

# Introduction to Microbiology

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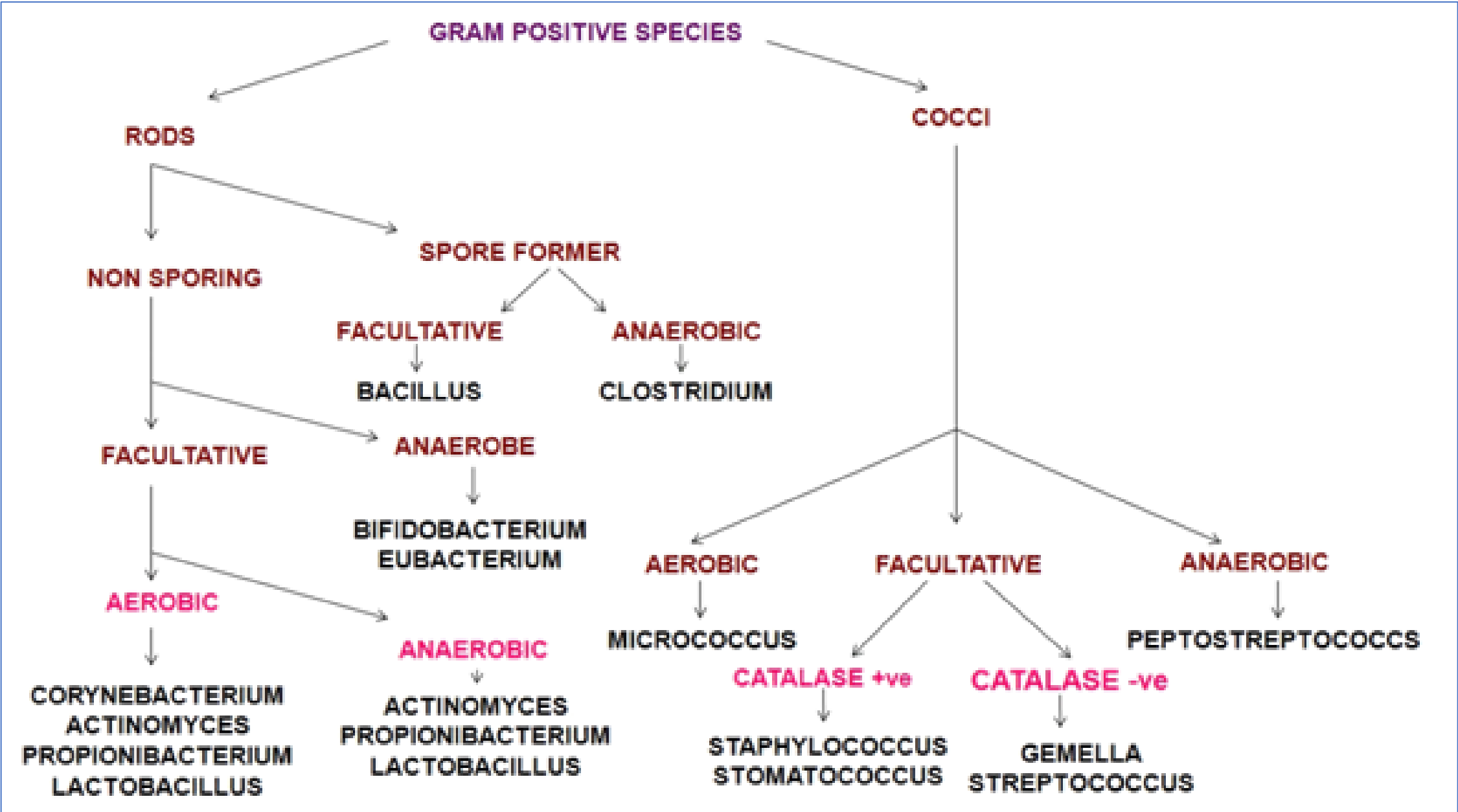
Anas Abu-Humaidan  
M.D. Ph.D.

# Overview

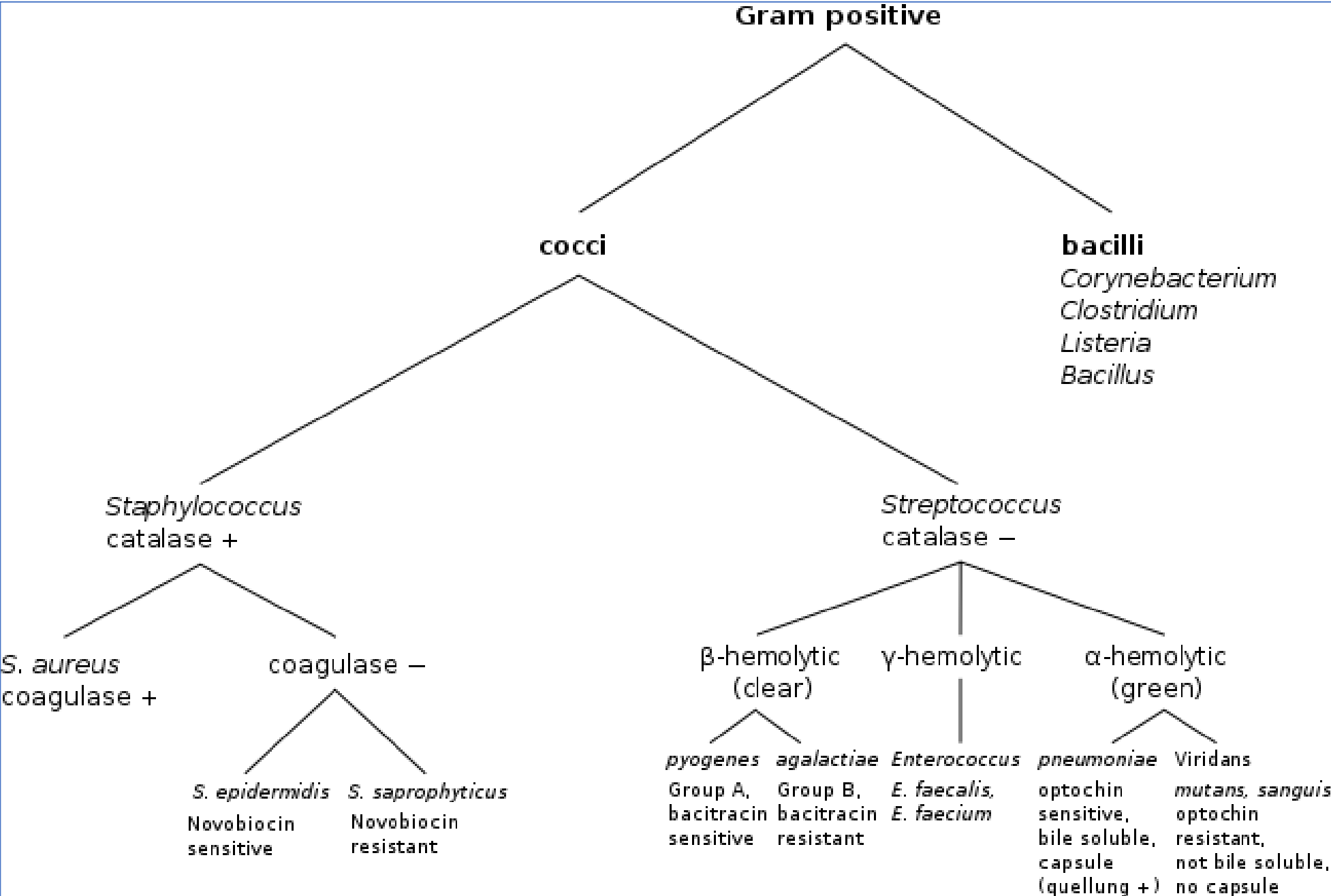
Pathogens that will be discussed this lecture are

- **Non-Spore-Forming Gram-Positive rods (aerobic and anaerobic),**
- **anerobic gram positive cocci,**
- **anaerobic gram-negative rods**

