Weak	Subject
1	*Staphylococcus aureus*
2	*STREPTOCOCCUS PYOGENES*
3	*STREPTOCOCCUS PNEUMONIAE*
4	*Neisseria gonorrhoeae (gonococci)*
5	*Neisseria meningitidis (meningococci*)
6	*Clostridium Species*
7	*BACILLUS ANTHRACIS , BACILLUS CEREU ,CORYNEBACTERIUM DIPHTHERIAE S*
8	*Examination*
9	Enteric Gram-Negative Rods (Enterobacteriaceae) *Escherichia coli, Proteus species ,klebsiella pneumoniaei*
10	*Shigellae species , Salmonellae Species*
11	*THE PSEUDOMONAD GROUP*
12	*Vibrio cholerae , H pylori*
13	*Haemophilus influenzae*
14	*THE BRUCELLAE Species*
15	*MYCOBACTERIUM TUBERCULOSIS*

References

1 -Jawetz, Melnick, & Adelberg's Medical Microbiology twenty-fourth edition Geo. F. Brooks, et al
2 -Medical Microbiology and Infection at a Glance Stephen H. et al
3 - SHERRIS MEDICAL MICROBIOLOGY AN INTRODUCTION TO INFECTIOUS DISEASES EDITORS KENNETH J. RYAN, M ,et al 4TH EDITION

The Staphylococci

The staphylococci are gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily on many types of media and are active metabolically, Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia.

The pathogenic staphylococci often :

1 - hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.

2 -The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin.

3 -Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems.

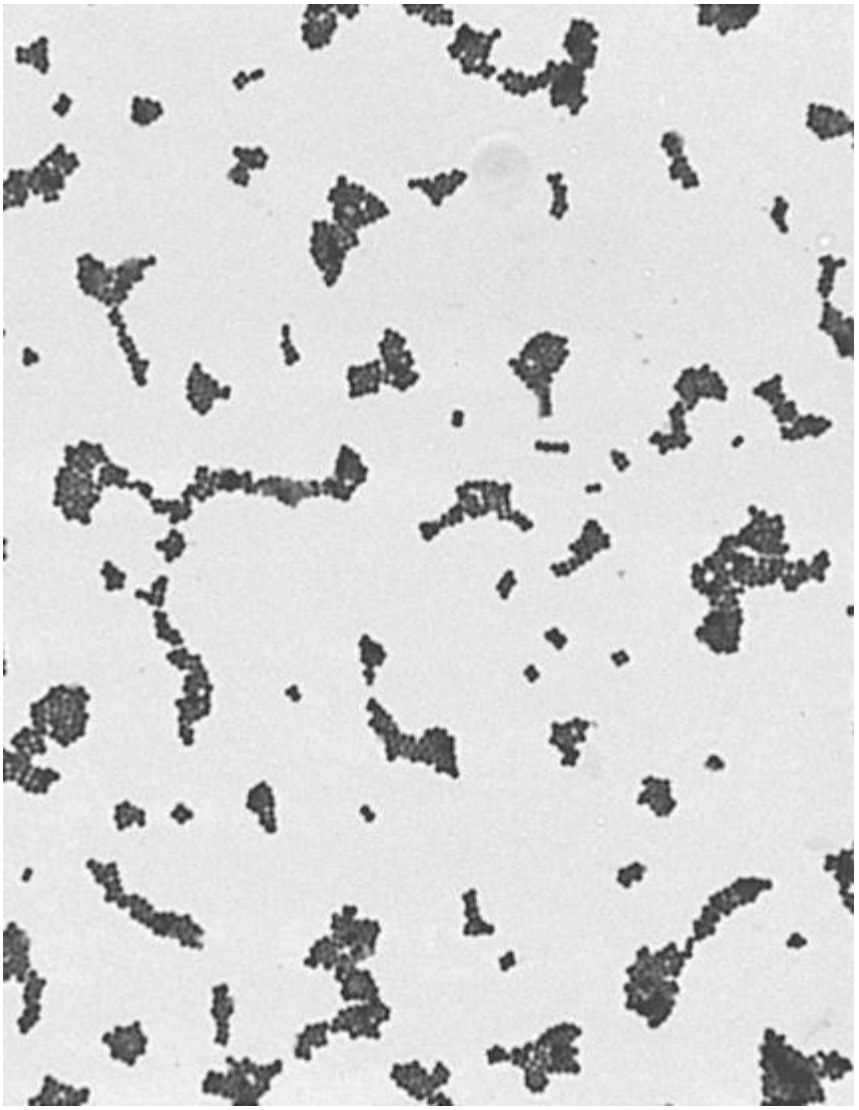
4 -The genus Staphylococcus has at least 35 species. The three main species of clinical importance *are Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus. Staphylococcus aureus* is coagulase-positive, which differentiates it from the other species. S aureus is a major pathogen for humans.

