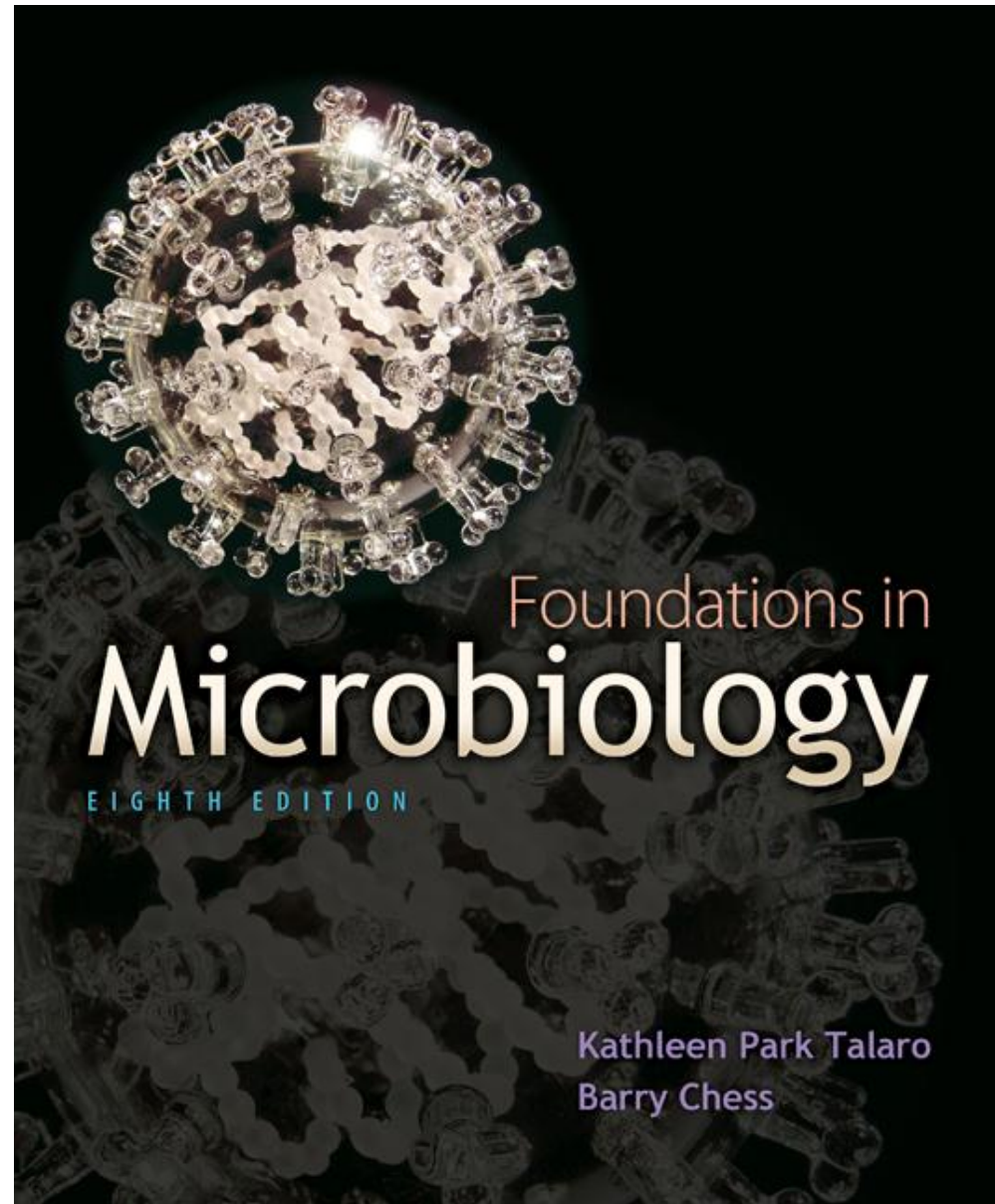


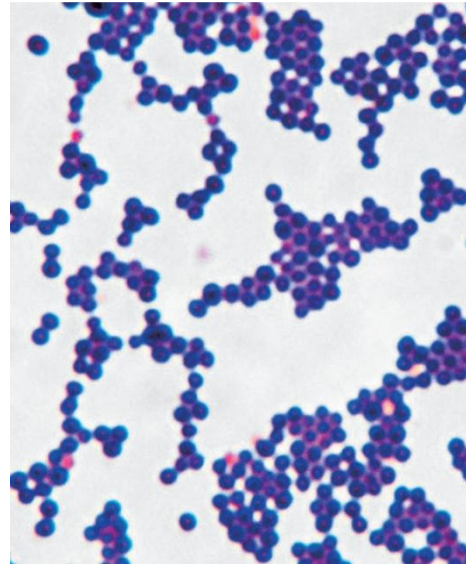
Chapter 18

The **Gram-Positive**
and **Gram-Negative**
Cocci of Medical
Importance

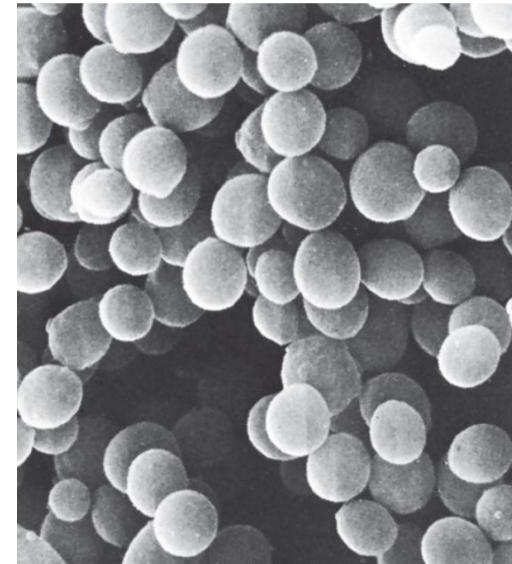


18.1 Staphylococci General Characteristics

- Common inhabitant of the skin and mucous membranes
- Spherical cells arranged in irregular clusters
- Gram-positive
- Lack spores and flagella
- May have capsules
- 31 species



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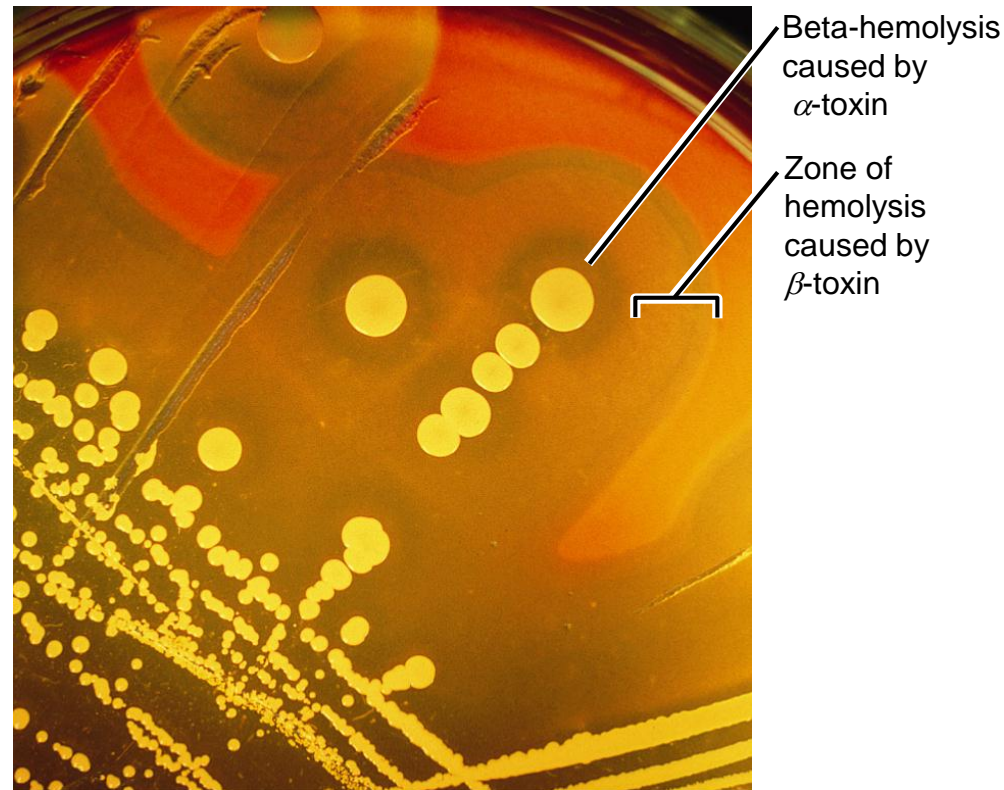
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Figure 18.1 Views of *S. aureus* shape and arrangement

Growth and Physical Characteristics of *Staphylococcus aureus*

- Grows in large, round, opaque colonies
- Optimum temperature of 37°C
- Facultative anaerobe
- Withstands high salt, extremes in pH, and high temperatures
- Produces many virulence factors

Figure 18.2 Blood agar plate showing *S. aureus*



Virulence factors of S. aureus

Enzymes:

- **Coagulase** – coagulates plasma and blood; produced by 97% of human isolates; *diagnostic*
- **Hyaluronidase** – digests connective tissue
- **Staphylokinase** – digests blood clots
- **DNase** – digests DNA
- **Lipases** – digest oils; enhances colonization on skin
- **Penicillinase** – inactivates penicillin

Virulence factors of S. aureus

Toxins:

- **Hemolysins** (α , β , γ , δ) – lyse red blood cells
- **Leukocidin** – lyses neutrophils and macrophages
- **Enterotoxin** – induce gastrointestinal distress
- **Exfoliative toxin** – separates the epidermis from the dermis
- **Toxic shock syndrome toxin** (TSST) – induces fever, vomiting, shock, systemic organ damage