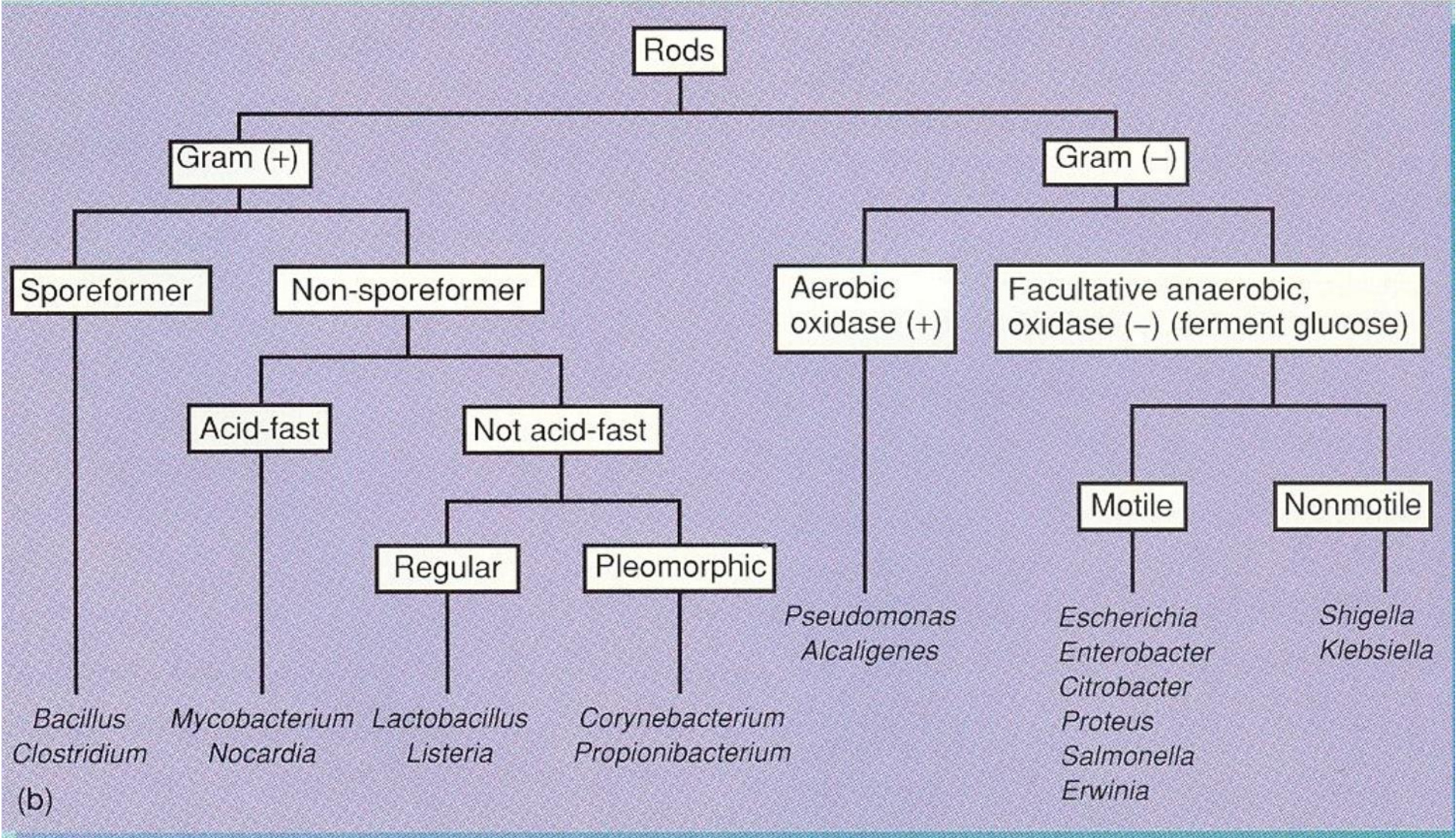
# Overview



# Overview



# Overview



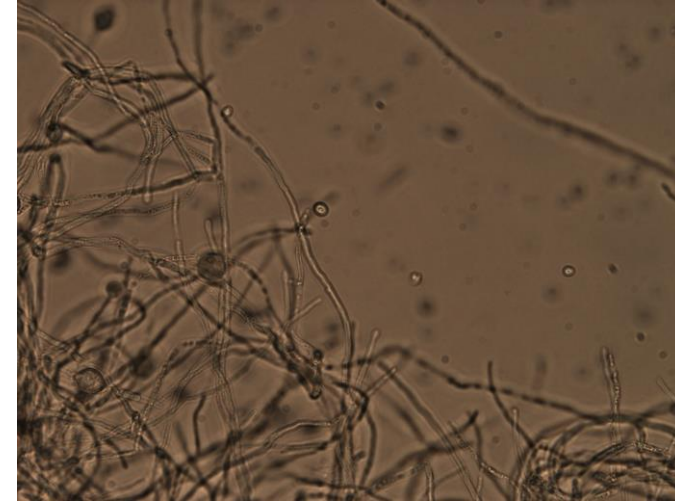
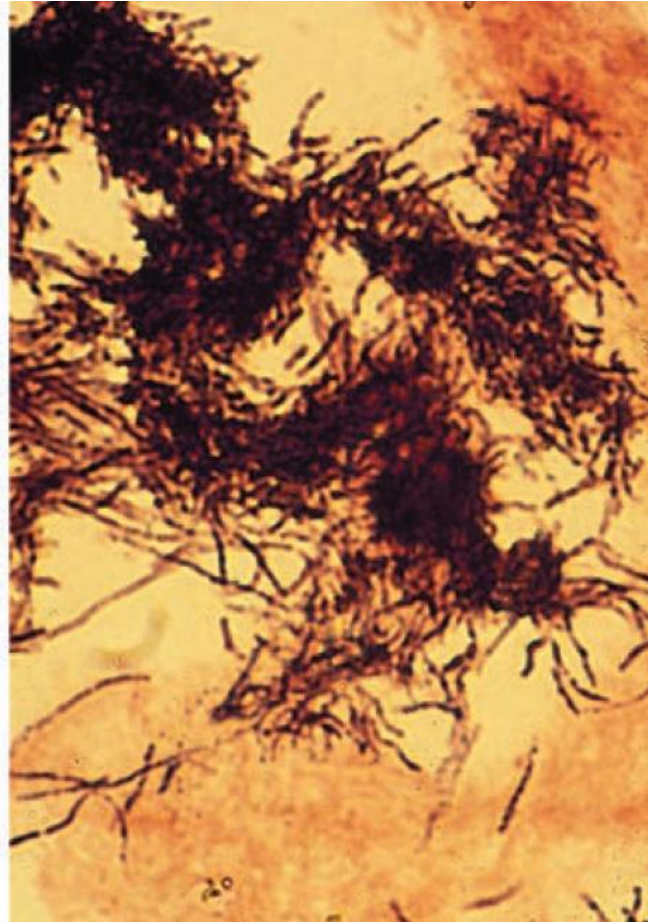
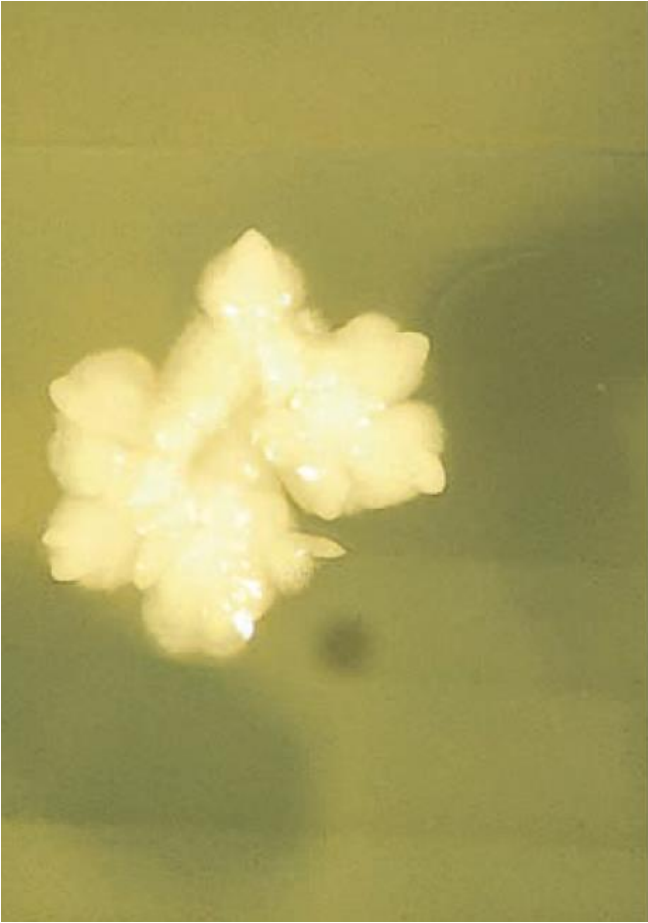
# ***NON-SPORE-FORMING BACTERIA***

- **Anaerobic Gram-Positive Rods** : The non-spore-forming gram-positive rods are a diverse collection of facultatively anaerobic or strictly anaerobic bacteria that colonize the skin and mucosal surfaces.
- ***Actinomyces, Mobiluncus, Lactobacillus, and Propionibacterium*** are well-recognized opportunistic pathogens, whereas other genera such as *Bifidobacterium* and *Eubacterium* can be isolated in clinical specimens but rarely cause human disease.

# Actinomyces

- *Actinomyces* organisms are **facultatively anaerobic or strictly anaerobic gram-positive rods**
- they grow slowly in culture, and they tend to produce **chronic, slowly developing infections**.
- *Actinomyces* organisms colonize the upper respiratory, GI, and female genital tracts but are **not normally present** on the skin surface.
- Infections caused by actinomycetes are **endogenous**, with no evidence of person-to-person spread or disease originating from an exogenous source. (specimens can be contaminated with *Actinomyces* that are part of the normal bacterial population on mucosal surfaces).