5 -The coagulase-negative staphylococci are normal human flora and sometimes cause infection

Morphology & Identification

A. TYPICAL ORGANISMS

Staphylococci are spherical cells about 1 μm in diameter arranged in irregular clusters . Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non motile and do not form spores.



B. CULTURE

Staphylococci grow readily on most bacteriologic media under aerobic or micro aerophilic conditions. They grow most rapidly at 37 °C but form pigment best at room temperature (20–25 °C). Colonies on solid media are round, smooth, raised, and glistening. *S aureus* usually forms gray to deep golden yellow colonies. *S epidermidis* colonies usually are gray to white on primary isolation; many colonies develop pigment only upon prolonged incubation. No pigment is produced an aerobically or in broth. Various degrees of hemolysis are produced by *S aureus* and occasionally by other species.

C. GROWTH CHARACTERISTICS

The staphylococci **produce catalase**, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas. Proteolytic activity varies greatly from one strain to another. Pathogenic staphylococci produce many extracellular substances, which are discussed below. Staphylococci are relatively resistant to drying, heat (they withstand 50 °C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene .**Staphylococci are variably sensitive to many antimicrobial drugs**

D. VARIATION

A culture of staphylococci contains some bacteria that differ from the bulk of the population in

1- expression of colony characteristics (colony size, pigment, hemolysis) 2 - in enzyme elaboration . 3-in drug resistance, and in pathogenic .

4 - In vitro, the expression of such characteristics is influenced by growth conditions: When nafcillin-resistant *S aureus* is incubated at 37 °C on blood agar, one in 107 organisms expresses nafcillin resistance; when it is incubated at 30 °C on agar containing 2–5% sodium chloride, one in 103 organisms expresses nafcillin resistance .

Antigenic Structure :

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure:

1- Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acid or exposure to lysozyme**. It is important in the pathogenesis of infection:** It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemo attractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement.

2 - Teichoic acids, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid

antibodies detectable by gel diffusion may be found in patients with active endocarditis due *to S aureus.*

3 - Protein A is a cell wall component of many *S aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic laboratory technology ,

4 - Some *S aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present.

5 - Most strains of S aureus have coagulase, or clumping factor, on the cell wall surface; coagulase binds non enzymatically to fibrinogen. yielding aggregation of the bacteria.

Enzymes & Toxins

Staphylococci can produce disease both through :

1 - their ability to multiply and spread widely in tissues.

2 - and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, though they may function as enzymes. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extrachromosomal control; and for others the mechanism of genetic control is not well defined.

A. 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.

B. COAGULASE AND CLUMPING FACTOR

***S aureus* produces**

1 - coagulase, an enzyme-like 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.

2 - Clumping factor : is a *surface S aureus* compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, S aureus forms clumps. Clumping factor is distinct from coagulase.

