protein A • Clumping factor (bound coagulase)	• Haemolysin • Panton-Valentine leukocidin • Enterotoxin • TSST • Exfoliatin toxin	• Coagulase • Catalase • Staphylokinase • DNAase • β-lactamase • lipase • Phospholipase • hyaluronidase • proteinase

1. Catalase

Staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

2. Coagulase

S. aureus produces an extracellular coagulase, an enzymelike protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential.

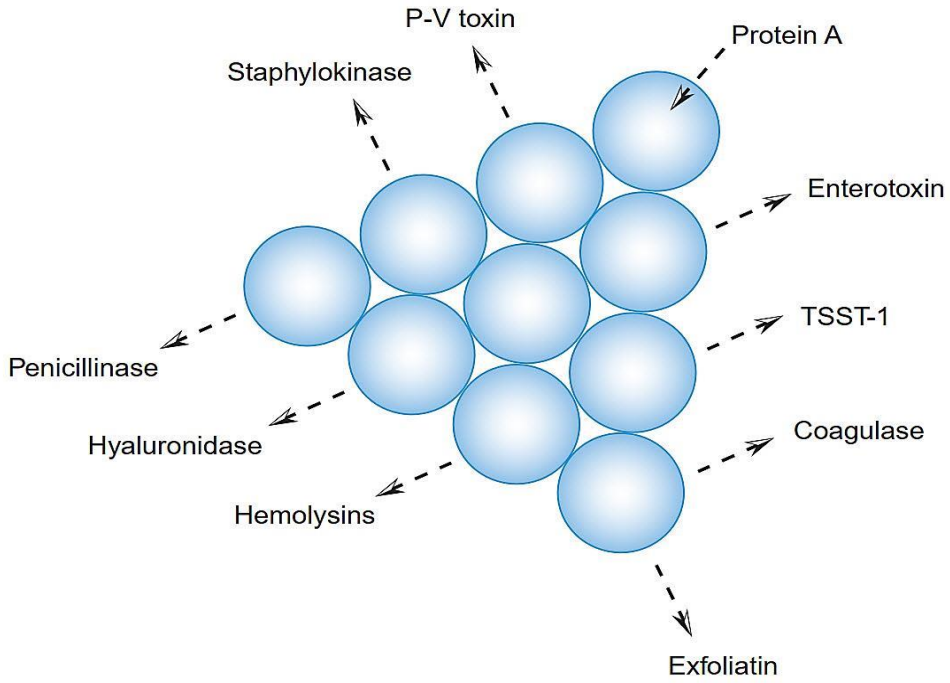


Figure. Virulence factors **of *Staphylococcus aureus*.** P-V, Panton-Valentine; TSST-1, toxic shock syndrome toxin 1.

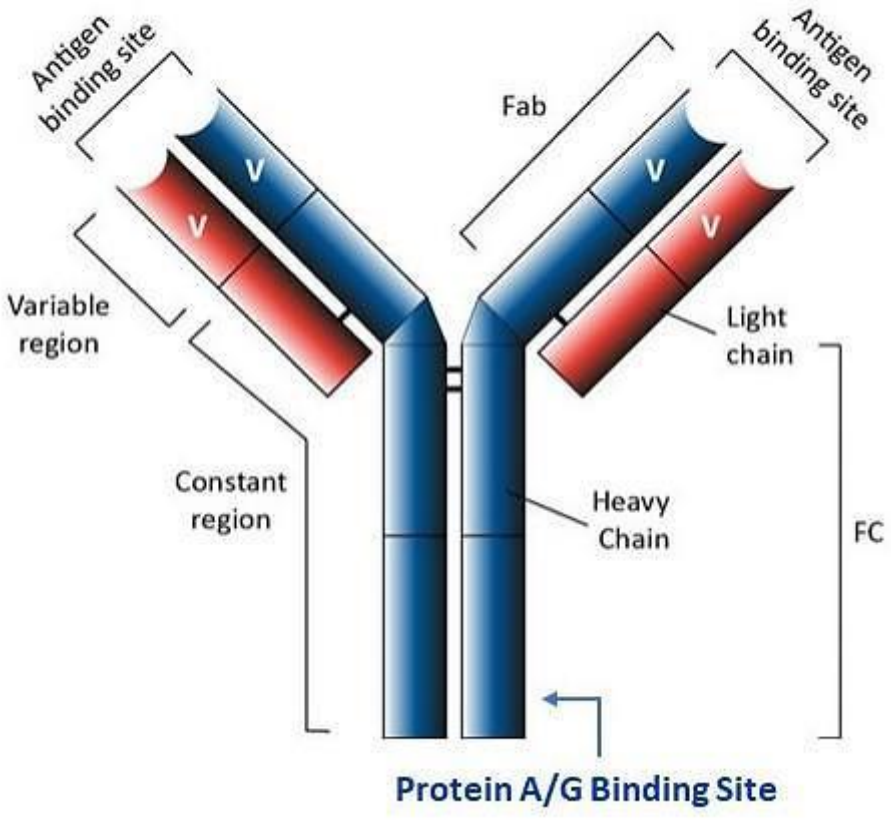
3. Cell wall virulence factors:

a. Capsule

The capsule layer is very thin but has been associated with increased resistance to phagocytosis. Clinical isolates produce capsule but expression is rapidly lost upon in vitro cultivation.

b. Protein A

Protein A is a major component of the *S. aureus* cell wall. It binds to the **Fc** region of IgG, exerting an anti-opsonin (and therefore strongly antiphagocytic) effect.



c. Fibronectin-binding protein: Fibrinectin-binding protein (**FnBP**) and other staphylococcal surface proteins promote binding to mucosal cells and tissue matrices.

d. Clumping factor A: is a virulence factor from *S. aureus* that binds to fibrinogen but does not convert fibrinogen to fibrin. When mixed with plasma, *S. aureus* forms clumps. Clumping factor is distinct from coagulase. Because clumping factor induces a strong immunogenic response in the host, it has been the focus of vaccine efforts. However, no human vaccines against this factor are available to date.

4. Hemolysins (Cytolytic exotoxins): *S. aureus* possesses four hemolysins that are regulated by ***agr***. α, β, γ, and δ Toxins attack mammalian cell (including red blood cell) membranes, and are often referred to as hemolysins. α Toxin is the best

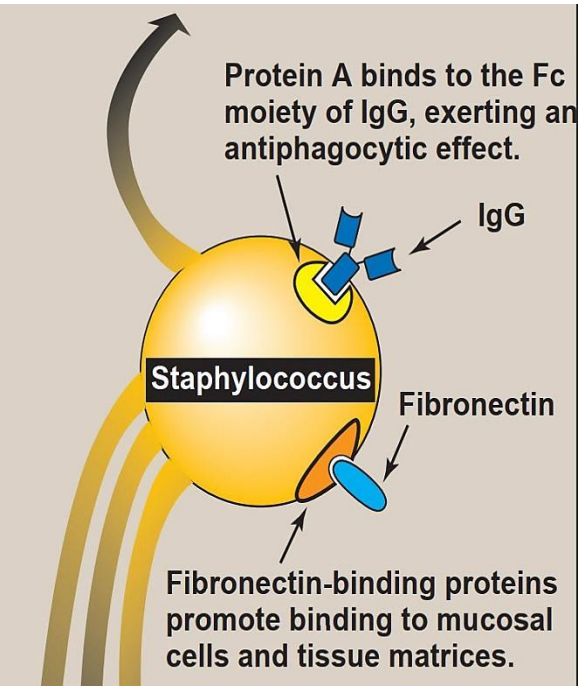
studied, and is chromosomally encoded. It polymerizes into tubes that pierce membranes, resulting in the loss of important molecules and, eventually, in osmotic lysis.