Epidemiology and Pathogenesis

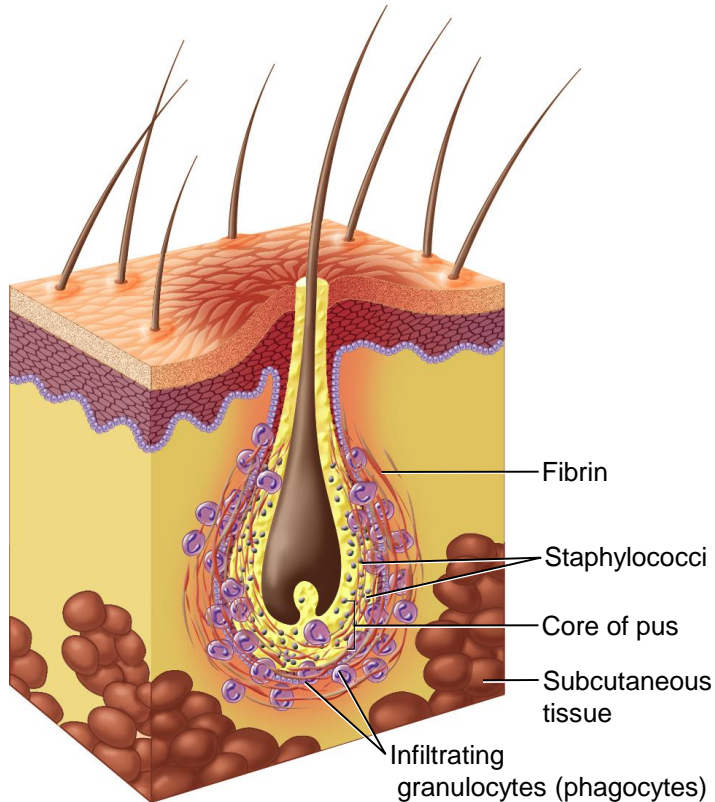
- Present in most environments frequented by humans
- Readily isolated from fomites
- Carriage rate for healthy adults is 20-60%
- Carriage is mostly in anterior nares, skin, nasopharynx, intestine
- Predisposition to infection include: poor hygiene and nutrition, tissue injury, preexisting primary infection, diabetes, immunodeficiency
- Increase in community acquired methicillin resistance - **MRSA**

The Scope of Staphylococcal Disease

Range from localized to systemic

- **Localized cutaneous infections** – invade skin through wounds, follicles, or glands
 - **Folliculitis**: superficial inflammation of hair follicle; usually resolved with no complications but can progress
 - **Furuncle**: boil; inflammation of hair follicle or sebaceous gland progresses into abscess or pustule
 - **Carbuncle**: larger and deeper lesion created by aggregation and interconnection of a cluster of furuncles
 - **Impetigo**: bubble-like swellings that can break and peel away; most common in newborns

Figure 18.3 Cutaneous Lesions of *S. aureus*



(a) Sectional view of a **boil or furuncle**, a single pustule that develops in a hair follicle or gland and is the classic lesion of the species. The inflamed infection site becomes abscessed when masses of phagocytes, bacteria, and fluid are walled off by fibrin.



© New Zealand Dermatological Society

(b) Appearance of **folliculitis** caused by *S. aureus*. Note the clusters of inflamed papules and pustules.



CDC

(c) An **abscess** on the knee caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

Miscellaneous Systemic Infections

- **Systemic infections**

- **Osteomyelitis**: infection is established in the metaphysis of bones, ankle or wrist; abscess forms
- **Bacteremia**: primary origin is bacteria from another infected site or medical devices; endocarditis possible

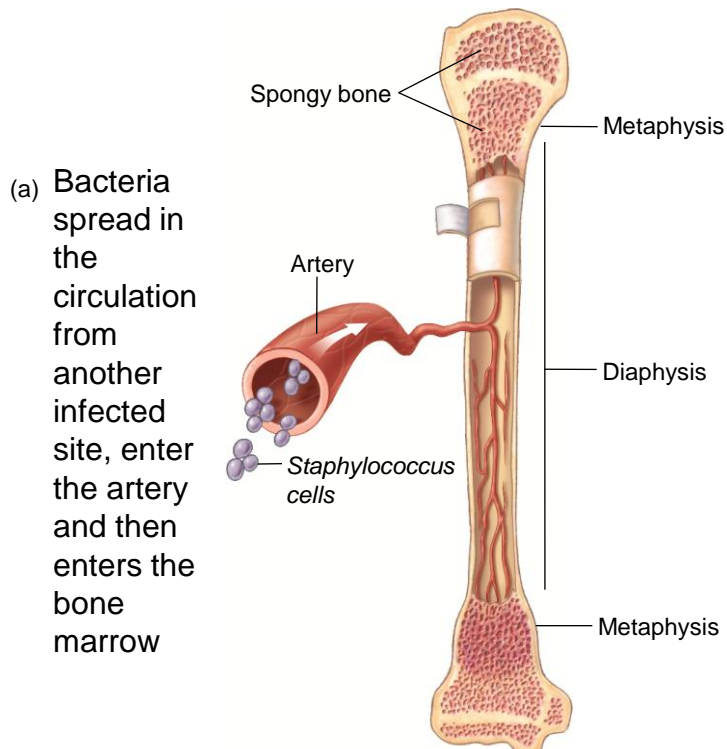
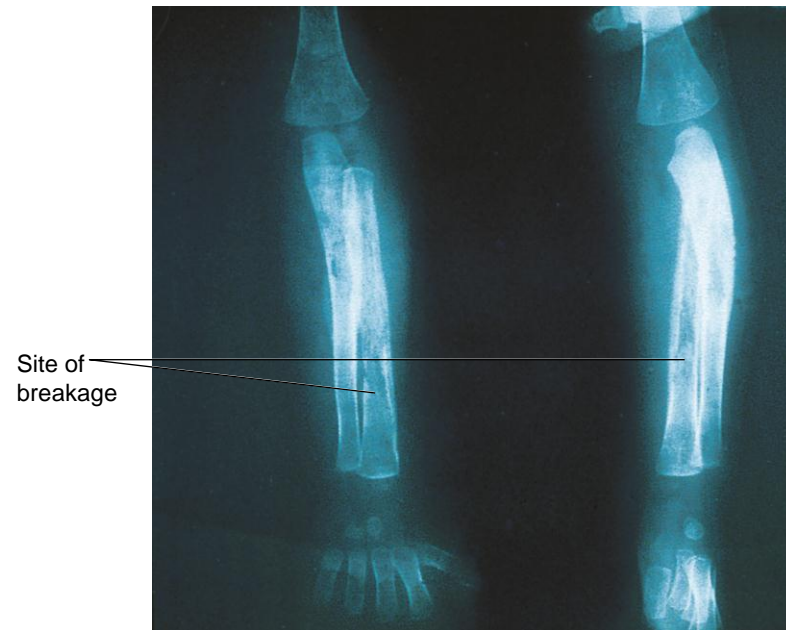


Figure 18.4 Staph osteomyelitis in a long bone



(b)

Toxigenic Staphylococcal Disease

- **Toxigenic disease**
 - **Food intoxication:** ingestion of heat stable enterotoxins; gastrointestinal distress
 - **Staphylococcal scalded skin syndrome:** toxin induces bright red flush, blisters, then desquamation of the epidermis
 - **Toxic shock syndrome:** toxemia leading to shock and organ failure

Figure 18.5 Effects of staphylococcal toxins on skin



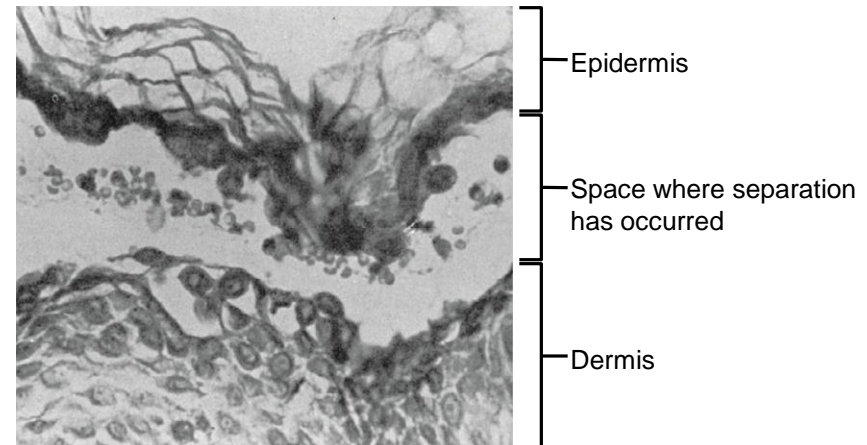
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(a)



© National Institute Slide Bank/The Wellcome Centre for Medical Sciences

(b)



From Braude, Infections Diseases in Medical Microbiology , 2/e, fig 3, page 1320 ©Saunders College Publishing

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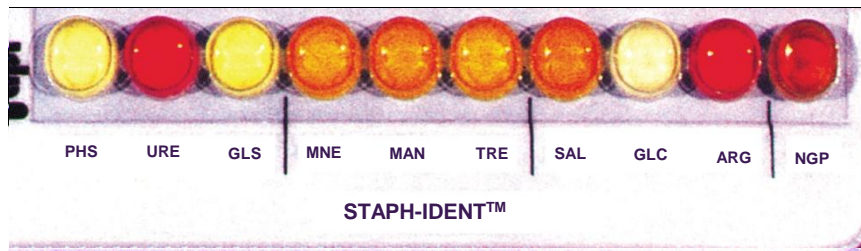
Other Important Staphylococci

Coagulase-negative staphylococcus; frequently involved in nosocomial and opportunistic infections

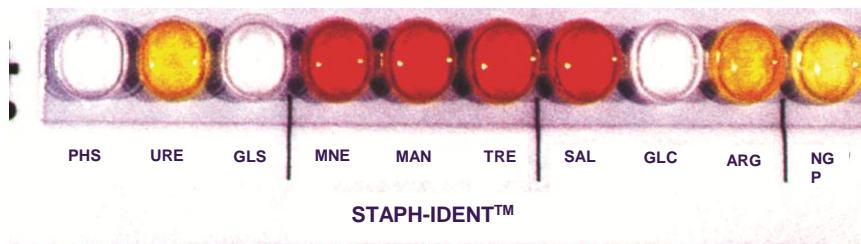
- *S. epidermidis* – lives on skin and mucous membranes; endocarditis, bacteremia, UTI
- *S. hominis* – lives around apocrine sweat glands
- *S. capitis* – live on scalp, face, external ear
- All 3 may cause wound infections by penetrating through broken skin
- *S. saprophyticus* – infrequently lives on skin, intestine, vagina; UTI

Identification of *Staphylococcus* Isolates in Clinical Samples

- Frequently isolated from pus, tissue exudates, sputum, urine, and blood
- Cultivation, catalase, biochemical testing, coagulase



All tests : **positive**



All tests : **negative**

Figure 18.6 Tests for differentiating the genus *Staphylococcus* from *Streptococcus* and for identifying *S. aureus*