C. 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.

D. EXOTOXINS

The α-toxin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes. The α- toxin is a potent hemolysin**. The β-toxin** degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells. **The δ-toxin** is heterogeneous and dissociates into subunits in nonionic detergents. It disrupts biologic membranes and may have a role *in S aureus* diarrheal diseases. **The γ** hemolysin refers to three proteins that interact with the two proteins comprising the Panton-Valentine leukocidin (see below) to form six potential two-component toxins**. All six of these protein toxins are capable of efficiently lysing white blood cells by causing pore formation in the cellular membranes that increase cation**

permeability.

E. LEUKOCIDIN

This toxin of *S aureus* has two components. It can kill white blood cells of humans and rabbits. The two components act synergistically on the white blood cell membrane as described above for γ toxin. This toxin is an important virulence factor in community associated methicillin resistant *S aureus* infections.

F. EXFOLIATIVE TOXINS

These epidermolytic toxins of *S aureus* are two distinct proteins of the same molecular weight. Epidermolytic toxin A is a chromosomal gene product and is heat-stable (resists boiling for 20 minutes). Epidermolytic toxin B is plasmid-mediated and heat-labile. The epidermolytic toxins yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

G. TOXIC SHOCK SYNDROME TOXIN

Most ***S aureus*** strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F. TSST-1 is the prototypical superantigen .

H. ENTEROTOXINS

There are multiple (A–E, G–I, K–M) enterotoxins. Approximately 50% *of S aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens. The enterotoxins are heat-stable and resistant to the action of gut enzymes. An important cause of food poisoning, enterotoxins are produced when S aureus grows in carbohydrate and protein foods. Ingestion of 25 µg of enterotoxin B results in vomiting and diarrhea.

The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.

The exfoliative toxins, TSST-1, and the enterotoxin genes are on a chromosomal element called a pathogenicity island. It interacts with accessory genetic elements—bacteriophages—to produce the toxins.

Pathogenesis

Staphylococci, particularly S epidermidis, are members of the normal flora of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S aureus* occurs in 20–50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments.

The pathogenic capacity of a given strain of *S aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain.

Pathology

The prototype of a staphylococcal lesion **is the furuncle** or other localized abscess. Groups *of S aureus* established in a hair follicle lead to tissue necrosis (dermonecrotic factor), **suppuration (abscess**) is typical of staphylococcal infections . From any one focus, organisms may spread via the lymphatics and bloodstream to other parts of the body.

Suppuration within veins, associated with **thrombosis**, is a common feature of such dissemination. In osteomyelitis, the primary focus of S *aureus* growth is typically in a terminal blood vessel of the metaphysis of a long bone**, leading to necrosis of bone and chronic suppurat**ion.

S aureus may **cause pneumonia, meningitis, empyema, endocarditis, or sepsis with suppuration in any organ**. Staphylococci of low invasiveness are involved **in many skin infections (eg, acne, pyoderma, or impetigo**). Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection. the scalded skin syndrome, is caused by the production of exfoliative toxins .

Diagnostic Laboratory Tests

A. SPECIMENS

Surface swab pus, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

B. SMEARS

Typical staphylococci appear as gram positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish saprophytic *(S epidermidis*) from pathogenic *(S aureus*) organisms on smears.

C. CULTURE

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37 °C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. *S aureus* but not other staphylococci ferment mannitol. Specimens contaminated with a mixed flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal flora but *not S aureus*. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers *of S aureus* and patients with cystic fibrosis.

D. CATALASE TEST

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test.

E. COAGULASE TEST

Citrated rabbit (or human) plasma diluted 1:5 is mixed with an equal volume

of broth culture or growth from colonies on agar and incubated at 37 °C. A tube of plasma mixed with sterile broth is included as a control. If clots

form in 1–4 hours, the test is positive. Coagulase-positive staphylococci are considered pathogenic .

F. SUSCEPTIBILITY TESTING

Broth micro dilution or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infections.

1- Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of S aureus produce β- lactamase

2 -.Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% *of S aureus* and approximately 75% of S epidermidis isolates. Nafcillin resistance correlates with the presence of mecA, the gene that codes for a penicillin-binding protein (PBP 2a) not affected by these drugs. The gene can be detected using the polymerase chain reaction.

Most clinical laboratories use a phenotypic method such as an oxacillin screening agar plate. Staphylococci that grow on Mueller- Hinton agar +9containing 4% NaCl and 6 µg/mL of oxacillin typically are mecA-po9+sitive and nafcillin-resistant. Alternatively, an assay for the mecA gene product, PBP 2a, is commercially available and is much more rapid than PCR for mecA or than testing for resistance using growth on oxacillin-containing salt agar.

Epidemiology & Control

Staphylococci are ubiquitous human parasites**. The chief sources** of infection are :

1- shedding human lesions, fomites contaminated from such lesions, and the human respiratory tract and skin.

2 - Contact spread of infection has assumed added importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant staphylococci in the nose or on the skin .

Although cleanliness, hygiene, and aseptic management of lesions can control the spread of staphylococci from lesions, few methods are

available to prevent the wide dissemination of staphylococci from carriers , In hospitals, the areas at highest risk for severe staphylococcal infections are the newborn nursery, intensive care units, operating rooms, and cancer chemotherapy wards. Massive introduction of "epidemic" *pathogenic S aureus* into these areas may lead to serious clinical disease.so that Personnel with active *S aureus* lesions and carriers must be :

1- have to be excluded from these areas.

2 - In such individuals, the application of topical antiseptics to nasal or perineal carriage sites may diminish shedding of dangerous organisms. Rifampin coupled with a second oral anti staphylococcal drug sometimes provides long-term suppression and possibly cure of nasal carriage; this form of therapy is usually reserved for major problems of staphylococcal carriage, because staphylococci can rapidly develop resistance to rifampin.

3 - Patients who test positive by culture or PCR are placed upon contact precautions so as to minimize spread on the hands of health care workers ,by wearing gloves and washing hands before and after patient contact .

The Streptococci

CLASSIFICATION OF STREPTOCOCCI

The classification of streptococci into major categories has been based on a series of observations over many years

(1) colony morphology and hemolytic reactions on blood agar;

(2) serologic specificity of the cell wall group-specific substance and other cell wall or capsular antigens;

(3) biochemical reactions and resistance to physical and chemical factors; and

(4) ecologic features. Molecular genetics have also been used to study the streptococci. Combinations of the above methods have permitted the classification of streptococci for purposes of clinical and epidemiologic convenience .

A. HEMOLYSIS

1 - Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β hemolysis.

2- Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α hemolysis.

3 - Other streptococci are non-hemolytic (sometimes called gamma hemolysis). The hemolysis patterns of the streptococci of medical importance .

B. GROUP-SPECIFIC SUBSTANCE (LANCEFIELD CLASSIFICATION)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups A–H and K–U. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar.

C - CAPSULAR POLYSACCHARIDES

The antigenic specificity of the capsular polysaccharides is used to classify *S pneumoniae* into over 90 types and to type the group B streptococci *(S agalactiae).*

D. BIOCHEMICAL REACTIONS

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymesand tests for susceptibility or resistance to certain chemical agent

Biochemical tests 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. For example, the viridans streptococci are α-hemolytic or nonhemolytic and do not react with the antibodies commonly used for the Lancefield classification .