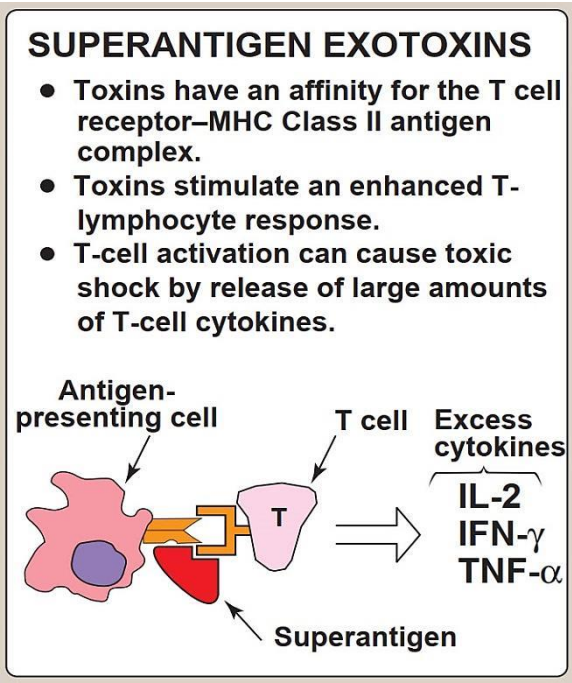
5. Other Enzymes

Other enzymes produced by staphylococci include a **hyaluronidase**, or spreading factor—a **staphylokinase** resulting in fibrinolysis but acting much more slowly than streptokinase, **proteinases**, **lipases**, and **β-lactamase**.

6. Panton-Valentine leukocidin: This pore-forming toxin lyses PMNs. Production of this toxin makes strains more virulent. This toxin is produced predominantly by community-acquired **methicillin-resistant *S. aureus*** (MRSA) strains.

7. Superantigen exotoxins: These toxins have an affinity for the T- cell receptor-major histocompatibility complex Class II antigen complex. They stimulate enhanced T-lymphocyte response (as many as 20 percent of T cells respond, compared with 0.01 percent responding to the usual processed antigens). This difference is a result of their ability to recognize a relatively conserved region of the T-cell receptor. This major T-cell activation can cause toxic shock syndrome, primarily by release into the circulation of inordinately large amounts of T-cell cytokines, such as interleukin-2 (IL- 2), interferon- γ (IFN- γ), and tumor necrosis factor-a (TNF- α).





a. Enterotoxins: Enterotoxins (six major antigenic types: A, B, C, D, E, and G) are produced by approximately half of all ***S. aureus*** isolates. When these bacteria contaminate food and are allowed to grow, they secrete enterotoxin, ingestion of which can cause food poisoning. [Note: The toxin stimulates the vomiting center in the brain by binding to neural receptors in the upper gastrointestinal (GI) tract.] Enterotoxins are superantigens that are even more heat-stable than ***S. aureus***. Therefore, organisms are not always recovered from incriminated food but the toxin may be recovered.

b. Toxic shock syndrome toxin (TSST-1): This is the classic cause of toxic shock syndrome (TSS). Because of similarities in molecular structure, it is sometimes referred to as staphylococcal enterotoxin F, although it does not cause food poisoning when ingested.

c. Exfoliatin (exfoliative toxin, ET) is also a superantigen. It causes scalded skin syndrome in children. The toxin cleaves desmoglein 1, which is a component of desmosomes (cell structures specialized for cell-to-cell adhesion). Cleavage results in loss of the superficial skin layer.

C. Clinical significance

S. aureus causes disease by infecting tissues, typically creating abscesses and/or by producing toxins (Figure). A common entry point into the body is a break in the skin, which may be a minute needlestick or a surgical wound. Another portal of entry is the respiratory tract. For example, staphylococcal pneumonia is an important complication of influenza.

The localized host response to staphylococcal infection is inflammation, characterized by swelling, accumulation of pus, and necrosis of tissue. Fibroblasts and their products may form a wall around the inflamed area, which contains bacteria and leukocytes. This creates a characteristic pus-filled boil or abscess. Serious consequences of staphylococcal infections occur when the bacteria invade the bloodstream. The resulting septicemia (the presence and persistence of pathogenic microorganisms or their toxins in the blood) may be rapidly fatal. Bacteremia (the presence of viable bacteria circulating in the bloodstream) may result in seeding internal abscesses, skin lesions, or infections in the lung, kidney, heart, skeletal muscle, or meninges.