# Actinomyces



They typically develop **delicate filamentous forms or hyphae** (resembling fungi) in clinical specimens or when isolated in culture, *Actinomyces* are **fastidious** and grow slowly under anaerobic conditions; it can take 2 weeks or more for the organisms to be isolated

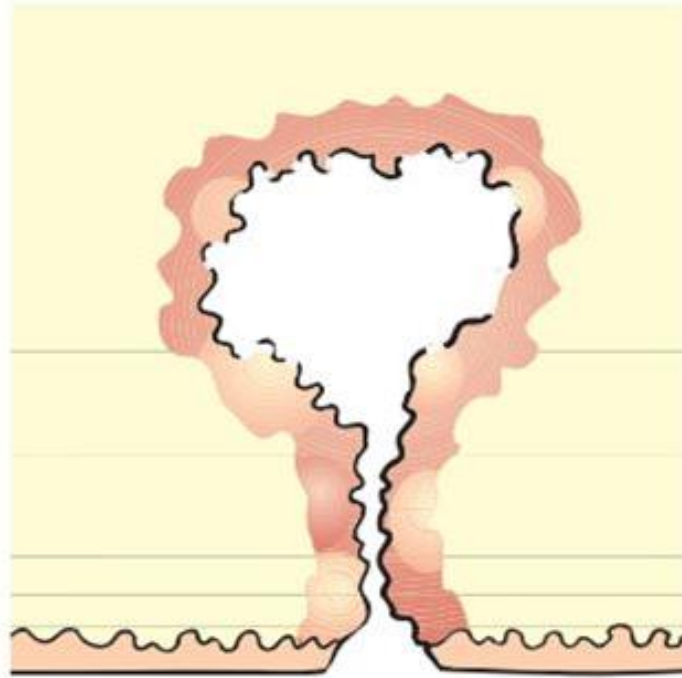
Fungal colonies and hyphae



# Actinomyces

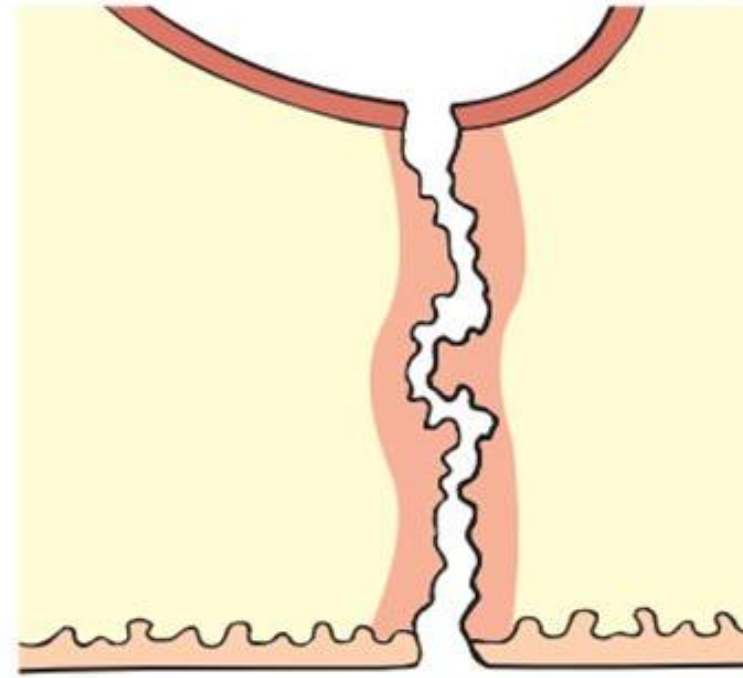
- Classic disease caused by *Actinomyces* is termed **actinomycosis**. Characterized by the development of chronic granulomatous lesions that become suppurative and form abscesses connected by sinus tracts.
- Most actinomycetes infections are **cervicofacial** (following invasive dental procedure or oral trauma).
- The finding of tissue swelling with fibrosis and scarring, as well as **draining sinus** tracts along the angle of the jaw and neck, should alert the physician to the possibility of actinomycosis
- The major sites of actinomycoses are cervicofacial, abdominopelvic, and thoracic
- Abdominal and pelvic infections are associated with abdominal surgery, tuboovarian abscess, ruptured appendicitis, and **intrauterine contraceptive devices (IUCD)**
- Treatment for actinomycosis involves the combination of drainage of a localized abscess or **surgical debridement** of the involved tissues, and **prolonged** administration of antibiotics.

Sinus



A sinus is a connection between a cavity lined with granulation tissue and an epithelial surface.

Fistula

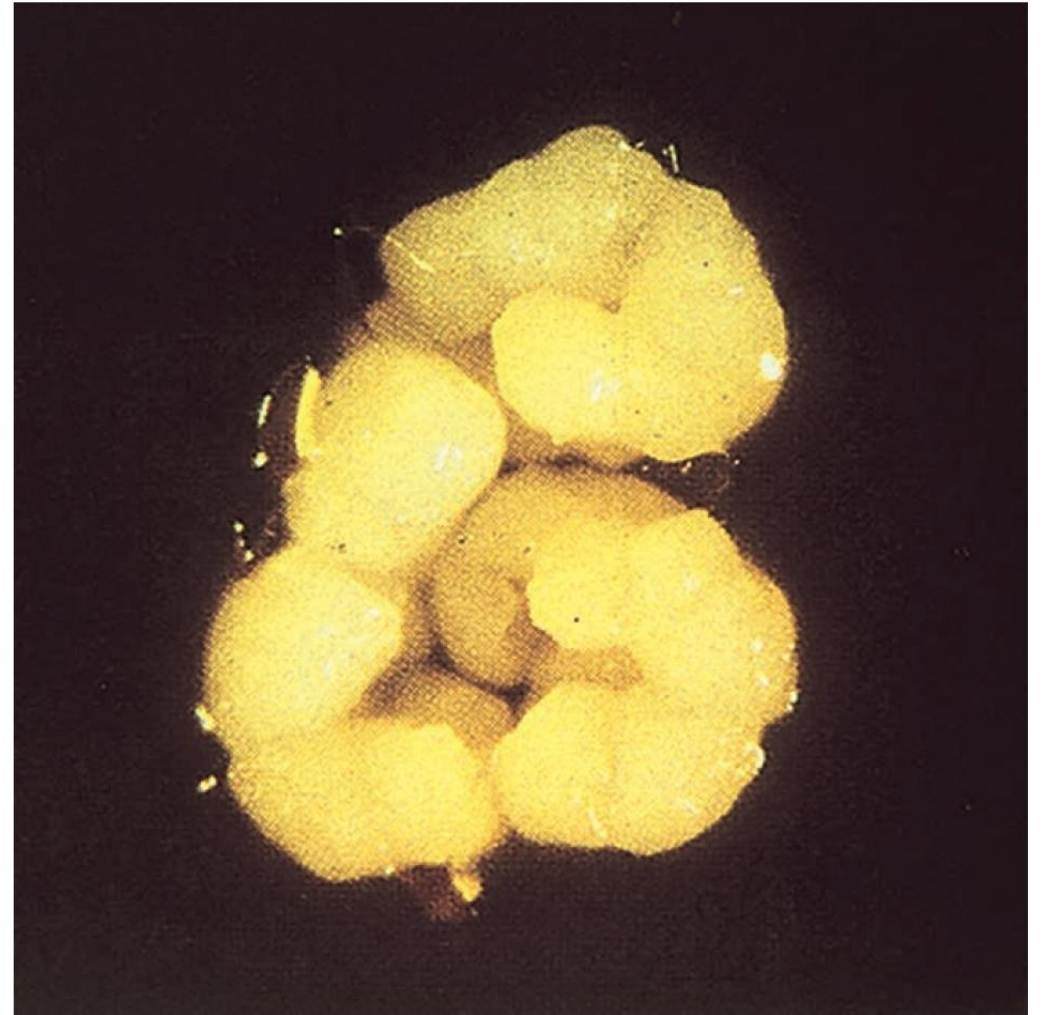


A fistula is a connection between two epithelial-lined surfaces.

A **fistula** is an abnormal pathway between two anatomic spaces **or** a pathway that leads from an internal cavity **or** organ to the surface of the body.  
A **sinus** tract is an abnormal channel that originates **or** ends in one opening.