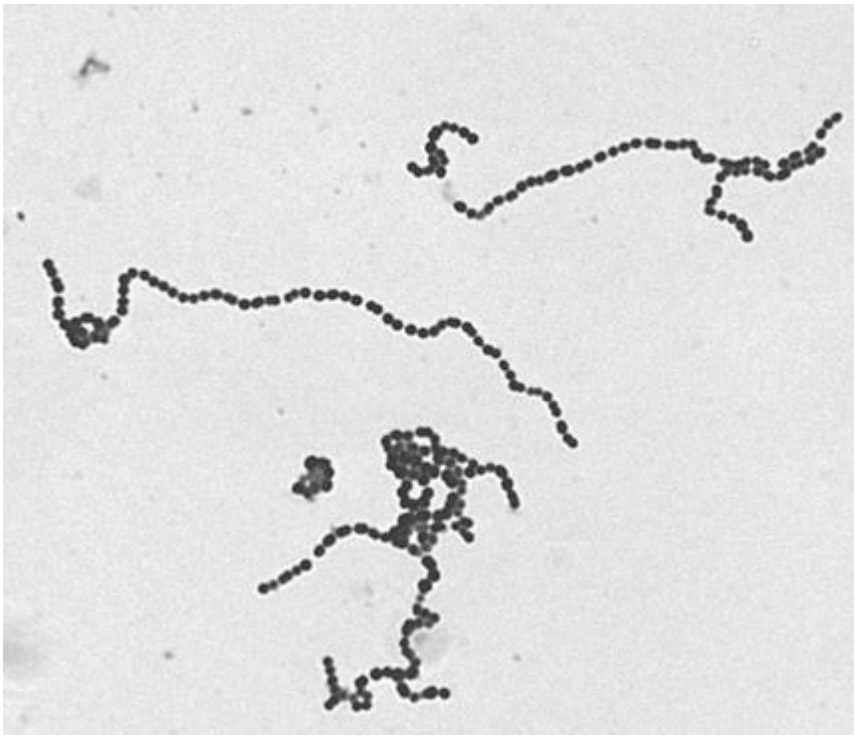
STREPTOCOCCUS PYOGENES

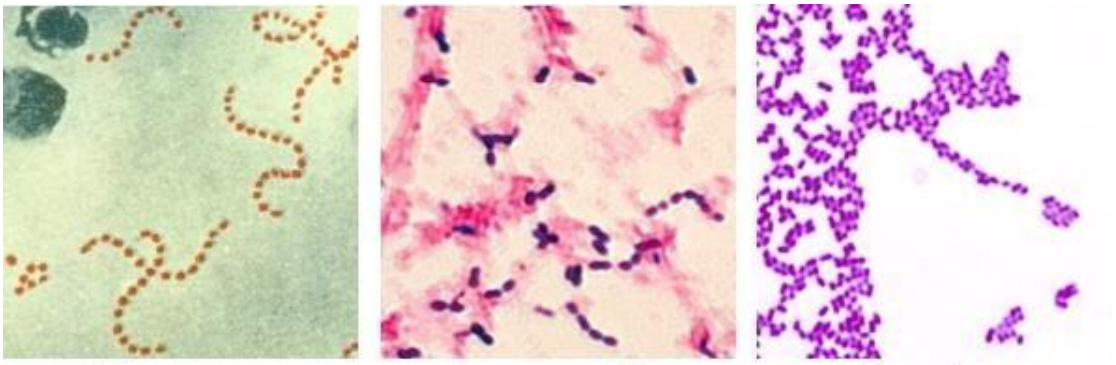
Most streptococci that contain the group A antigen *are S pyogenes*. It is a prototypical human pathogen, *S pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders.

Morphology & Identification

A. TYPICAL ORGANISMS

Individual cocci are spherical or 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 diplococcal appearance, and rod-like forms are occasionally seen. The lengths of the chains vary widely and are conditioned by environmental factors. Streptococci are **gram-positive**; however, as a culture ages and the bacteria die, they lose their gram-positivity and can appear to be gram-negative; for some streptococci, this can occur after overnight incubation. **Most group A strains produce capsules composed of hyaluronic acid. The capsules are most noticeable in very young cultures. They impede phagocytosis.** Capsules of other streptococci (eg, *S agalactiae and S pneumonia*e) are different. **The *S pyogenes* cell wall contains 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 protein and are covered with lipoteichoic 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.