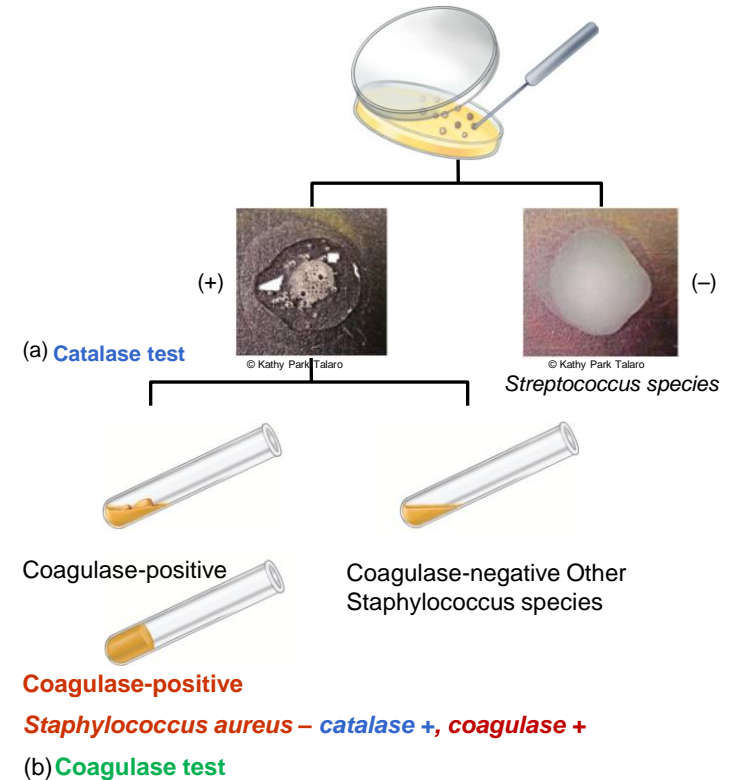
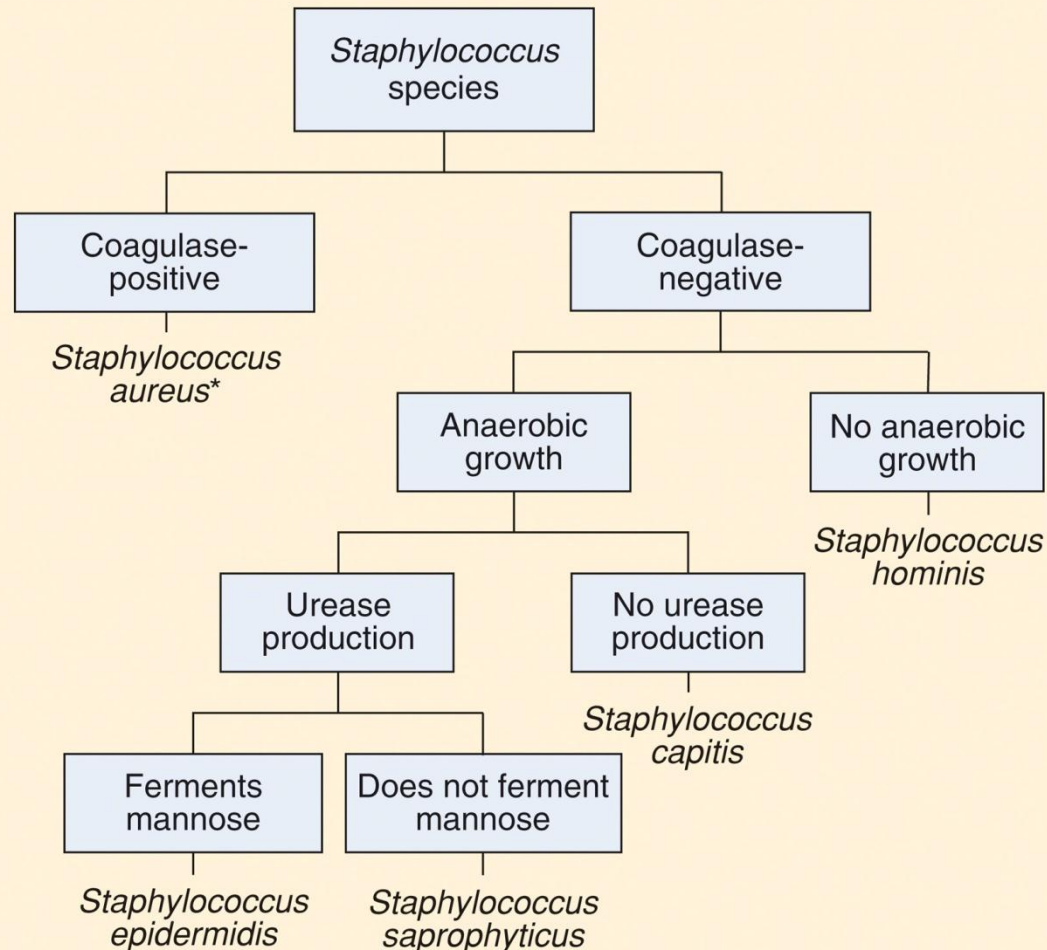


Figure 18.7 Mini test system used in further identification of *Staph* isolates

Species of Staphylococcus

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TABLE 18.2 Separation of Clinically Important Species of *Staphylococcus*



Clinical Concerns in Staphylococcal Infections

- 95% have penicillinase and are resistant to penicillin and ampicillin
- **MRSA** (methicillin-resistant *S. aureus*) : carry multiple resistance
 - Some strains have resistance to all major drug groups except vancomycin
- Abscesses have to be surgically perforated
- Systemic infections require intensive lengthy therapy

Prevention of Staphylococcal Infections

- Universal precautions by healthcare providers to prevent nosocomial infections
- Hygiene and cleansing –
- **WASH YOUR HANDS!!!**
- **WEAR GLOVES!!!**

18.2 General Characteristics of the Streptococci and Related Genera

- **Gram-positive** spherical/ovoid cocci arranged in long chains; commonly in pairs
- Non-spore-forming, nonmotile
- Can form capsules and slime layers
- Facultative anaerobes
- Do not form catalase, but have a peroxidase system
- Most parasitic forms are fastidious and require enriched media
- Small, nonpigmented colonies
- Sensitive to drying, heat, and disinfectants

Figure 18.8 Freshly Isolated *Streptococcus*

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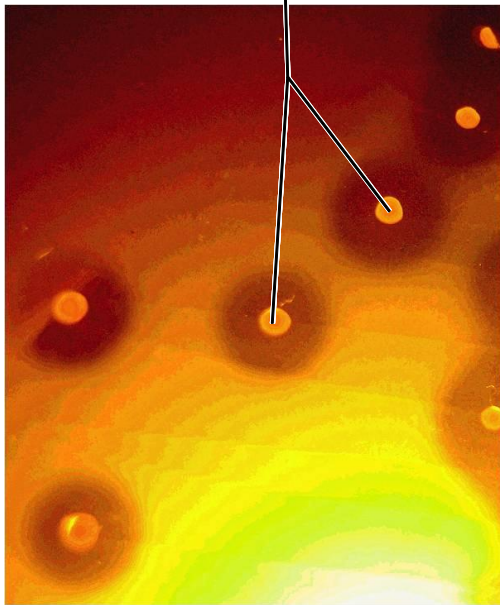
long chains



Streptococci

- Lancefield classification system based on cell wall Ag – 17 groups (A, B, C,.....)
- Another classification system is based on **hemolysis**
 - β -hemolysis – A, B, C, G and some D strains
 - α – hemolysis – *S. pneumoniae* and *viridans*

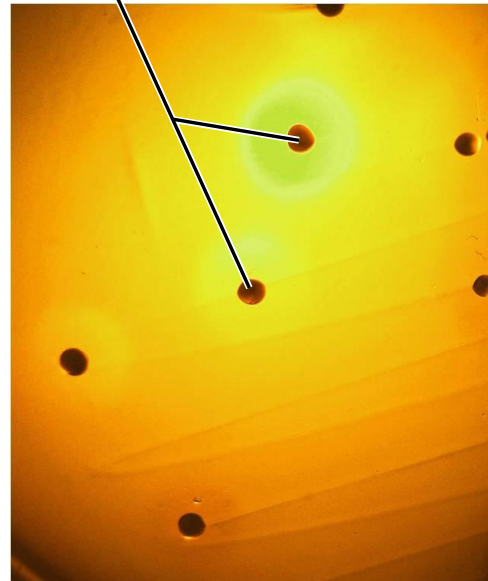
Streptococcus pyogenes
with zones of β -hemolysis



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(a)

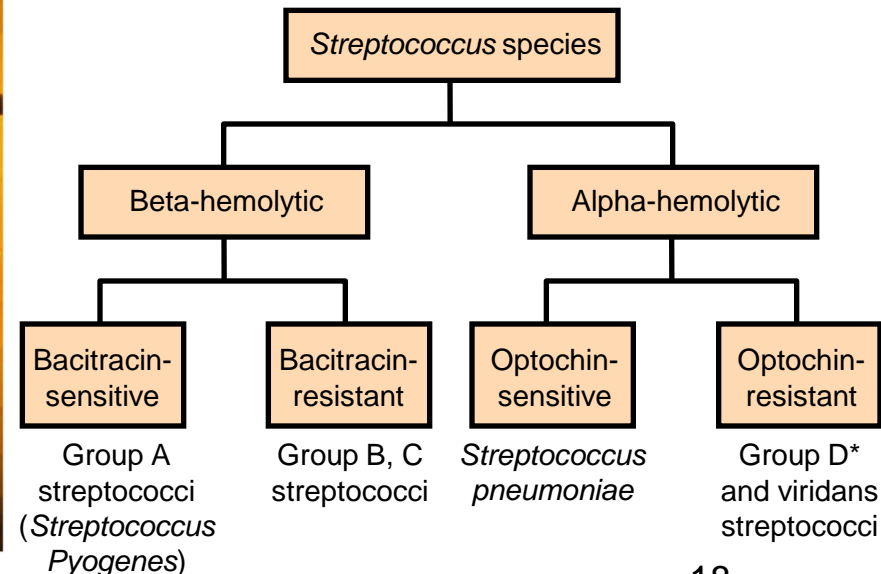
Streptococcus pneumoniae
Displaying α -hemolysis



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(b)

Figure 18.9 Hemolysis patterns on blood agar may be used to separate streptococci into major subgroups



(c)

Streptococcus and Related Genera

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TABLE 18.3 Major Species of *Streptococcus* and Related Genera