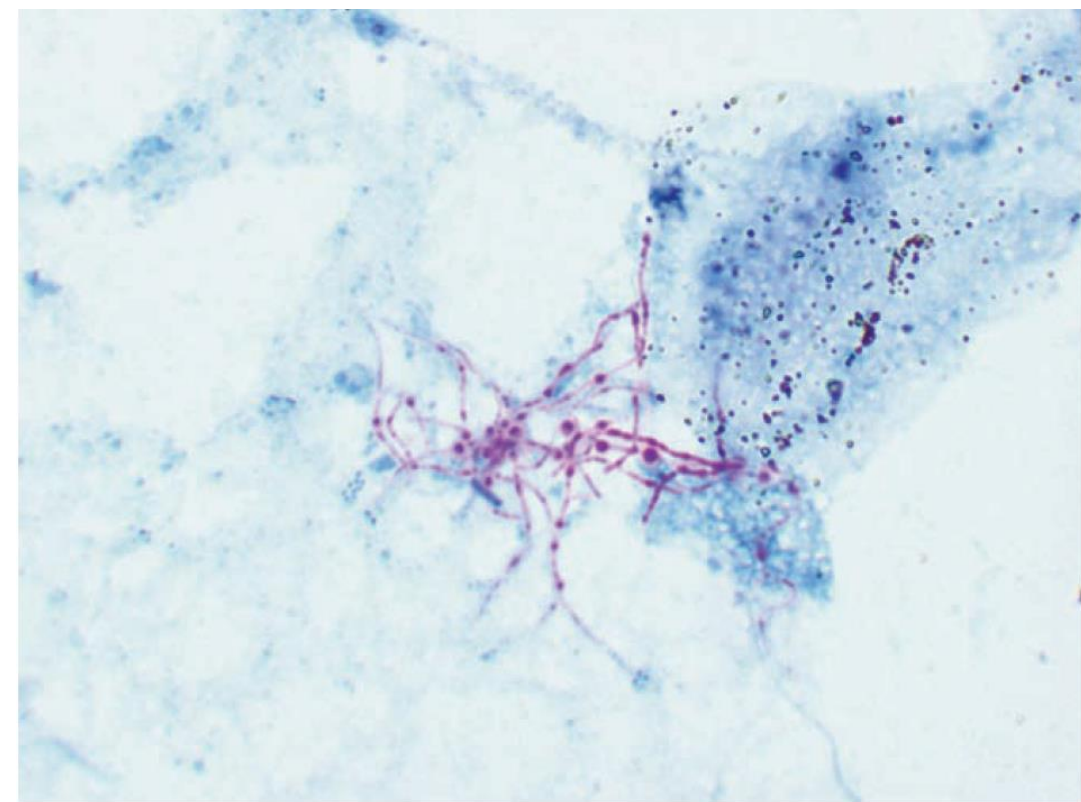
**FIGURE 31-4** Patient suffering from cervicofacial actinomycosis. Note the draining sinus tract (*arrow*).



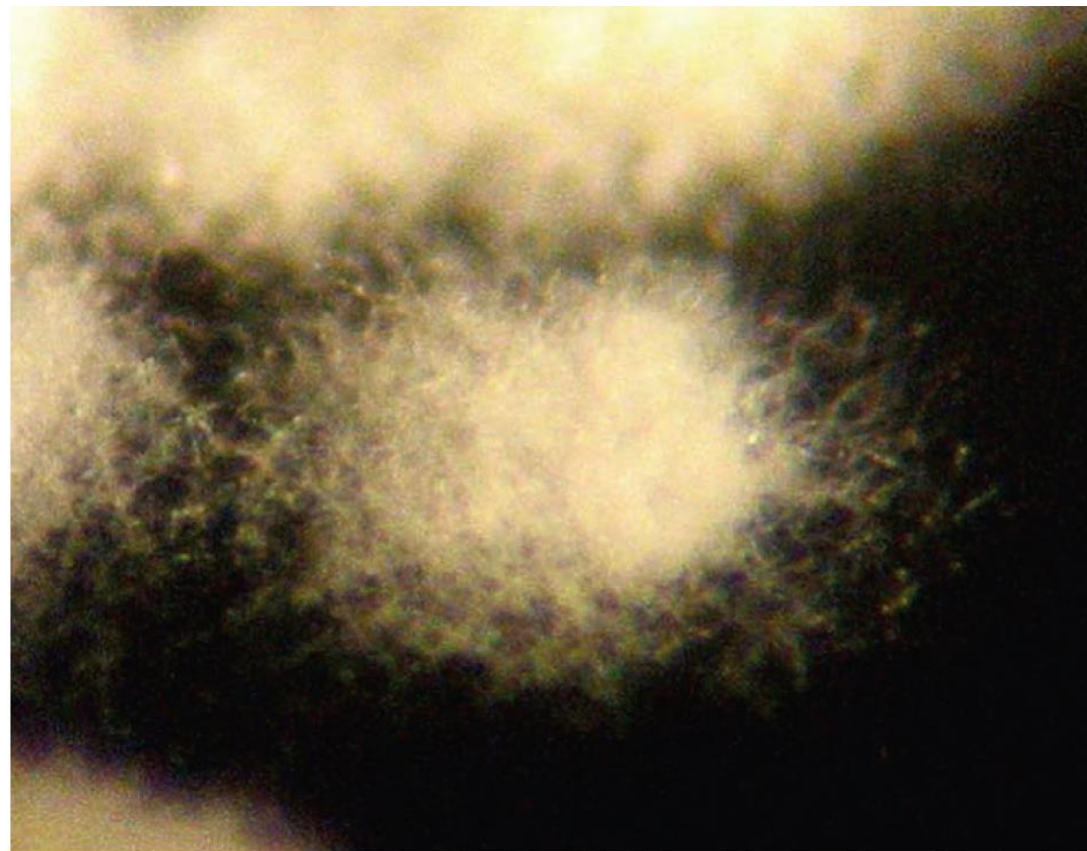
**FIGURE 31-6** Molar tooth appearance of *Actinomyces israelii* after incubation for 1 week. This colonial morphology serves as a reminder that the bacteria are normally found in the mouth.

# Nocardia (added here for similarity to actinomyces)

- Nocardiae are **strict aerobic rods** that form branched filaments in tissues and culture.
- *Nocardia* is described as “**weakly acid-fast**”; that is, a weak decolorizing solution of hydrochloric acid must be used to demonstrate the acid-fast property of nocardiae. **This distinguish it from the similar Actinomyces.**
- Growth is slow, requiring 3 to 5 days of incubation before colonies may be observed on the culture plates.
- *Nocardia* infections are **exogenous** (i.e., caused by organisms not normally part of the normal human flora). The ubiquitous presence of the organism in soil rich with organic matter and the increasing numbers of immunocompromised individuals living in communities have led to dramatic increases in disease caused by this organism.



**FIGURE 22-10** Acid-fast stain of *Nocardia* species in expectorated sputum. In contrast with the mycobacteria, members of the genus *Nocardia* do not uniformly retain the stain (“partially acid-fast”).



**FIGURE 22-12** Aerial hyphae of *Nocardia*.

The combination of both **presence of aerial hyphae and acid-fastness is unique to the genus *Nocardia*** and can be used as a rapid test for identification of the genus

# Nocardia

- It would appear that the primary factor associated with virulence is the ability of pathogenic strains to **avoid phagocytic killing**. Through :  
Secretion of **catalase** and **superoxide dismutase** that counter **hydrogen peroxide and superoxide released by phagocytic cells**, preventing fusion of the phagosome-lysosome (mediated by **cord factor**) and preventing acidification of the phagosome.
- **Bronchopulmonary disease** develops after the initial colonization of the upper respiratory tract by inhalation and then aspiration of oral secretions into the lower airways, occurs **almost always in immunocompromised patients**.
- **Primary cutaneous nocardiosis** develops after traumatic introduction of organisms into subcutaneous tissues, can present in the form of ***Mycetoma*** is characterized by a triad of painless subcutaneous mass, multiple sinuses and discharge containing grains.
- As many as one third of all patients with *Nocardia* infections have dissemination to the brain, most commonly involving the formation of single or multiple **brain abscesses**.

# Nocardia

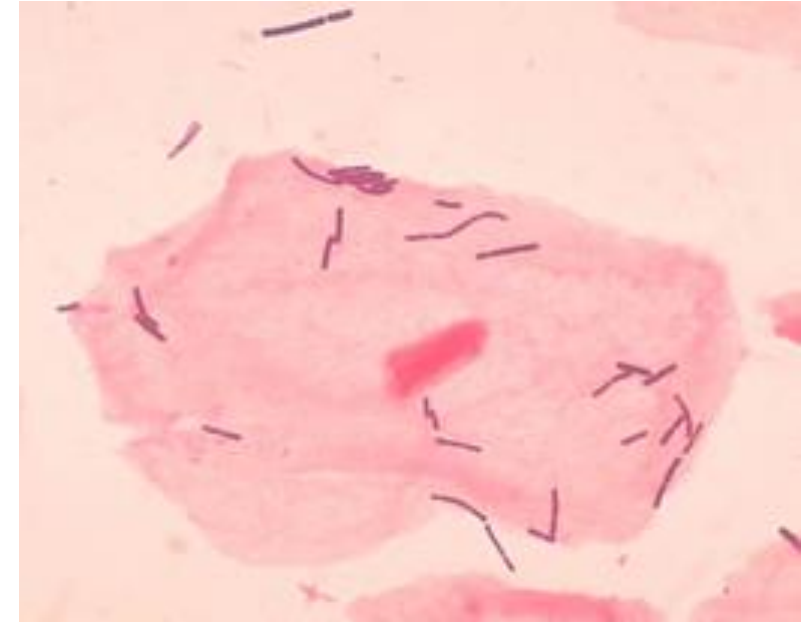


**Mycetoma** is a chronic suppurative disease of the skin and subcutaneous tissue, characterized by a symptomatic triad: tumor, fistulas and grains. It can be caused by fungi (eumycetoma) and bacteria (actinomycetoma), with similar clinical features.

Given its **slow progression, painless nature**, massive lack of health education and scarcity of medical and health facilities in endemic areas, many patients present late with advanced infection where amputation may be the only available treatment.