C. GROWTH CHARACTERISTICS

Energy is obtained principally from the utilization of glucose with lactic acid as the end product. 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% CO2. Most pathogenic hemolytic streptococci grow best at 37 °C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.

D. VARIATION

Variants of the same streptococcus strain may show different colony forms. This is particularly marked *among S pyogenes* strains, giving rise to either matte or glossy , Streptococci grown in broth showing gram-positive cocci in chains. colonies**. Matte colonies** consist of organisms that produce much M protein and generally are virulent. The *S pyogenes* **in glossy colonie**s tend to produce little M protein and are often not virulent.

Antigenic Structure

A. M PROTEIN This substance is a major virulence factor of group A *S pyogenes*. M protein appears as hair-like projections of the streptococcal cell wall. When M protein is present, the streptococci are virulent, and in the absence of M type specific antibodies, they are able to resist phagocytosis by polymorphonuclear leukocytes. *S pyogenes* that lack M protein are not virulent.

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

C. NUCLEOPROTEINS Extraction of streptococci with weak alkali yields mixtures of proteins and other substances of little serologic specificity, called P substances, which probably make up most of the streptococcal cell body.

Toxins & Enzymes

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

A. STREPTOKINASE (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.

B. STREPTODORNASE (streptococcal deoxyribonuclease) depolymerizes DNA. Mixtures 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. An antibody to DNAse develops after streptococcal infections (normal limit = 100 units), especially after skin infections.

C. HYALURONIDASE splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).

D. PYROGENIC EXOTOXINS (ERYTHROGENIC TOXIN) Pyrogenic exotoxins are elaborated by S pyogenes. There are three antigenically distinct streptococcal pyrogenic exotoxins: A, B, and C. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever.

E. DIPHOSPHOPYRIDINE NUCLEOTIDASE This enzyme is may be related to the organism's ability to kill leukocytes**. Proteinases and amylase** are produced by some strains.

F. HEMOLYSINS The β-hemolytic group A *S pyogenes* elaborates two hemolysins (streptolysins).

A - Streptolysin O is a protein (MW 60,000) that is hemolytically active in the reduced state (available –SH groups) 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. It combines quantitatively with antistreptolysin O, 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 *S pyogenes* or persistently high antibody levels due to an exaggerated immune response to an earlier exposure in a hypersensitive person.**

B - Streptolysin S is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates. It is elaborated in the presence of serum—hence the name streptolysin S. It is not antigenic .

STREPTOCOCCUS PYOGENES infections :

A variety of distinct disease processes are associated *with S pyogenes* infections. The infections can be divided into several categories.

A. DISEASES ATTRIBUTABLE TO INVASION BY S PYOGENES, β- HEMOLYTIC GROUP A STREPTOCOCCI

1. Erysipelas—If the portal of entry is the skin, erysipelas results, with massive brawny edema and a rapidly advancing margin of infection

2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues. Cellulitis is differentiated from erysipelas by two clinical findings: In cellulitis, the lesion is not raised, and the line between the involved and uninvolved tissue is indistinct.

3. Necrotizing Fasciitis (Streptococcal Gangrene)— This is infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues .

4. Puerperal Fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).

5. Bacteremia/Sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which rapidly can be fatal. S pyogenes bacteremia can also follow skin infections, such as cellulitis and rarely pharyngitis.

B. DISEASES ATTRIBUTABLE TO LOCAL INFECTION WITH S PYOGENES AND THEIR BY-PRODUCTS

1. Streptococcal Sore Throat—The most common infection due to β-hemolytic *S pyogenes* is streptococcal sore throat or pharyngitis

2. Streptococcal Pyoderma—Local infection of superficial layers of skin, especially in children, is called impetigo

C. INVASIVE GROUP A STREPTOCOCCAL INFECTIONS, STREPTOCOCCAL TOXIC SHOCK SYNDROME, AND SCARLET FEVER Fulminant, invasive *S pyogenes i*nfections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients.