Species	Lancefield Group	Hemolysis Type	Habitat	Pathogenicity to Humans
<i>S. pyogenes</i>	A	Beta (β)	Human throat	Skin, throat infections, scarlet fever
<i>S. agalactiae</i>	B	β	Human vagina, cow udder	Neonatal, wound infections
<i>S. equisimilis</i>	C	β	Swine, cows, horses	Pharyngitis, endocarditis
<i>S. equi</i> , <i>S. zooepidemicus</i>	C	β	Various mammals	Rare, in abscesses
<i>S. dysgalactiae</i>	C	β	Cattle	Rare
<i>Enterococcus faecalis</i>	D	α , β , N	Human, animal intestine	Endocarditis, UTI*
<i>E. faecium</i> , <i>E. durans</i>	D	Alpha (α)	Human, animal intestine	Similar to <i>E. faecalis</i>
<i>S. bovis</i>	D	N	Cattle	Subacute endocarditis, bacteremia
<i>S. anginosus</i>	F, G, L	β	Humans, dogs	Endocarditis, URT** infections
<i>S. sanguis</i>	H	α	Human oral cavity	Endocarditis
<i>S. salivarius</i>	K	N	Human saliva	Endocarditis
<i>Lactococcus lactis</i>	N	V	Dairy products	Very rare
<i>S. mutans</i>	NI***	N	Human oral cavity	Dental caries
<i>S. uberis</i> , <i>S. acidominimus</i>	NI	V	Domestic mammals	Rare
<i>S. mitior</i>	O, M	α	Human oral cavity	Tooth abscess, endocarditis
<i>S. milleri</i>	F	N	URT	Endocarditis, organ abscess
<i>S. pneumoniae</i>	NI	α	Human RT	Bacterial pneumonia

Note: Species in bold type are the most significant sources of human infection and disease. N = none; V = varies

*Urinary tract infection **Upper respiratory tract ***No group C carbohydrate identified

Human Streptococcal Pathogens

- ***S. pyogenes***
- ***S. agalactiae***
- **Viridans streptococci**
- ***S. pneumoniae***
- ***Enterococcus faecalis***

β -hemolytic *S. pyogenes*

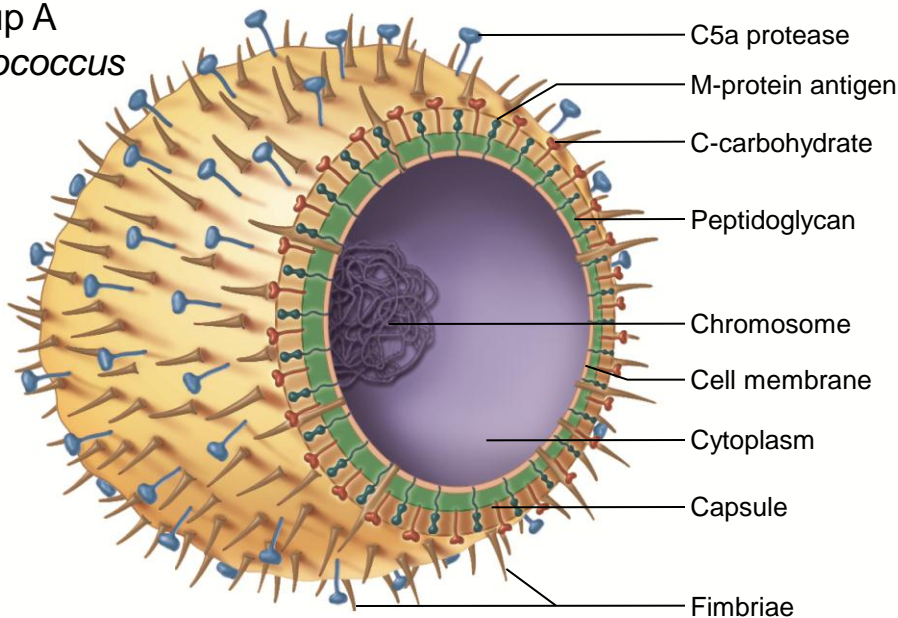
- Most serious streptococcal pathogen
- Strict parasite
- Inhabits throat, nasopharynx, occasionally skin

Cell Surface Antigens and Virulence Factors of *S. pyogenes*

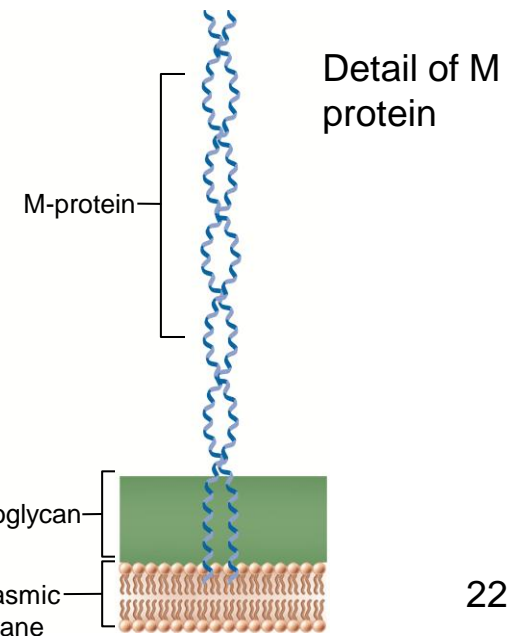
Produces surface antigens:

- **C-carbohydrates**: protect against lysozyme
- **Fimbriae**: adherence
- **M-protein**: contributes to resistance to phagocytosis
- Hyaluronic acid **capsule**: provokes no immune response
- C5a protease hinders complement & neutrophil response

Cut away view
of group A
Streptococcus



(a)



(b)

Virulence Factors of S. pyogenes

Extracellular toxins:

Streptolysins: hemolysins; streptolysin O (SLO) and streptolysin S (SLS) – both cause cell and tissue injury

Erythrogenic toxin (pyrogenic): induces fever and typical red rash

Superantigens: strong monocyte and lymphocyte stimulants; cause the release of tissue necrotic factor

Virulence Factors of S. pyogenes

Extracellular enzymes

Streptokinase – digests fibrin clots

Hyaluronidase – breaks down connective tissue

DNase – hydrolyzes DNA

Epidemiology and Pathogenesis of Streptococcus pyogenes

- Humans only reservoir
- Inapparent carriers
- Transmission – contact, droplets, food, fomites
- Portal of entry generally skin or pharynx
- Children predominant group affected for cutaneous and throat infections
- Systemic infections and progressive sequelae possible if untreated

Scope of Clinical Disease – *S. pyogenes*

Skin infections:

- **Impetigo (pyoderma)**: superficial lesions that break and form highly contagious crust; often occurs in epidemics in school children; also associated with insect bites, poor hygiene, and crowded living conditions
- **Erysipelas**: pathogen enters through a break in the skin and eventually spreads to the dermis and subcutaneous tissues; can remain superficial or become systemic

impetigo



© Kenneth E. Greer/Visuals Unlimited

erysipelas



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Scope of Clinical Disease – *S. pyogenes*

Throat infections:

- **Streptococcal pharyngitis** – strep throat

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Figure 18.12 The appearance of the throat in pharyngitis and tonsillitis

Scope of Clinical Disease

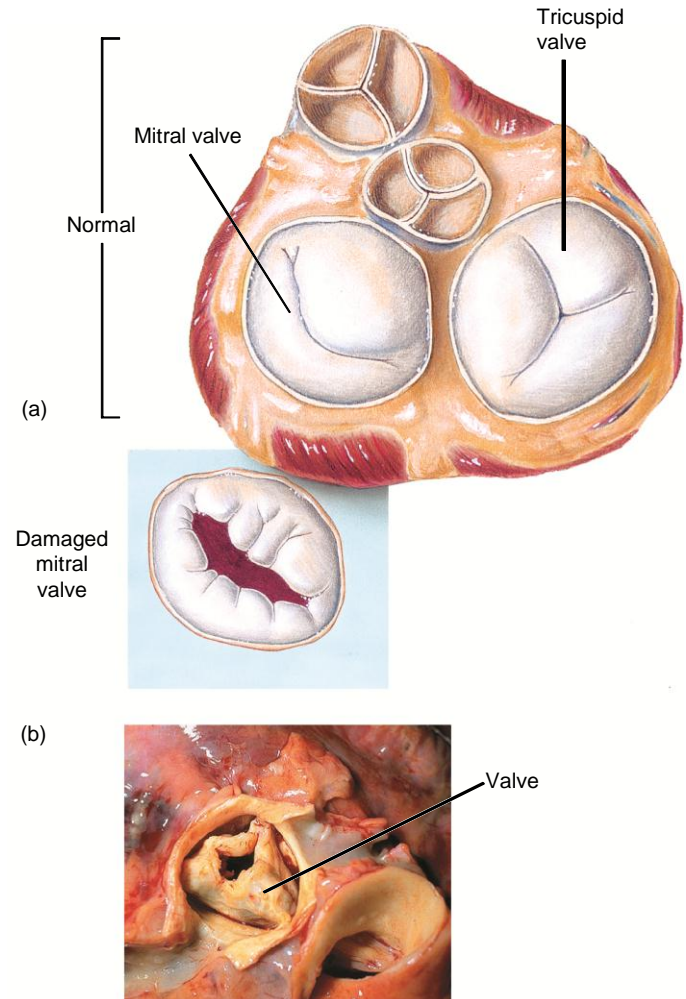
Systemic infections:

- **Scarlet fever** – strain of *S. pyogenes* carrying a prophage that codes for erythrogenic toxin; can lead to sequelae
- Septicemia
- Pneumonia
- Streptococcal toxic shock syndrome

Long-Term Complications of Group A Infections

- **Rheumatic fever** – follows overt or subclinical pharyngitis in children; carditis with extensive valve damage possible, arthritis, chorea, fever
- **Acute glomerulonephritis** – nephritis, increased blood pressure, occasionally heart failure; can become chronic leading to kidney failure

Figure 18.13 The cardiac complications of rheumatic fever



Dr. Edwin P. Ewing, Jr./CDC

Group B: *Streptococcus agalactiae*

- Regularly resides in human vagina, pharynx, and large intestine
- Can be transferred to infant during delivery and cause severe infection
 - Most prevalent cause of neonatal pneumonia, sepsis, and meningitis
 - Pregnant women should be screened and treated
- Wound and skin infections and endocarditis in debilitated people

Group D Enterococci and Groups C and G Streptococci

- **Group D:**

- *Enterococcus faecalis*, *E. faecium*, *E. durans*
- Normal colonists of human large intestine
- Cause opportunistic urinary, wound, and skin infections, particularly in debilitated persons