# Lactobacillus

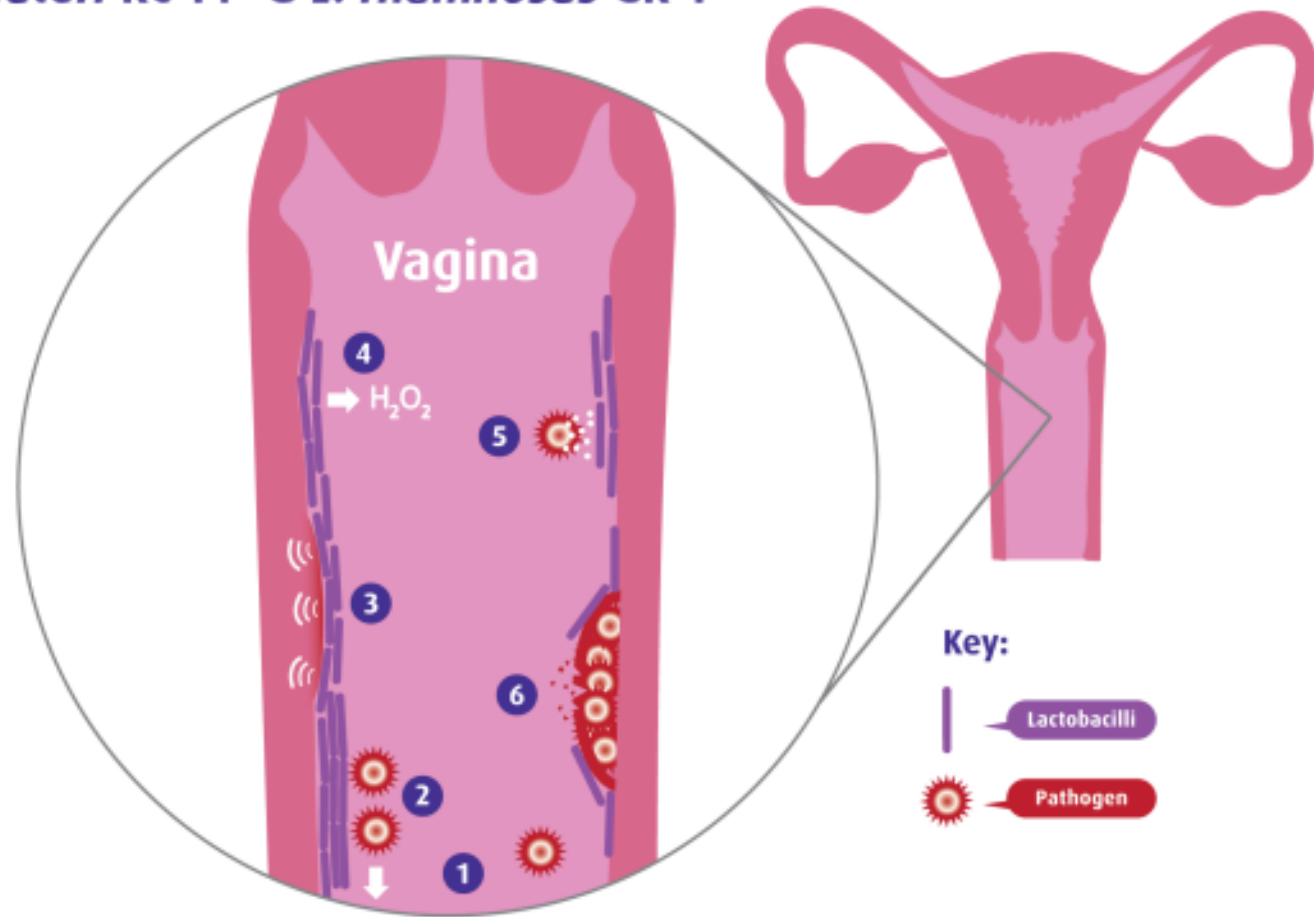
- *Lactobacillus* species are facultatively anaerobic or strictly anaerobic rods that **ferment to yield lactic acid**.
- They are found as part of the **normal flora** of the mouth, stomach, intestines, and genitourinary tract. In around 70% of women, a *Lactobacillus* species is dominant in the **female genital tract**.
- **Rarely cause infections.**
- Commonly found in **probiotics**.
- Some *Lactobacillus* species are used as starter cultures in **industry for controlled fermentation** in the production of yogurt, cheese, sauerkraut, pickles, beer, cider.
- Invasion into blood occurs in one of the following three settings: (1) **transient bacteremia** from a genitourinary source (e.g., after childbirth or a gynecologic procedure), (2) **endocarditis** and (3) **opportunistic septicemia** in an immunocompromised patient.





## Mechanisms of action of *L. reuteri* RC-14® & *L. rhamnosus* GR-1®

- 1 Restores a healthy pH < 4.5
- 2 Competitive inhibition
- 3 Modulates cytokines to decrease inflammation
- 4 Produces  $H_2O_2$  (kills pathogens and lowers pH)
- 5 Bacteriocin production (kills pathogens)
- 6 Produces biosurfactants, which breakdown pathogen biofilms



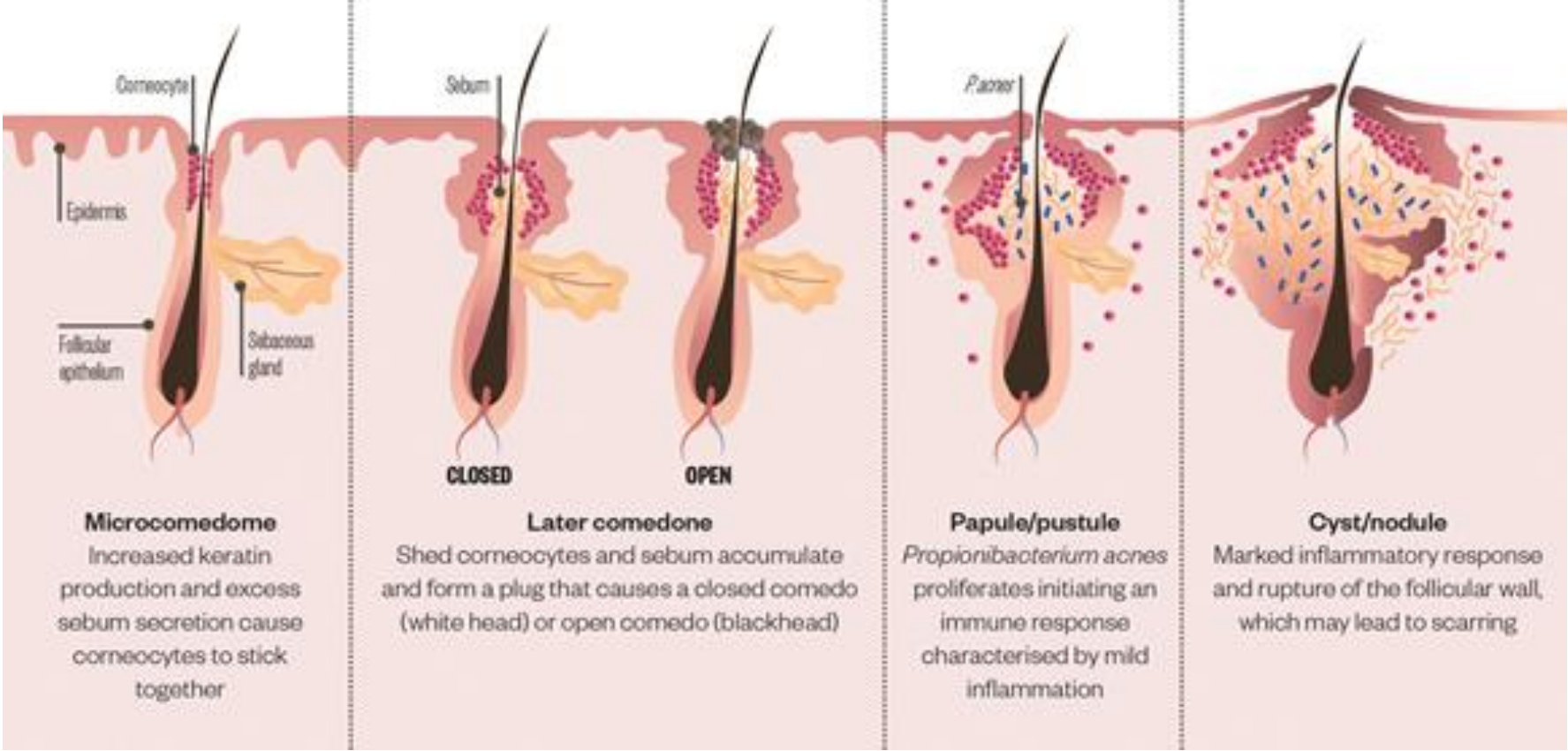
Taken from a website promoting probiotic therapy, proceed with caution!

# Propionibacterium

- Propionibacteria are small gram-positive rods often arranged in short chains or clumps, commonly **found on the skin** (in contrast with the *Actinomyces*), conjunctiva, and external ear, and in the oropharynx and female genital tract.
- The most commonly isolated species is *Propionibacterium acnes*. *P. acnes* is responsible for two types of infections: (1) **acne vulgaris** in teenagers and young adults and (2) **opportunistic infections** in patients with prosthetic devices or intravascular lines.
- *P. acnes* apparently only triggers the disease (acne vulgaris) when it meets favorable dermatophysiological terrain; *P. acnes* colonization of the skin is therefore **necessary but not sufficient for the establishment of the pathology**.