D. POSTSTREPTOCOCCAL DISEASES (RHEUMATIC FEVER, GLOMERULONEPHRITIS)

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1. Acute Glomerulonephritis—This sometimes develops 3 weeks after S *pyogenes* skin infection (pyoderma, impetigo). Glomerulonephritis may be initiated by antigen-antibody complexes on the glomerular basement membrane. The most important antigen is probably in the streptococcal protoplast membranee majority recover completely.

2. Rheumatic Fever—This is the most serious sequela *of S pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens. Sera from patients with rheumatic fever contain antibodies to these antigens .

Diagnostic Laboratory Tests

A. SPECIMENS to be obtained depend upon the nature of the streptococcal infection. A throat swab, pus, or blood is obtained for culture. Serum is obtained for antibody determinations.

B. SMEARS from pus often show single cocci or pairs rather than definite chains. Cocci are sometimes gram-negative because the organisms are no longer viable and have lost their ability to retain the blue dye (crystal violet) and be gram-positive. If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected. Smears of throat swabs are rarely contributory, because viridans streptococci are always present and have the same appearance as group A streptococci on stained smears.

C. CULTURE Specimens suspected of containing streptococci are cultured on blood agar plates. If anaerobes are suspected, suitable anaerobic media must also be inoculated. Incubation in 10% CO2 often speeds hemolysis. Slicing the inoculum into the blood agar has a similar effect, because oxygen does not readily diffuse through the medium to the deeply embedded organisms, and it is oxygen that inactivates streptolysin O. Blood cultures will grow hemolytic group A streptococci (eg, in sepsis) within hours or a few days. Certain α-hemolytic streptococci and enterococci may grow slowly, so blood cultures in cases of suspected endocarditis occasionally do not turn positive for a few days. The degree and kind of hemolysis (and colonial appearance) may help place an organism in a definite group. *S pyogenes* can be identified by rapid tests specific for the presence of the group A-specific antigen and by the PYR test. Streptococci belonging to group A may be presumptively identified by inhibition of growth by bacitracin, but this should be used only when more definitive tests are not available

D. ANT IGEN DETECTION TESTS Several commercial kits are available for rapid detection of group A streptococcal antigen from throat swabs.

E. SEROLOGIC TESTS A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include antistreptolysin O (ASO), particularly in respiratory disease; anti-DNase and antihyaluronidase, particularly in skin infections; antistreptokinase;

anti-M type-specific antibodies; and others. Of these, the antiASO titer is most widely used.

Control procedures are directed mainly at the human source:

(1) Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci.

(2) Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever .

(3) Eradication of *S pyogenes* from carriers. This is especially important when carriers are in areas such as obstetric delivery rooms, operatin

VIRIDANS STREPTOCOCCI

The viridans streptococci include *S mitis, S mutans, S salivarius, S sangui*s, and others. Typically they are α- hemolytic, but they may be nonhemolytic.

Their growth is not inhibited **by Optochin,** and colonies are not soluble in bile (deoxycholate).

The viridans streptococci are the most prevalent members of the normal flora of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves.

Some viridans streptococci (eg, *S mutans*) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

STREPTOCOCCUS PNEUMONIAE

The pneumococci (*S pneumoniae*) are gram-positive diplococci, often lancet-shaped or arranged in chains, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, and other infectious processes.

Morphology & Identification

A. TYPICAL ORGANISMS

The typical gram-positive, lancet-shaped diplococci , are often seen in specimens of young cultures. In sputum or pus, single cocci or chains are also seen. With age, the organisms rapidly become gram-negative and tend to lyse spontaneously. Autolysis of pneumococci is greatly enhanced by surface-active agents**. Lysis of pneumococci occurs in a few minutes when ox bile (10%) or sodium deoxycholate (2%) is added to a broth culture or suspension of** organisms at neutral pH.