1. Localized skin infections: The most common ***S. aureus*** infections are small, superficial abscesses involving hair follicles (folliculitis) or sweat or sebaceous glands (see Figure 8.12). For example, the common sty (external hordeolum) is created by infection of an eyelash follicle. Subcutaneous abscesses called furuncles (boils) often form around foreign bodies such as splinters. These generally respond to local therapy, that is, removal of the foreign body, soaking, and drainage as indicated. Carbuncles are larger, deeper, multiloculated skin infections that can lead to bacteremia and require antibiotic therapy and debridement. Impetigo is usually a localized, superficial, spreading crusty skin lesion generally seen in children. It can be caused by *S. aureus*, although more commonly by *Streptococcus pyogenes*, or both organisms together. Human staphylococcal infections usually remain localized at the portal of entry by normal host defenses.

2. Deep, localized infections: These may be metastatic from superficial infections or skin carriage or may result from trauma. ***S. aureus*** is the most common cause of acute and chronic infection of bone marrow. ***S. aureus*** is also the most common cause of acute infection of joint space in children (septic joint). [Note: Septic joints are medical emergencies because pus can rapidly cause irreparable cartilage damage. They must be treated promptly with drainage and an antibiotic.]

3. Acute endocarditis: Generally associated with intravenous drug abuse, acute endocarditis is caused by injection of contaminated preparations or by needles contaminated with ***S. aureus***. ***S. aureus*** also colonizes the skin around the injection site, and if the skin is not sterilized before injection, the bacteria can be introduced into soft tissues and the bloodstream, even when a sterilized needle is used. An abscess in any organ or tissue is cause to suspect ***S. aureus***, although many other bacteria can cause abscesses.

4. Septicemia is a generalized infection with sepsis or bacteremia that may be associated with a known focus (for example, a septic joint) or not (an occult focus).

5. Pneumonia: ***S. aureus*** is a cause of severe, necrotizing pneumonia.

6. Nosocomial infections: ***S. aureus*** is one of the most common causes of hospital-associated infections, often of wounds (surgical, decubital) or bacteremia associated with catheters (see Figure 8.10). Progression to septicemia is often a terminal event.

7. **Toxinoses**: These are diseases caused by the action of a toxin, frequently when the organism that secreted the toxin is undetectable. Toxinoses caused by *S. aureus* include:

a. Toxic shock syndrome: TSS results in high fever, rash (resembling a sunburn, with diffuse erythema followed by desquamation), vomiting, diarrhea, hypotension, and multiorgan involvement (especially GI, renal, and/or hepatic damage). An outbreak of TSS occurred in the late 1970s among menstruating women. It was shown to be related to the use of hyperabsorbant tampons by women who happened to be vaginally colonized by toxic shock syndrome toxin-(TSST)-positive strains of *S. aureus*. [Note: These tampons stimulated TSST expression, resulting in entry of the toxin into the circulation in the absence of true infection.] The incidence has decreased markedly since such tampons were removed from the market. Of the few cases of TSS that occur currently, approximately half are associated with ordinary *S. aureus* infections. Of the remainder, many result from a circulating enterotoxin rather than TSST. Figure 8.6 shows the desquamation (peeling or scaling of the skin) seen in TSS.

b. Staphylococcal gastroenteritis: This is caused by ingestion of food contaminated with enterotoxin-producing *S. aureus*. Often contaminated by a food handler, these foods tend to be protein rich (for example, egg salad or cream pastry) or salty, like ham (*S. aureus* is salt tolerant), and improperly refrigerated. These heat-resistant toxins are able to withstand subsequent reheating. Symptoms, such as nausea, vomiting, and diarrhea, are acute following a short incubation period (less than 6 hours) and are triggered by local actions of the toxin on the GI tract rather than from infection. The short incubation period of staphylococcal food poisoning occurs because the toxin in the food has already been formed by the staphylococci before the food is ingested.

c. Scalded skin syndrome: This involves the appearance of superficial bullae resulting from the action of an exfoliative toxin that attacks the intercellular adhesive of the stratum granulosum, causing marked epithelial desquamation (see Figure 8.12). The bullae may be infected or may result from toxin produced by organisms infecting a different site.