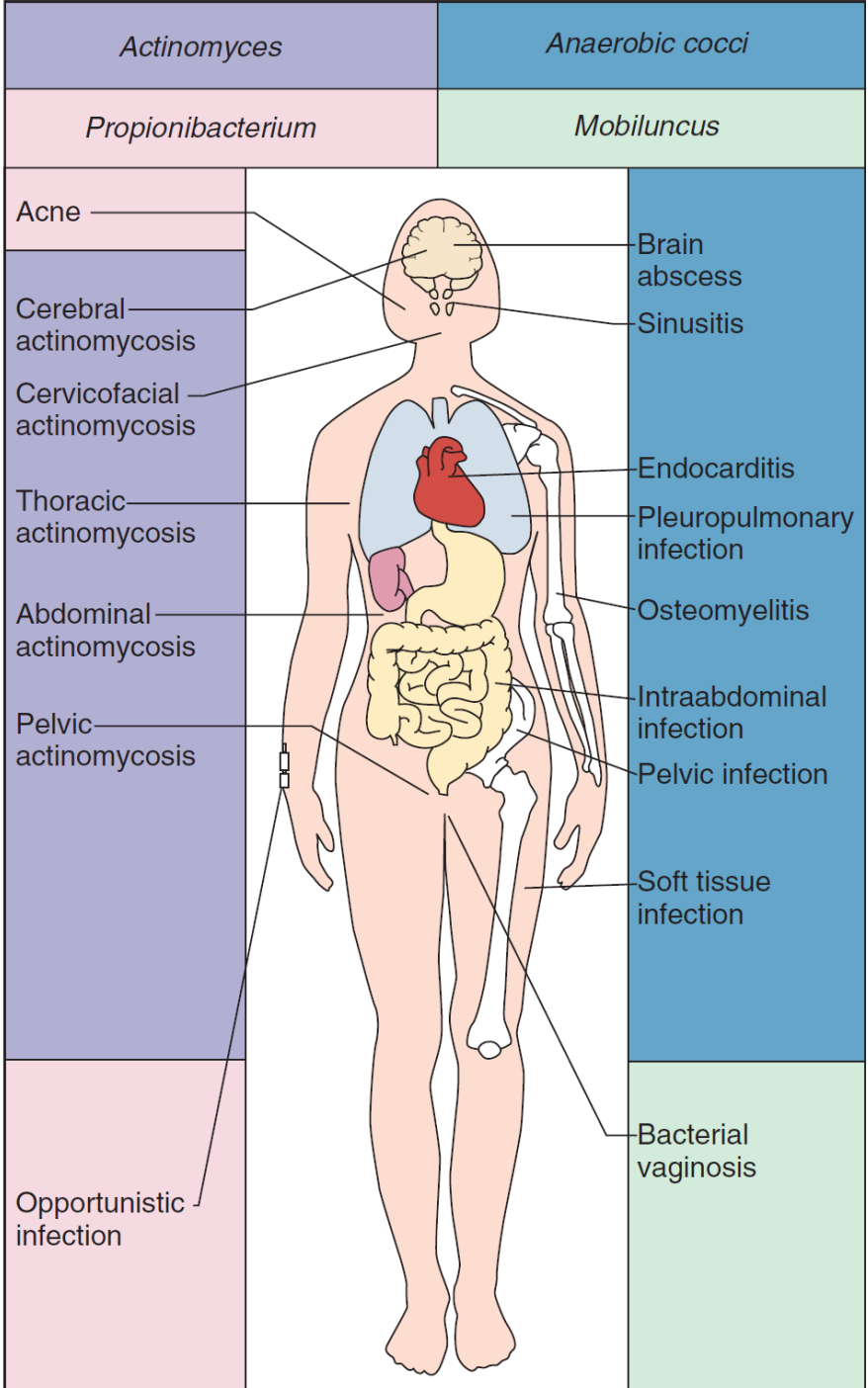


# Propionibacterium



# Other non-spore-forming anaerobic gram-positive rods

- ***Mobiluncus***: Members of the genus *Mobiluncus* are obligate anaerobic, gram-variable or gram-negative, curved rods with tapered ends. ***But classified as gram positive.*** because they (1) have a gram-positive cell wall, (2) lack endotoxin, and (3) are susceptible to vancomycin, clindamycin, erythromycin, and ampicillin but resistant to colistin. ***M. curtisii*** is rarely found in the vaginas of healthy women but is abundant in women with **bacterial vaginosis**.
- ***Bifidobacterium* and *Eubacterium*** : commonly found in the oropharynx, large intestine, and vagina. Usually represent clinically insignificant contaminants



# Overview

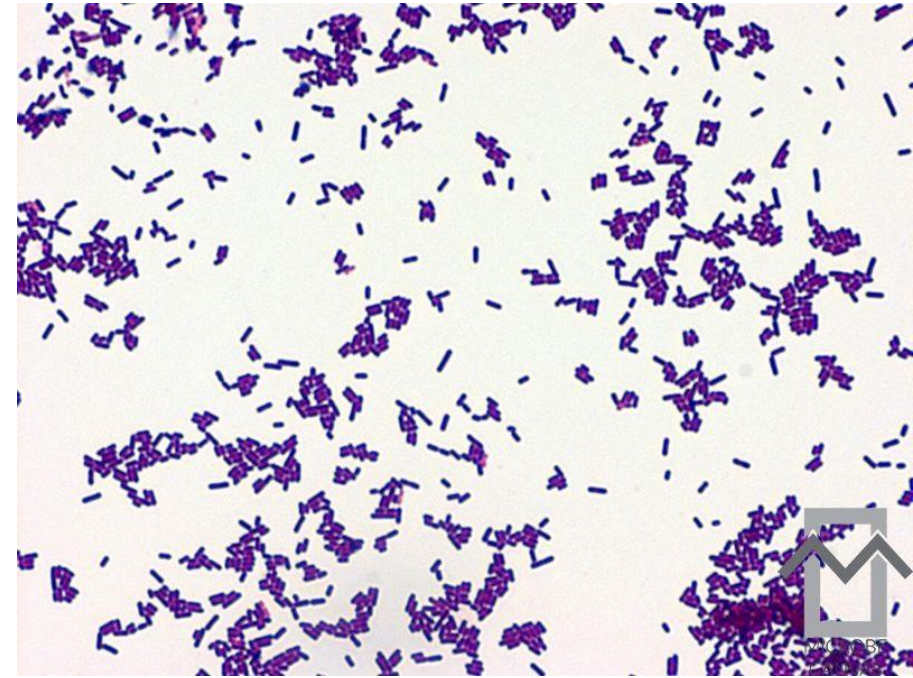


# Non-spore forming Aerobic Gram-Positive Rods

- Heterogeneous group of bacteria.
- Some are well-recognized **human pathogens** (e.g., *Listeria monocytogenes*, *Corynebacterium diphtheriae*);
- Others are **primarily animal pathogens** that can cause human disease (e.g., *Erysipelothrix rhusiopathiae*);
- And some are opportunistic pathogens that typically infect hospitalized or immunocompromised patients (e.g., *Corynebacterium jeikeium*)

# Listeria monocytogenes

- *L. monocytogenes* is a short (0.4 to 0.5 × 0.5 to 2 μm), nonbranching, gram-positive, **facultatively anaerobic rod**. The **short rods** appear singly, in pairs, or in short chains and can be mistaken for *Streptococcus pneumoniae*.
- The organisms are **motile** at room temperature but less so at 37° C, and they exhibit a characteristic end-over-end tumbling motion when a drop of broth is examined microscopically. exhibits **weak β-hemolysis** when grown on sheep blood agar plates.
- These differential characteristics (i.e., **Gram-stain morphology, motility, β-hemolysis**) are useful for the preliminary identification of *Listeria*.
- Although the bacteria are widely distributed in nature, human disease is uncommon and is restricted primarily to several well-defined populations: **neonates, the elderly, pregnant women, and patients with defective cellular immunity**