- **Groups C and G:**

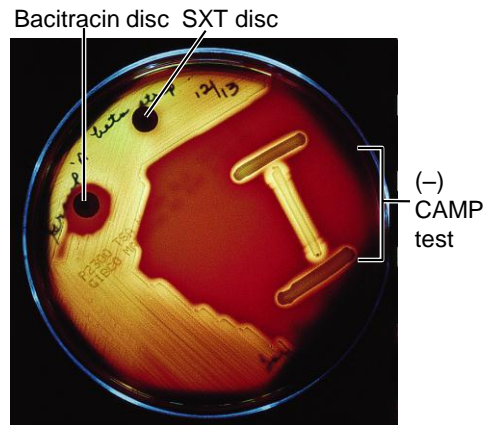
- Common animal flora, frequently isolated from upper respiratory; pharyngitis, glomerulonephritis, bacteremia

Laboratory Identification of Streptococci

- Cultivation and diagnosis ensure proper treatment to prevent possible complications
- Rapid diagnostic tests based on monoclonal antibodies that react with C-carbohydrates:

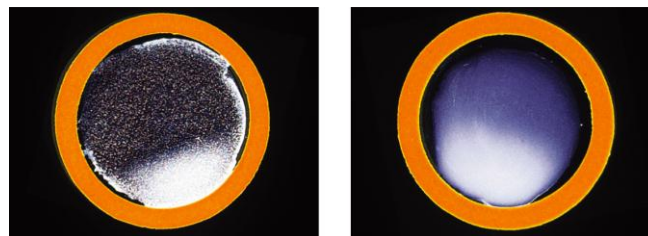
Figure 18.14
Streptococcal tests

a. Bacitracin –
sensitive to minute
concentrations



(a) Courtesy D. David Schlaes

b. Rapid, direct test
kit for group A strep



© Diagnostic Products Corporation

(b)

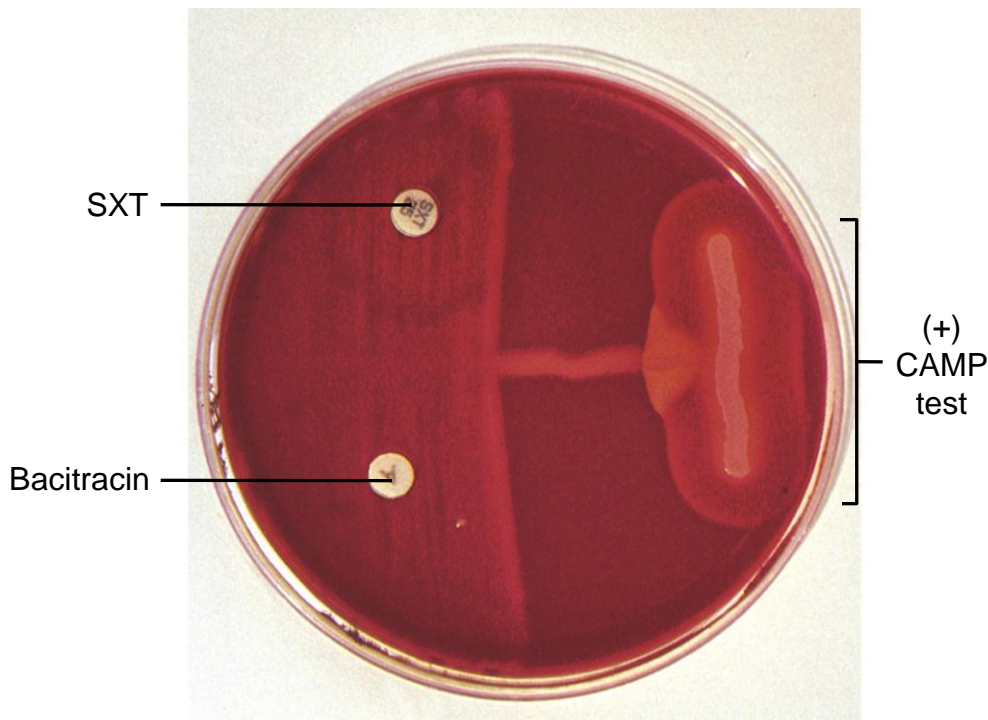
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Negative reaction

Identification of Streptococci

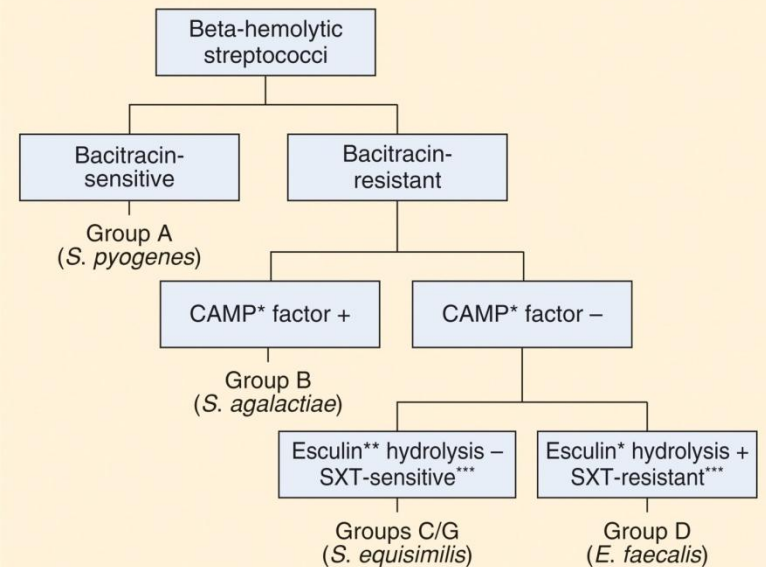
- Culture using bacitracin disc test, CAMP test, Esculin hydrolysis

Figure 18.15 Test for β -hemolytic strep



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TABLE 18.4 Scheme for Differentiating Beta-Hemolytic Streptococci



Treatment and Prevention of Group A, B, and D Streptococcal Infections

- Groups A and B are treated with penicillin
- Long-term penicillin prophylaxis for people with a history of rheumatic fever or recurrent strep throat
- Enterococcal treatment usually requires combined therapy

α -Hemolytic Streptococci: Viridans Group

- Large complex group
 - *Streptococcus mutans*, *S. oralis*, *S. salivarius*,
S. sanguis, *S. milleri*, *S. mitis*
- Most numerous and widespread residents of the gums and teeth, oral cavity, and also found in nasopharynx, genital tract, skin
- Not very invasive; dental or surgical procedures facilitate entrance

Viridans Group

- Bacteremia, meningitis, abdominal infection, tooth abscesses
- Most serious infection: **subacute endocarditis** – Blood-borne bacteria settle and grow on heart lining or valves
- Persons with preexisting heart disease are at high risk
- Colonization of heart by forming biofilms

Figure 18.16 Effects of streptococcal colonization

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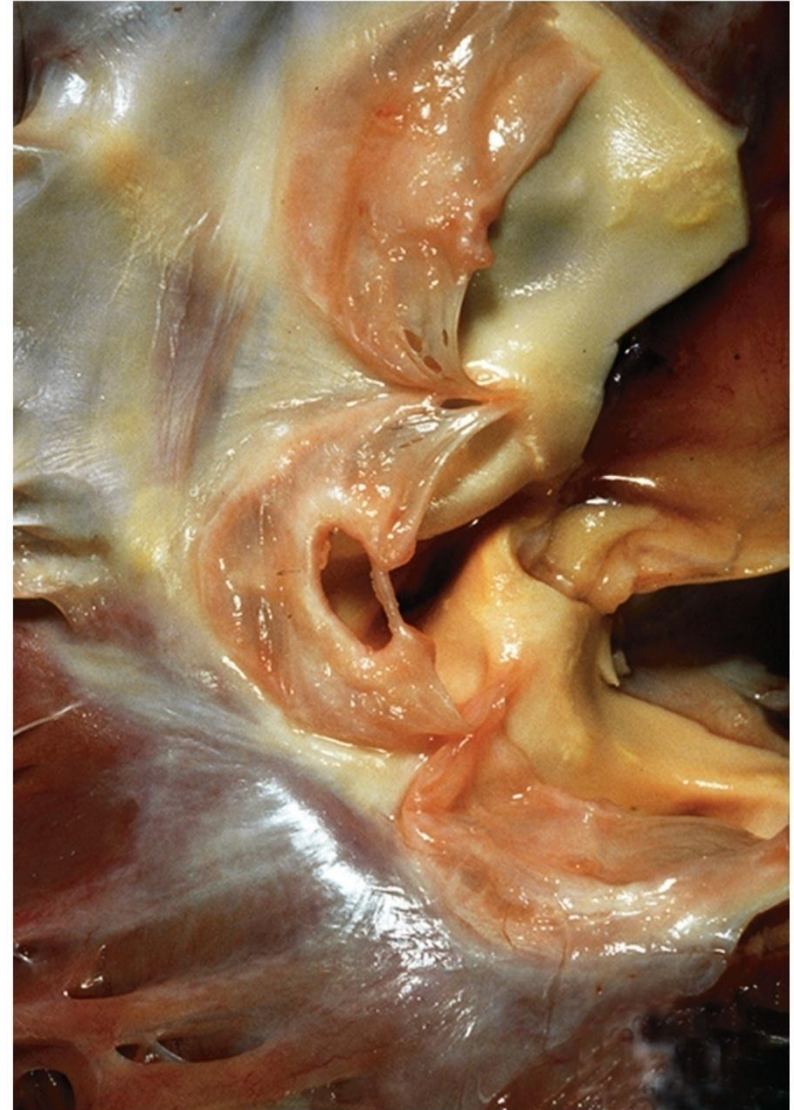


Image reprinted with permission from eMedicine.com, 2010. Available at: <http://emedicine.medscape.com/article/1612368-overview>

Viridans Group

- *S. mutans* produce slime layers that adhere to teeth, basis for **plaque**
- Involved in **dental caries**
- Persons with preexisting heart conditions should receive prophylactic antibiotics before surgery or dental procedures

Streptococcus pneumoniae: The Pneumococcus

- Causes 60-70% of all **bacterial pneumonias**
- Small, lancet-shaped cells arranged in pairs and short chains
- Culture requires blood or chocolate agar & growth improved by 5-10% CO₂
- Lack catalase and peroxidases – cultures die in O₂