D. Laboratory identification

Identification of an isolate as a staphylococcus relies largely on microscopic and colony morphology and catalase positivity. Bacteria stain strongly gram-positive, and are frequently seen in grapelike clusters. *S. aureus* is distinguished from the coagulase-negative staphylococci primarily by coagulase positivity. In addition, S. aureus colonies tend to be yellow (hence "aureus," meaning golden) and hemolytic (see Figure 8.12), rather than gray and nonhemolytic like the coagulase-negative staphylococci. *S. aureus* is also distinguished from most coagulase-negative staphylococci by being mannitol-positive.

E. Immunity

S. aureus infections do not elicit strong or long-lasting immunity, as demonstrated by the continuing susceptibility of individuals to *S. aureus* infections throughout life.

F. Treatment

Serious *S. aureus* infections require aggressive treatment, including incision and drainage of localized lesions, as well as systemic antibiotics. Choice of antibiotics is complicated by the frequent presence of acquired antibiotic resistance determinants. Virtually all community and hospital-acquired *S. aureus* infections are now resistant to penicillin G due to penicillinase-encoding plasmids or transposons. This has required the replacement of the initial agent of choice, penicillin G, by β-lactamase-resistant penicillins, such as methicillin or oxacillin. However, increased use of methicillin and related antibiotics has resulted in *S. aureus* that is resistant to a number of β-lactam antibiotics, such as methicillin, oxacillin and amoxicillin (Figure 8.8). These strains are known as methicillin-resistant *S. aureus* (MRSA).

1. Hospital-acquired methicillin-resistant *S. aureus* (MRSA)

In recent decades, a high percentage (often in the range of 50 percent) of hospital *S. aureus* isolates has been found to be also resistant to methicillin or oxacillin. Antibiotic resistance is caused by chromosomal acquisition of the gene for a distinct penicillin¬binding protein, PBP-2a. This protein codes for a new peptidoglycan transpeptidase with a low affinity for all currently available β-lactam antibiotics, and thus renders infections with MRSA unresponsive to β-lactam therapy. Compared with methicilllin-sensitive *S. aureus*, MRSA infections are associated with worse outcomes, including longer hospital and

intensive care unit stays, longer durations of mechanical ventilation, and higher mortality rates. MRSA strains are also frequently resistant to many other antibiotics, some being sensitive only to glycopeptides such as vancomycin.

2. Community-acquired MRSA (CA-MRSA)

Community acquired MRSA infections were documented in the mid-1990s, occurring in individuals who had no previous risk factors for MRSA infections, such as exposure to hospital. The most common clinical manifestations of CA-MRSA are skin and soft tissue infections such as abscesses or cellulitis (Figure). Less commonly, CA-MRSA can also cause severe diseases such as necrotizing pneumonia, osteomyelitis, and septicemia.

DRUG	HA-MRSA (Hospital strain)	CA-MRSA (Community strain)
Characteristics of patients	Patients are typically elderly, debilitated, and/or chronically ill.	Patients are typically young and healthy. Children students, athletes, and military service personnel are at risk.
Infection site	Bacteremia commonly occurs with no obvious infection site. Infection of surgical wounds, open ulcer, intravenous line, and urinary catheters often occur.	Infections often occur in skin and soft tissues, producing cellulitis and abscesses. Infections include necrotizing community pneumonia, septic shock, and bone and joint infections.
Transmission	Transmission occurs within health care settings. Only rarely is transmission among household contacts.	Transmission occurs in the community. May spread in families, sport teams, and other risk groups.
Medical history	Infections more likely in patients with a history of MRSA infections, recent surgery, admission to a hospital or nursing home. Antibiotic use, dialysis and permanent indwelling catheters are risk factors.	Patients show no significant medical history or health care contact.
Virulence of infecting strain	Spread of infection in the community is limited. PVL genes are usually absent.	Spread of infection in the community readily occurs. PVL genes are often present, predisposing to necrotising soft tissue or lung infections.
Antibiotic susceptibility	Multidrug antibiotic resistance often occurs, resulting in a limited choice of effective therapeutic agents.	CA-MRSA strains are often more virulent than HA-MRSA, but they tend to be susceptible to a broader array of antibiotics.