On solid media, the growth of pneumococci is inhibited around a disk of Optochin; viridans streptococci are not inhibited by Optochin. Other identifying points include almost uniform virulence for mice when injected intraperitoneally and **the "capsule swelling test," or quellung reaction .**

B. CULTURE Pneumococci form small round colonies, at first domeshaped and later developing a central plateau with an elevated rim. Pneumococci are **α-hemolytic on blood agar**. Growth is enhanced by 5–10% CO2 .

c -VARIATION Pneumococcal isolates that produce large amounts of capsules produce large mucoid colonies.

Antigenic Structure

A. COMPONENT STRUCTURES The pneumococcal cell wall has peptidoglycan and teichoic acid, like other streptococci. The capsular polysaccharide is covalently bound to the peptidoglycan and to the cell wall polysaccharide. The capsular polysaccharide is immunologically distinct for each of the more than 90 types.

B. QUELLUNG REACTION When pneumococci of a certain type are mixed with specific anti polysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by cross linking of the antibodies**. 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 all of the types (“omniserum”), is a good reagent for rapid microscopic determination of whether or not pneumococci are present in fresh sputum.

A. TYPES OF PNEUMOCOCCI

In adults, types 1–8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all fatalities in pneumococcal bacteremia; in children, types 6, 14, 19, and 23 are frequent causes.

B. PRODUCTION OF DISEASE

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

C. LOSS OF NATURAL RESISTANCE

Since 40–70% of humans are at some time carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus

(1) Viral and other respiratory tract infections that damage surface cells; abnormal accumulations of mucus (eg, allergy), which protect pneumococci from phagocytosis; bronchial obstruction (eg, atelectasis); and respiratory tract injury due to irritants disturbing its mucociliary function.

(2) Alcohol or drug intoxication, which depresses phagocytic activity, depresses the cough reflex, and facilitates aspiration of foreign material.
(3) Abnormal circulatory dynamics (eg, pulmonary congestion, heart failure).

(4) Other mechanisms, eg, malnutrition, general debility, sickle cell anemia, hyposplenism, nephrosis, or complement deficiency.

Diagnostic Laboratory Tests

Blood is drawn for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. Serum antibody tests are impractical. Sputum may be examined in several ways

A. STAINED SMEARS A Gram-stained film of rusty-red sputum shows typical organisms, many polymorphonuclear neutrophils, and many red cells.

B. CAPSULE SWELLING TESTS Fresh emulsified sputum mixed with antiserum causes capsule swelling (the quellung reaction) for identification of pneumococci .

C. CULTURE The culture is created by sputum cultured on blood agar and incubated in CO2 or a candle jar. A blood culture is also taken. Immunity Immunity to infection with pneumococci is type-specific and depends both on antibodies to capsular polysaccharide and on intact phagocytic function.

ENTEROCOCCI

The enterococci have the group D group-specific substance and were previously classified as group D streptococci. Because the group D cell wall specific antigen is a teichoic acid, it is not an antigenically good marker; enterococci are usually identified by characteristics other than immunologic reaction with group-specific antisera.

They are part of the normal enteric flora. They are usually nonhemolytic, but occasionally α-hemolytic. Enterococci are PYR-positive. They grow in the presence of bile and hydrolyze esculin (bile esculin-positive). They grow in 6.5% NaCl. They grow well at between 10
°C and 45 °C whereas streptococci generally grow at a much narrower temperature range. They are more resistant to penicillin G than the streptococci, and rare isolates have plasmids that encode for β-lactamase.

Enterococci are transmitted from one patient to another primarily on the hands of hospital personnel, some of whom may carry the enterococci in their gastrointestinal tracts. Enterococci occasionally are

transmitted on medical devices. In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood.

Enterococci may cause meningitis and bacteremia in neonates. In adults, enterococci can cause endocarditis. However, in intra-abdominal, wound, urine, and other infections .