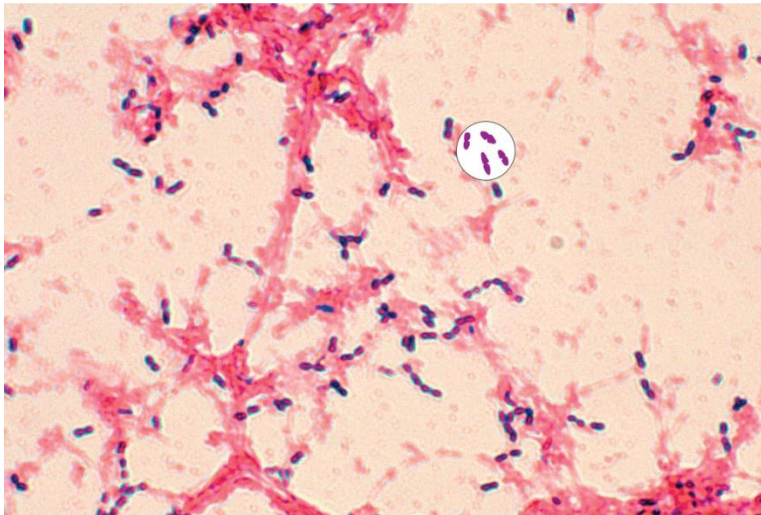


Figure 18.17 Gram stain of *Streptococcus pneumoniae*

S. pneumoniae

- All pathogenic strains form large **capsules** – major virulence factor
- Specific soluble substance (SSS) varies among types
- 90 different capsular types have been identified
- Causes pneumonia and otitis media

Epidemiology and Pathology of the Pneumococcus

- 5-50% of all people carry it as normal flora in the nasopharynx; infections are usually endogenous
- Very delicate, does not survive long outside of its habitat
- Young children, elderly, immune compromised, those with other lung diseases or viral infections, persons living in close quarters are predisposed to pneumonia
- Pneumonia occurs when cells are aspirated into the lungs of susceptible individuals
- Pneumococci multiply and induce an overwhelming inflammatory response
- Gains access to middle ear by way of eustachian tube

Figure 18.18 *S. pneumoniae* and Pneumonia – The course of bacterial pneumonia

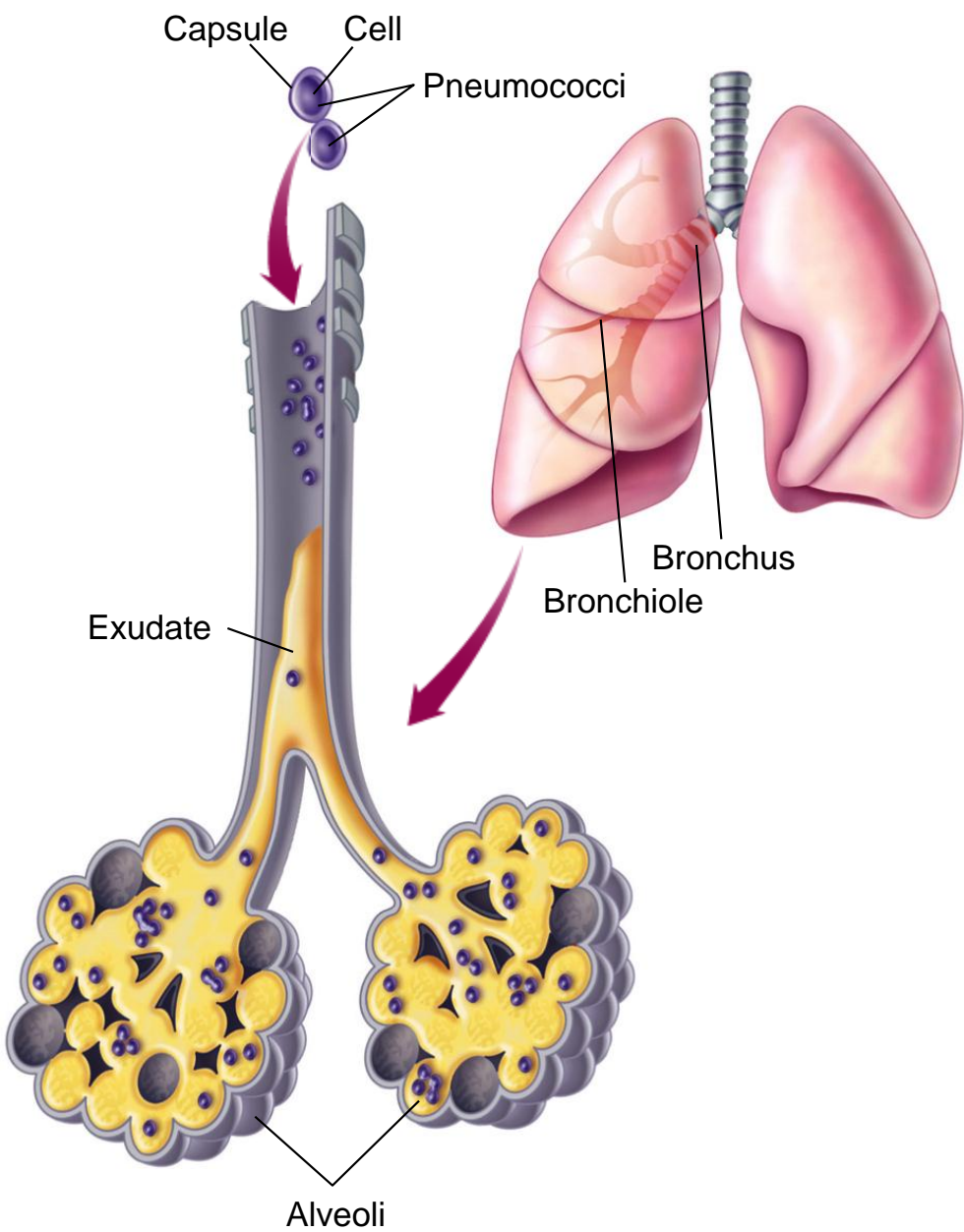
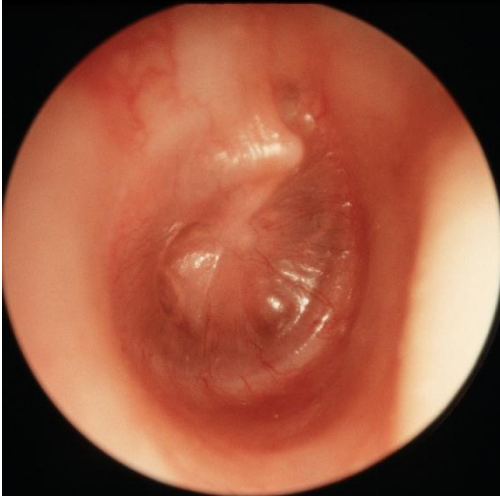
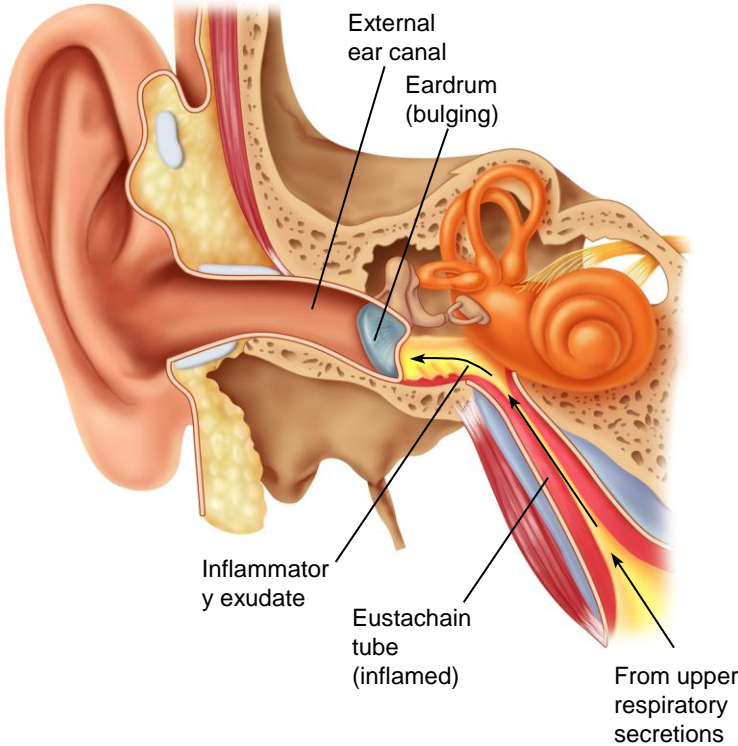


Figure 18.20 *S. pneumoniae* and Otitis Media



© Clinica Claros/Phototake
(a) a. Acute inflammation



b. Chronic infection

(b) © Clinica Claros/Phototake

Laboratory Cultivation and Diagnosis

- Gram stain of specimen – presumptive identification
- **Quellung test** or capsular swelling reaction
- α -hemolytic; optochin sensitivity, bile solubility, inulin fermentation

Treatment and Prevention of Pneumococcal Infections

- Traditionally treated with penicillin G or V
- Increased drug resistance
- Two vaccines available for high risk individuals:
 - Capsular antigen vaccine for older adults and other high risk individuals – effective 5 years
 - Conjugate vaccine for children 2 to 23 months

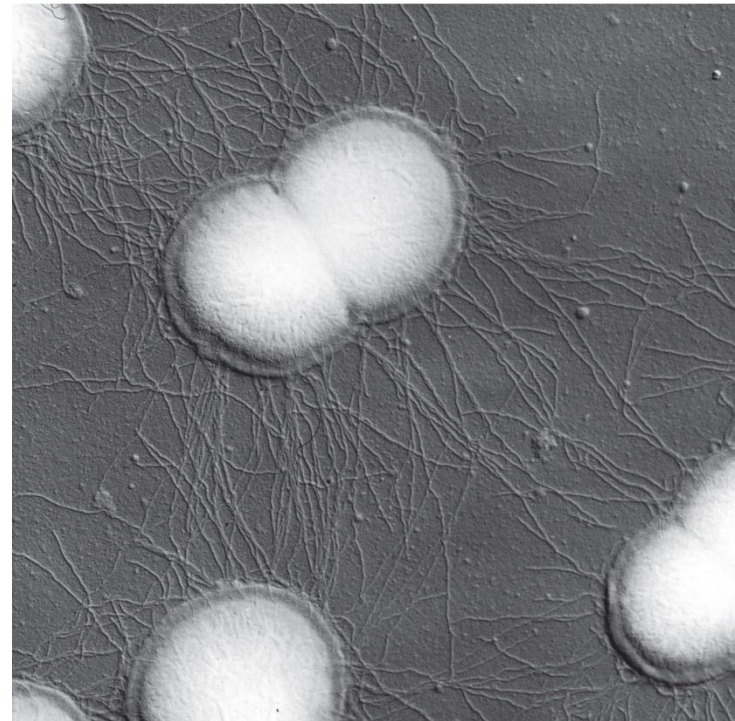
18.3 The Family Neisseriaceae: **Gram-negative Cocci**