Figure Comparison of **hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA)** with **community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). PVL** = Panton-Valentine leukocidin.

Community-acquired MRSA has a number of characteristics that help distinguish it from hospital- associated MRSA. For example, CA-MRSA has a characteristic pattern of DNA fragments obtained upon enzymic cleavage and electrophoresis, and it produces specific toxins. CA-MRSA also exhibits a unique antibiotic

resistance pattern, that is, CA-MRSA is sensitive to many antibiotics that do not show much activity against hospital-associated MRSA. These antibiotics include **ciprofloxacin** and **clindamycin**, with some CA-MRSA even sensitive to **erythromycin**, **gentamicin**, **rifampin**, **tetracycline**, and/or **trimethoprim-sulfamethoxazole**.

Note: Emerging antibiotic-resistant strains of ***S. aureus*** that infect otherwise healthy individuals (community-acquired infections) are often more virulent than the more common strains that originate in hospitals.

3**. Vancomycin resistance**

Vancomycin has been the agent of choice for empiric treatment of life-threatening MRSA S. aureus infections. Unfortunately, in 1997, several MRSAs were isolated that had also acquired low-level vancomycin resistance. The incidence of vancomycin resistance has increased steadily, prompting the use of alternative drugs such as **quinupristin-dalfopristin**, **linezolid**, and **daptomycin**. These agents have good in vitro activity against MRSA and most other clinically important gram-positive bacterial pathogens.

G. Prevention

There is no effective vaccine against ***S. aureus***. Infection control procedures, such as barrier precautions and disinfection of hands and fomites, are important in the control of nosocomial *S. aureus* epidemics.

II. Coagulase-Negative Staphylococci

Of 12 coagulase-negative staphylococcal species that have been recovered as normal commensals of human skin and anterior nares, the most abundant and important is *S. epidermidis*. For this reason some clinical laboratories designate all coagulase-negative staphylococci as *S. epidermidis*, a practice that is not encouraged. The second most important coagulase-negative staphylococcus is *S. saprophyticus*, which has a special medical niche. Coagulase-negative staphylococcal species are important agents of hospital-acquired infections associated with the use of implanted prosthetic devices and catheters.

A. *Staphylococcus epidermidis*

S. epidermidis is present in large numbers as part of the normal flora of the skin. As such, it is frequently recovered from blood cultures, generally as a contaminant from skin. Despite its low virulence, it is a common cause of infection of implants such as heart valves and catheters (Figure). Acquired drug resistance by *S. epidermidis* is even more frequent than by *S. aureus*. Vancomycin sensitivity remains the rule, but vancomycin-resistant isolates have been reported. *S. epidermidis* produces an extracellular polysaccharide material called polysaccharide intercellular adhesin (sometimes called "slime"), that facilitates adherence to bioprosthetic material surfaces, such as intravenous catheters, and acts as a barrier to antimicrobial agents.

B. *Staphylococcus saprophyticus*

This organism is a frequent cause of cystitis in women, probably related to its occurrence as part of normal vaginal flora. It tends to be sensitive to most antibiotics, even penicillin G. *S. saprophyticus* can be distinguished from *S. epidermidis* and most other coagulase-negative staphylococci by its natural resistance to novobiocin (Figure). [Note: Urinary coagulase-negative staphylococcus is often presumed to be *S. saprophyticus*; but **novobiocin** resistance can be used for confirmation.].

Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation	Novobiocin resistance
S. aureus	**Common**	+	**Golden Yellow**	+	-
S. epidermidis	**Common**	-	**White**	-	-
S. saprophyticus	**Occasional**	-	**Variable**	-	+

Figure Summary of various species of staphylococci.