# Listeria monocytogenes

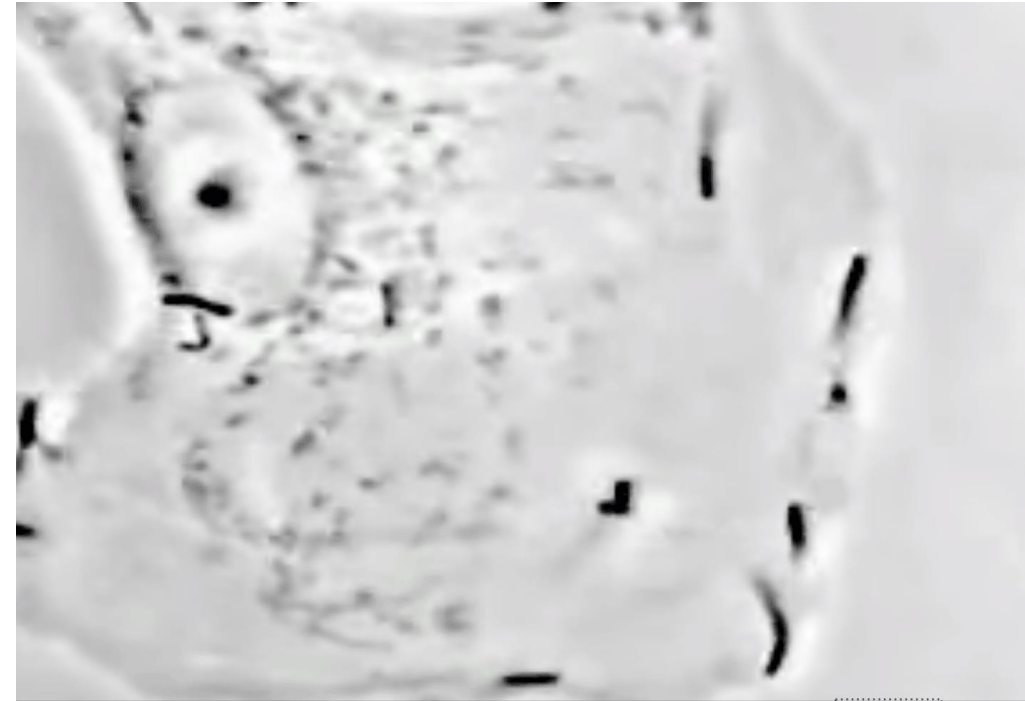


## Clinical Case 21-1 *Listeria* Meningitis in Immunocompromised Man

The following patient described by Bowie and associates (*Ann Pharmacother* 38:58–61, 2004) illustrates the clinical presentation of *Listeria* meningitis. A 73-year-old man with refractory rheumatoid arthritis was brought by his family to the local hospital because he had a decreased level of consciousness and a 3-day history of headache, nausea, and vomiting. His current medications were infliximab, methotrexate, and prednisone for his rheumatoid arthritis. On physical examination, the patient had a stiff neck and was febrile, had a pulse of 92 beats/min, and had a blood pressure of 179/72 mm Hg. Because meningitis was suspected, blood and cerebrospinal fluid (CSF) were collected for culture. The Gram stain of the CSF was negative, but *Listeria* grew from both blood and CSF cultures. The patient was treated with vancomycin, the infliximab was discontinued, and he made an uneventful recovery despite using less-than-optimal antimicrobial therapy. Infliximab has been associated with a dose-dependent monocytopenia. Because monocytes are key effectors for clearance of *Listeria*, this immunocompromised patient was specifically at risk for infection with this organism. Failure to detect *Listeria* in CSF by Gram stain is typical of this disease because the bacteria fail to multiply to detectable levels.

# Listeria monocytogenes

- *L. monocytogenes* is a **facultative intracellular pathogen**. Following ingestion of contaminated food, *L. monocytogene* **adhere to host cells** via the interaction of proteins on the surface of the bacteria (**internalin A**) with glycoprotein receptors on the host cell surface (e.g., **epithelial cadherin**)
- After **penetration into the cells**, the acid pH of the phagolysosome that surrounds the bacteria activates a bacterial pore-forming cytolysin (**listeriolysin O**) and two different **phospholipase C** enzymes, leading to **release of the bacteria into the cell cytosol**.
- This movement is mediated by a bacterial protein, **ActA** that coordinates **assembly of actin**.
- These bacteria can replicate in **macrophages** and move within cells, thus avoiding antibody-mediated clearance. Patients with defects in **cellular immunity**, but not in humoral immunity, are particularly susceptible to severe infections

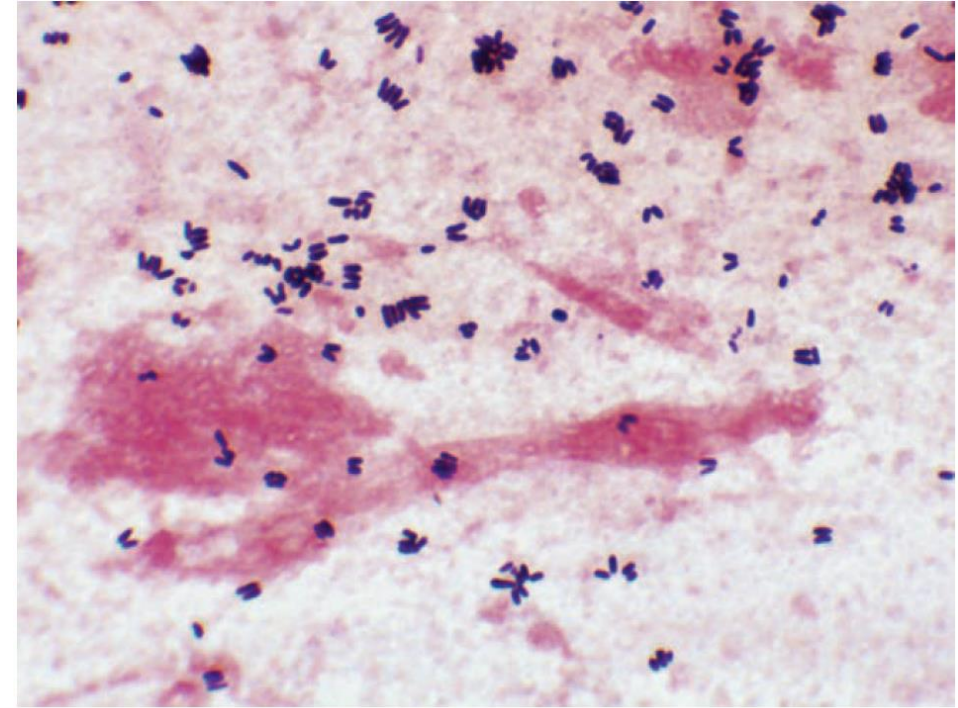


# Listeria monocytogenes

- The primary source of infection with this organism is **consumption of contaminated food**; causing **Foodborne Listeriosis**.
- Human-to-human transmission can occur primarily from **mother to child in utero or at birth**.
- **Neonatal Disease** (1) **early-onset disease**, acquired **transplacentally** in utero, and (2) **late-onset disease**, acquired at or soon after birth. Early-onset disease can result in **abortion, stillbirth, or premature birth**. Late-onset disease occurs 2 to 3 weeks after birth in the form of **meningitis or meningoencephalitis** with **septicaemia**.
- Most infections in pregnant women occur during the third trimester when **cellular immunity is most impaired**.
- Disease in **Healthy Adults** is self limited and **asymptomatic** or in the form of a mild influenza-like illness.

# Corynebacterium diphtheriae

- *C. diphtheriae* is an irregularly staining, pleomorphic rod (0.3 to 0.8 × 1.0 to 8.0 μm).
- Corynebacteria are **aerobic or facultatively anaerobic**, nonmotile, and catalase positive.
- Corynebacteria are **ubiquitous** in plants and animals, and they **normally colonize** the skin, upper respiratory tract, gastrointestinal tract, and urogenital tract in humans.
- The most famous of these is *C. diphtheriae*, the etiologic agent of **diphtheria**
- **Humans** are the **only known reservoir** for this organism. **Respiratory droplets or skin contact** transmits it from person to person.



**FIGURE 21-4** Gram stain of *Corynebacterium* species in sputum specimen.

# Corynebacterium diphtheriae



## Clinical Case 21-3 Respiratory Diphtheria

Lurie and associates (*JAMA* 291:937–938, 2004) reported the last patient with respiratory diphtheria seen in the United States. An unvaccinated 63-year-old man developed a sore throat while on a week-long trip in rural Haiti. Two days after he returned home to Pennsylvania, he visited a local hospital with complaints of a sore throat and difficulties in swallowing. He was treated with oral antibiotics but returned 2 days later with chills, sweating, difficulty swallowing and breathing, nausea, and vomiting. He had diminished breath sounds in the left lung, and radiographs confirmed pulmonary infiltrates as well as enlargement of the epiglottis. Laryngoscopy revealed yellow exudates on the tonsils, posterior pharynx, and soft palate. He was admitted to the intensive care unit and treated with azithromycin, ceftriaxone, nafcillin, and steroids, but over the next 4 days he became hypotensive with a low-grade fever. Cultures were negative for *Corynebacterium diphtheriae*. By the eighth day of illness, a chest radiograph showed infiltrates in the right and left lung bases, and a white exudate consistent with *C. diphtheriae* pseudomembrane was observed over the supraglottic structures. Cultures at this time remained negative for *C. diphtheriae*, but polymerase chain reaction testing for the exotoxin gene was positive. Despite aggressive therapy the patient continued to deteriorate, and on the seventeenth day of hospitalization he developed cardiac complications and died. This patient illustrates (1) the risk factor of an unimmunized patient traveling to an endemic area, (2) the classic presentation of severe respiratory diphtheria, (3) delays associated with diagnosis of an uncommon disease, and (4) the difficulties most laboratories would now have isolating the organism in culture.