- Gram-negative cocci
- Residents of mucous membranes of warm-blooded animals
- Genera include *Neisseria*, *Branhamella*, *Moraxella*
- 2 primary human pathogens:
 - *Neisseria gonorrhoeae*
 - *Neisseria meningitidis*

Genus *Neisseria*

- Gram-negative, bean-shaped, diplococci
- None develop flagella or spores
- **Capsules** on pathogens
- **Pili**
- Strict parasites, do not survive long outside of the host
- Aerobic or microaerophilic
- Produce **catalase** and cytochrome oxidase
- Pathogenic species require enriched complex media

Figure 18.21 TEM of *Neisseria*



© Charles C. Brinton, Jr., John A. Tainer, Michael E. Piques and Lisa Craig

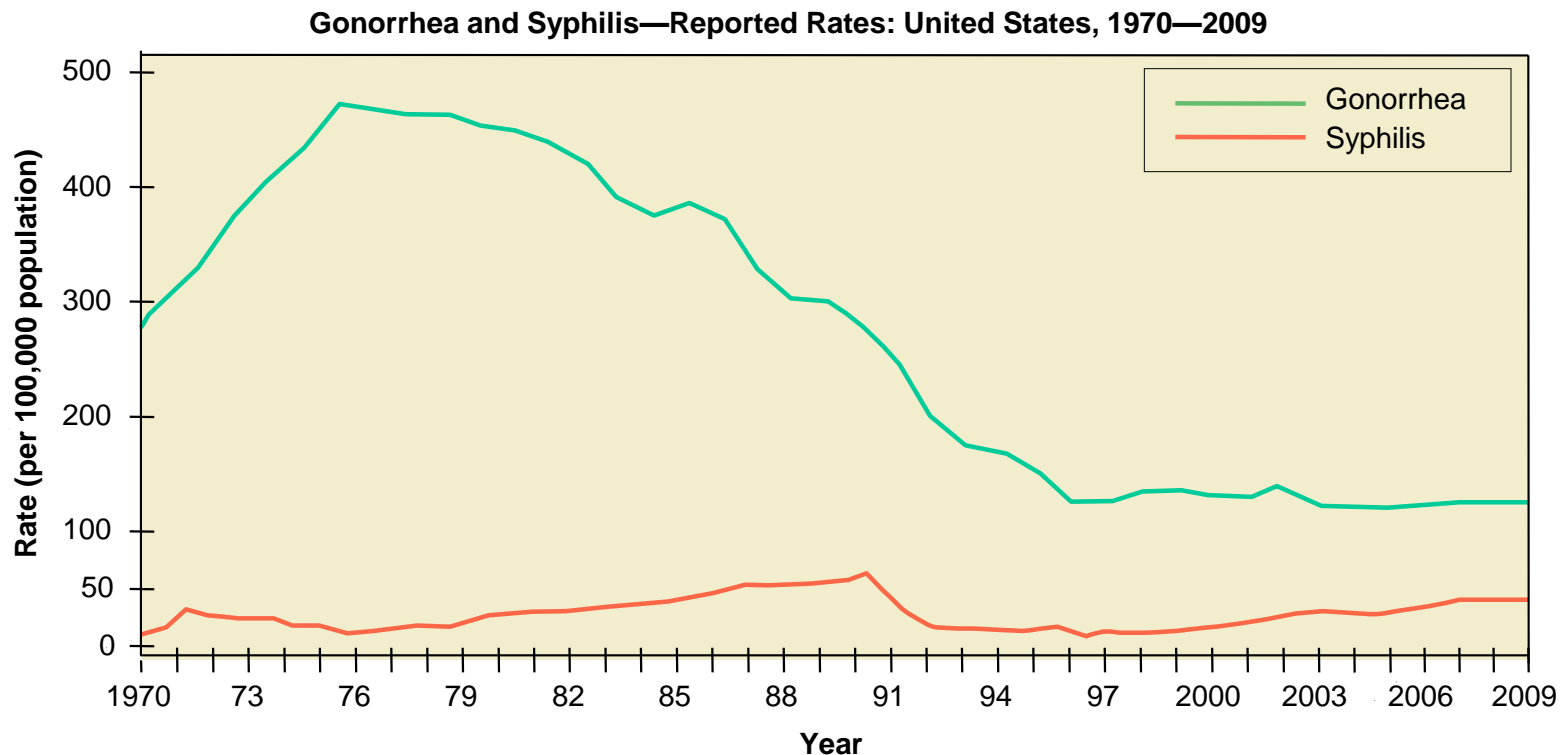
Neisseria gonorrhoeae: The Gonococcus

- Causes **gonorrhea**, an STD
- Virulence factors:
 - **Fimbriae**, other surface molecules for attachment; slows phagocytosis
 - **IgA protease**: cleaves secretory IgA

Epidemiology and Pathology of Gonorrhea

- Strictly a human infection
- In top 5 STDs
- Infectious dose 100-1,000
- Does not survive more than 1-2 hours on fomites

Figure 18.22 Comparative incidence of two reportable infectious STDs



Gonorrhea

- Males – **urethritis**, yellowish discharge, scarring, and infertility
 - 10% of males are asymptomatic

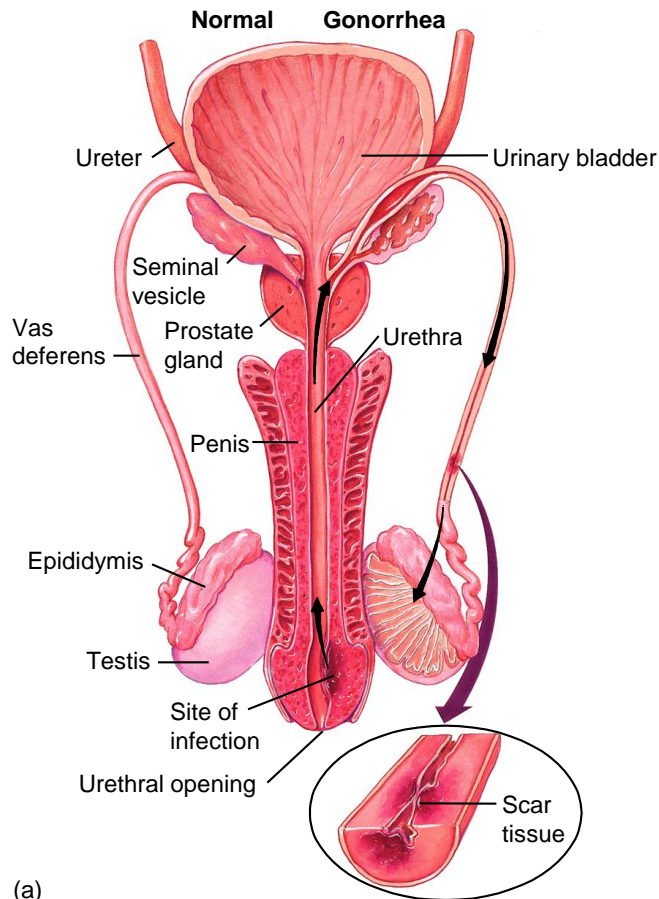


Figure 18.23 Gonorrhea damage to the male reproductive tract



(b) Image courtesy of the Centers for Disease Control and Prevention, Renelle Woodfall

Gonorrhoea

- Females – **vaginitis, urethritis, salpingitis** (PID) mixed anaerobic abdominal infection, common cause of sterility and ectopic tubal pregnancies
 - 50% of females are asymptomatic

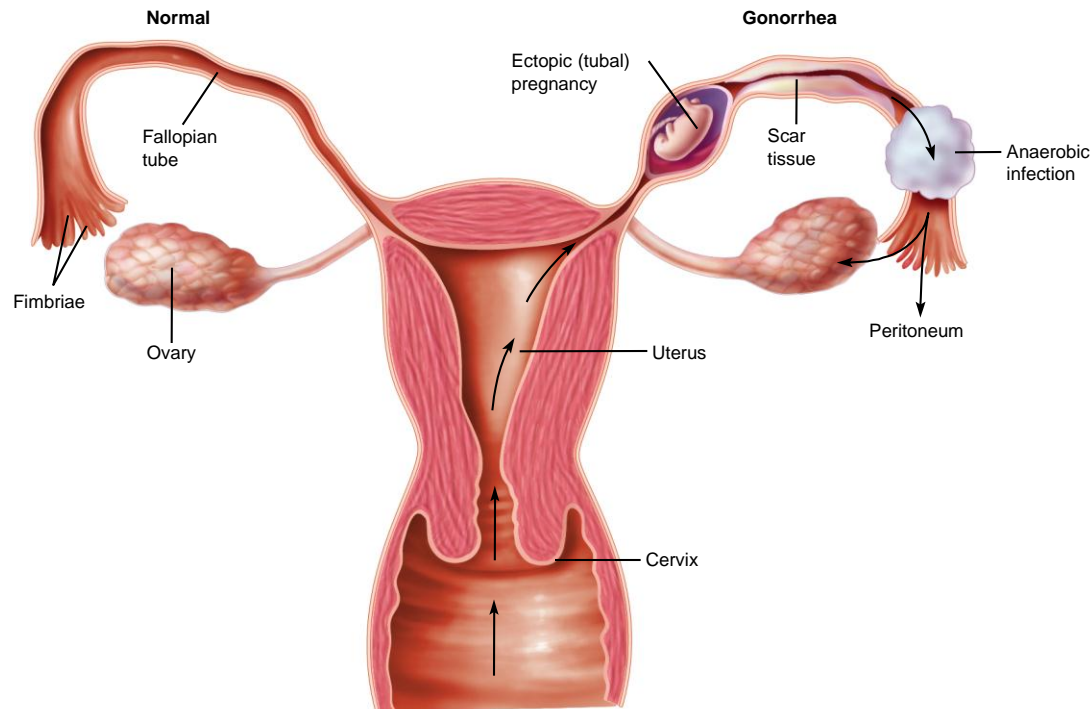


Figure 18.24
Ascending
gonorrhoea in
women

Gonorrhea in Newborns

- Infected as they pass through birth canal
- **Eye inflammation**, blindness
- Prevented by prophylaxis immediately after birth

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Figure 18.25 Gonococcal ophthalmia neonatorum in an infant infected during birth