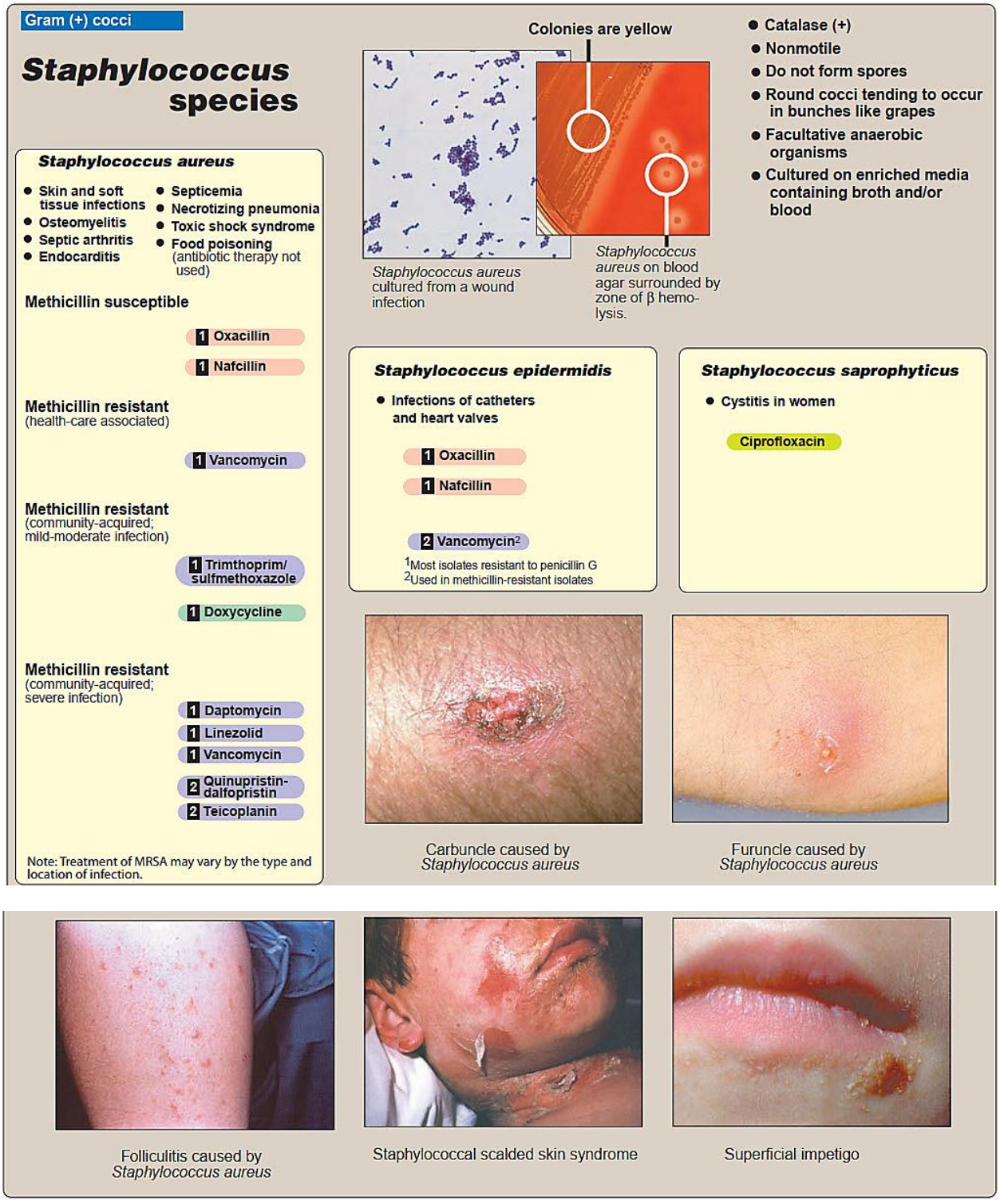


Figure -Summary of staphylococcal disease. 1 Indicates first-line drugs; 2 indicates alternative drugs.

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