Clinical Diagnosis and Control of Gonococcal Infections

- Gram stain – **Gram-negative** intracellular (neutrophils) diplococci from urethral, vaginal, cervical, or eye exudate – presumptive identification
- 20-30% of new cases are penicillinase-producing **PPNG** or tetracycline resistant **TRNG**
- Combined therapies indicated
- Recurrent infections can occur
- Reportable infectious disease

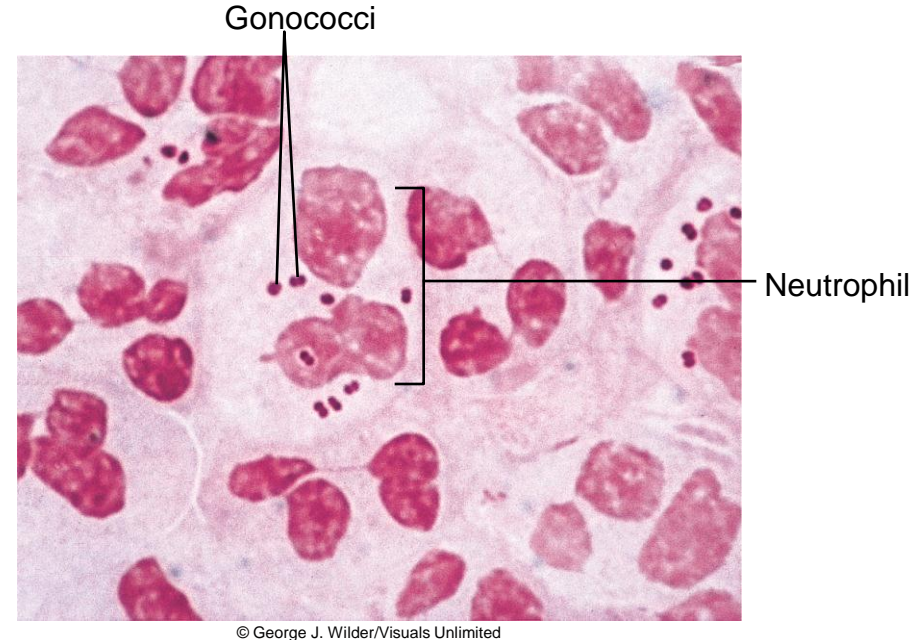


Figure 18.26 Gram stain of urethral pus from a patient with gonorrhea

Neisseria meningitidis: The Meningococcus

Virulence factors:

- **Capsule**
 - **Adhesive fimbriae**
 - **IgA protease**
 - **Endotoxin**
-
- 12 strains; serotypes A, B, C cause most cases

Epidemiology and Pathogenesis of Meningococcal Disease

- Prevalent cause of **meningitis**; sporadic or epidemic
- Human reservoir – nasopharynx; 3-30% of adult population; higher in institutional settings
- High risk individuals are those living in close quarters, children 6 months-3 years, children and young adults 10-20 years
- **Disease begins when bacteria enter bloodstream, cross the blood-brain barrier, permeate the meninges, and grow in the cerebrospinal fluid**
- Very rapid onset; neurological symptoms; endotoxin causes hemorrhage and shock; can be fatal

Meningococcus Infection

Figure 18.27 Dissemination of the meningococcus from a nasopharyngeal infection

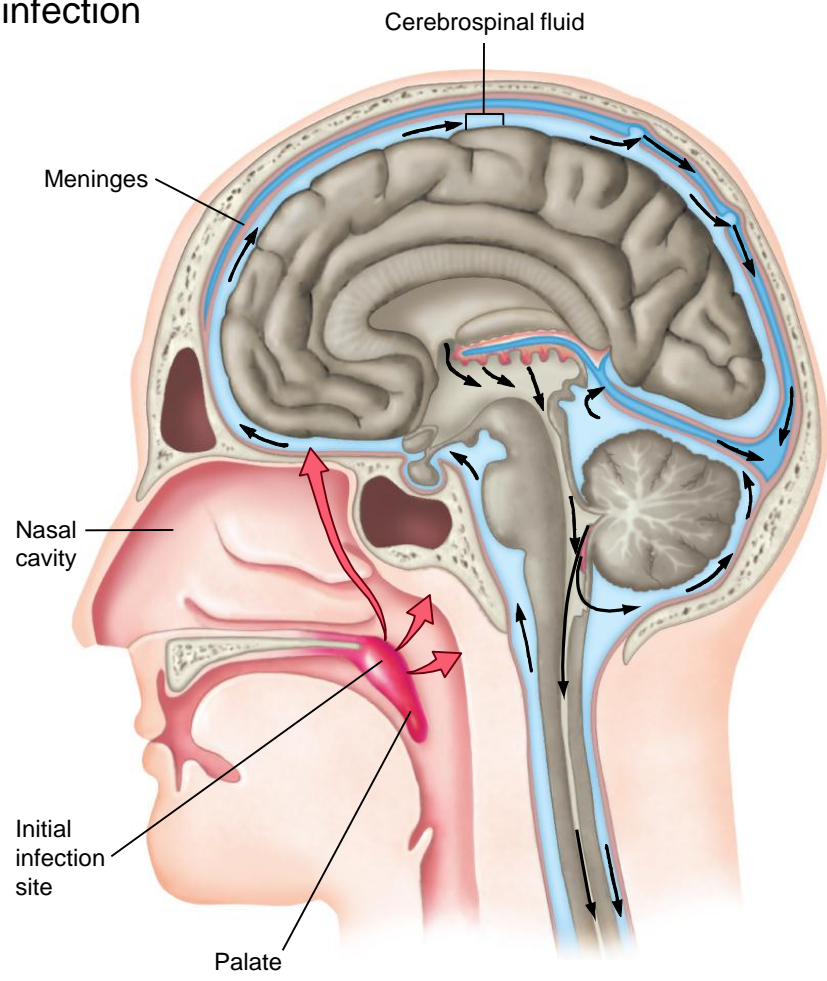


Figure 18.28 One clinical sign of meningococemia

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Clinical Diagnosis of Meningococcal Disease

- Gram stain CSF, blood, or nasopharyngeal sample
- Culture for differentiation
- Rapid tests for capsular polysaccharide

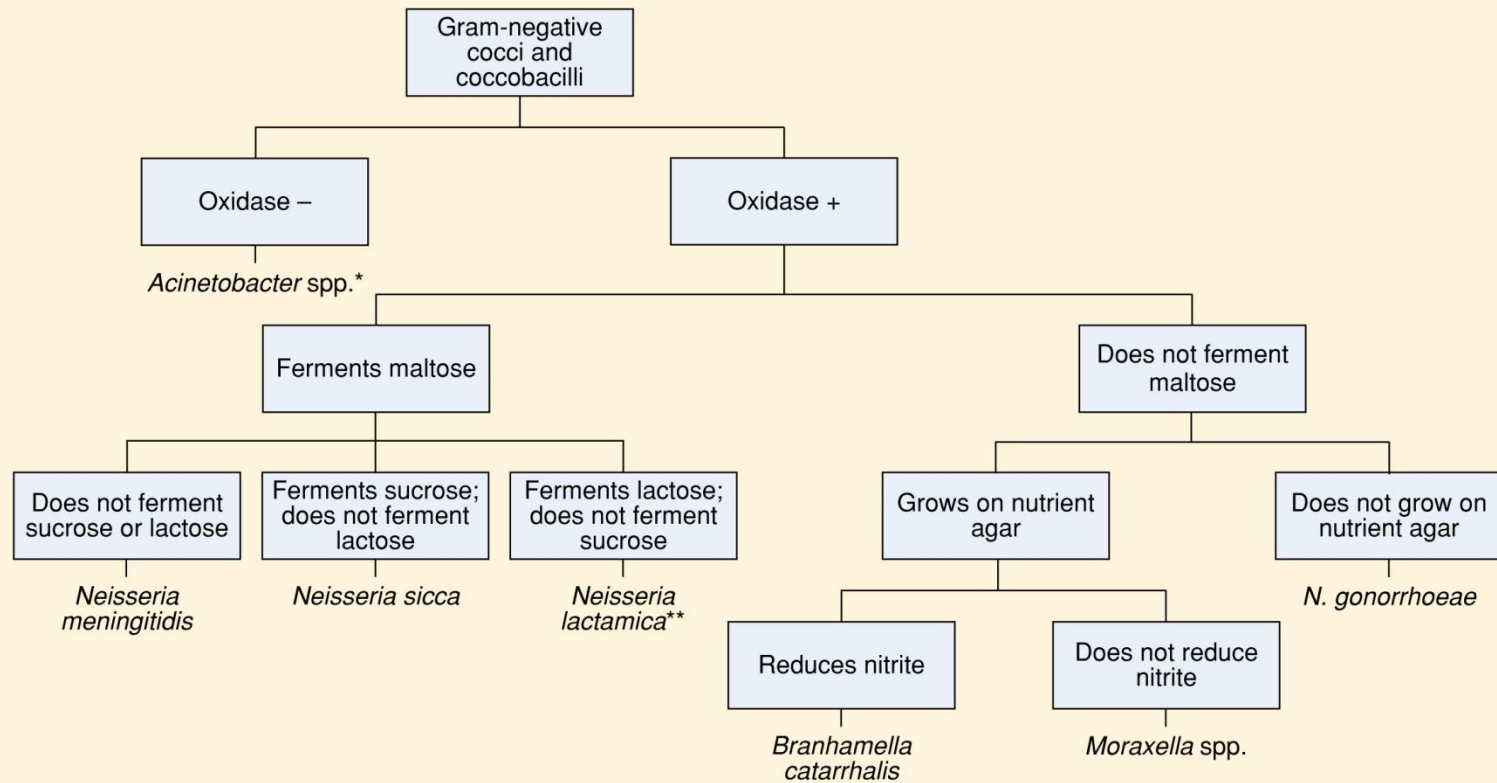
Treatment and Prevention

- Treated with IV penicillin G, cephalosporin
- Prophylactic treatment of family members, medical personnel, or children in close contact with patient
- Primary vaccine contains specific purified capsular antigens

The Gram-Negative Cocci

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TABLE 18.5 Scheme for Differentiating Gram-Negative Cocci and Coccobacilli



*See chapter 20.

**A weak pathogen, found in the nasopharynx of children and easily mistaken for *N. meningitidis*.

Other Gram-Negative Cocci and Coccobacilli

- Genus ***Branhamella***
 - *Branhamella catarrhalis* – found in nasopharynx: significant opportunist in cancer, diabetes, alcoholism
- Genus ***Moraxella***
 - Bacilli – found on mucous membranes; associated with endocarditis