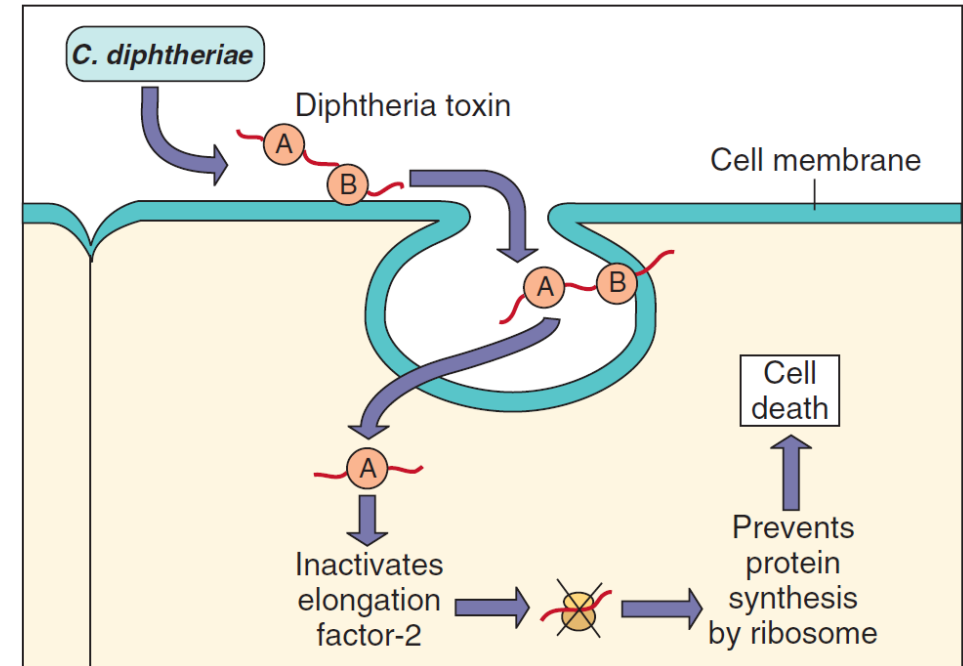
# Corynebacterium diphtheriae

- The clinical presentation of diphtheria is determined by the (1) site of infection, (2) immune status of the patient, and (3) virulence of the organism. Exposure to *C. diphtheriae* may result in **asymptomatic colonization in fully immune people**.
- Diphtheria toxin is produced at the site of the infection and then **disseminates through the blood** to produce the **systemic signs** of diphtheria.
- **Respiratory Diphtheria**, The symptoms of diphtheria involving the **respiratory tract** develop after a 2- to 4-day incubation period.
- Evidence of **myocarditis** can be detected in the majority of patients .
- **Cutaneous Diphtheria** A papule develops first and then evolves into a **chronic, nonhealing ulcer**

# Corynebacterium diphtheriae

- **Diphtheria toxin** is the major virulence factor of *C. diphtheriae*. An example of the classic **A-B exotoxin**.
- A **catalytic region** on the **A subunit**.  
And a **receptor-binding region** and a **translocation region** on the **B subunit**.
- The toxin binds to heparin-binding epidermal growth factor precursor (**HB-EGF**) present on many epithelial membranes. And is endocytosed by the cell. **A subunit** is translocated to the **cytosol**.
- A subunit ADP-ribosylates host eEF-2. **eEF-2** is required for **protein synthesis**; when it is inactivated by the toxin, the host cannot make protein and thus dies

A Inhibition of protein synthesis

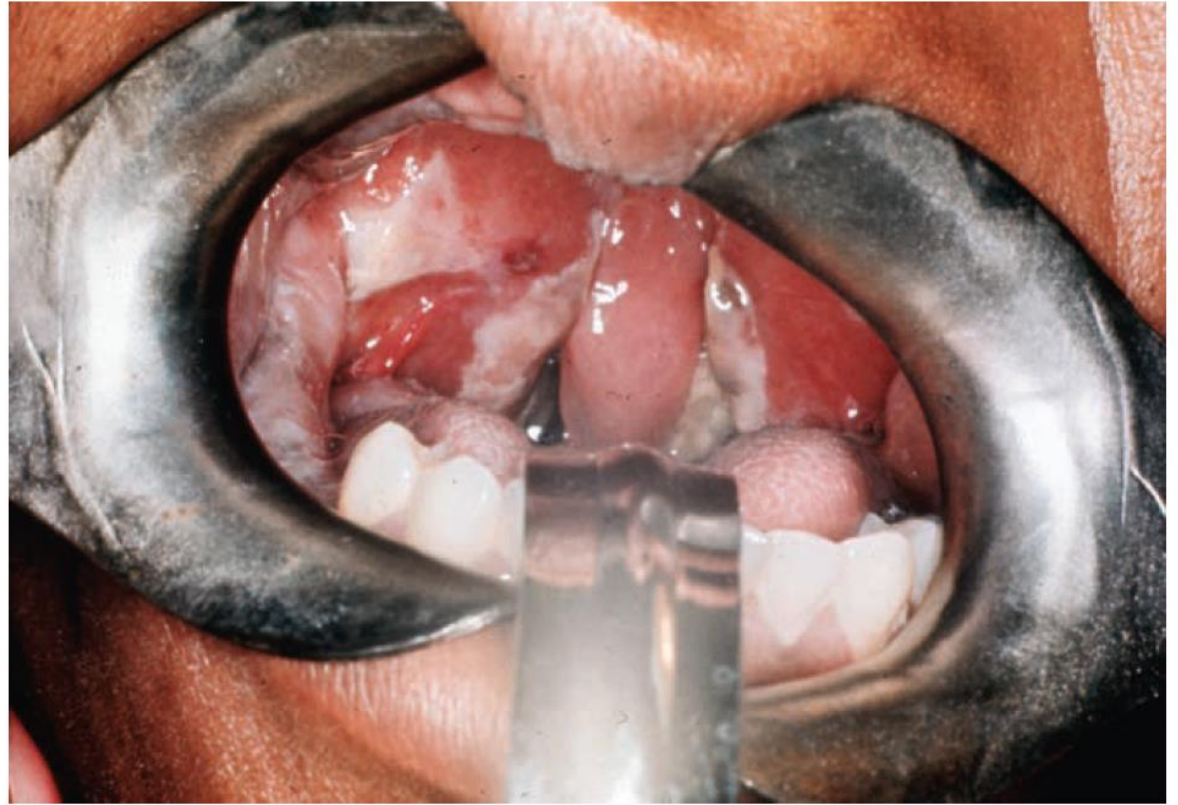


# Corynebacterium diphtheriae

## Respiratory Diphtheria

The onset is sudden, with malaise, sore throat, **exudative pharyngitis**, and a low-grade fever. The exudate evolves into a thick **pseudomembrane** composed of bacteria, lymphocytes, plasma cells, fibrin, and dead cells that can cover the tonsils, uvula, and palate and can extend up into the nasopharynx or down into the larynx

**Diphtheria** has become **uncommon** in the United States because of an **active immunization program**, as shown by the fact that more than 200,000 cases were reported in 1921 but **no cases have been reported since 2003**.

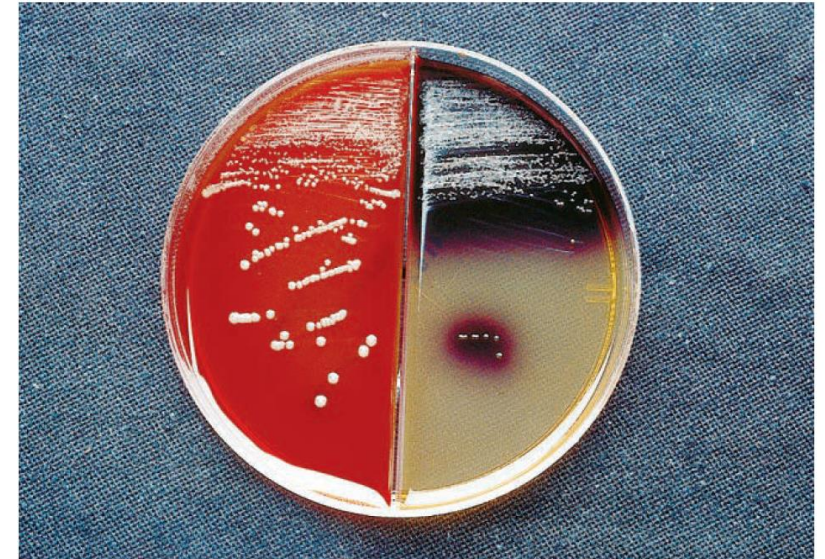


**FIGURE 21-5** Pharynx of a 39-year-old woman with bacteriologically confirmed diphtheria. The photograph was taken 4 days after the onset of fever, malaise, and sore throat. Hemorrhage caused by removal of the membrane by swabbing appears as a dark area on the left. (From Mandell G, Bennett J, Dolin R: *Principles and practice of infectious diseases*, ed 8, Philadelphia, 2015, Elsevier.)



# Anaerobic Gram-Negative Rods

- These anaerobes are the **predominant bacteria on most mucosal surfaces, outnumbering aerobic bacteria 10- to 1000-fold**. Despite the abundance and diversity of these bacteria, most infections are caused by relatively few species
- ***Bacteroides*, *Fusobacterium*, *Parabacteroides*, *Porphyromonas*, and *Prevotella***
- Characteristically, *Bacteroides* growth is **stimulated by bile**. Other anaerobic gram-negative rods are fastidious. *Bacteroides* species are **pleomorphic in size and shape** and resemble a mixed population of organisms in a casually examined Gram stain.
- *Bacteroides* have a typical gram-negative cell wall structure, which can be surrounded by a **polysaccharide capsule**
- *Bacteroides* LPS has **little endotoxin activity**, probably due to **structural differences to pathogen LPS**.



**FIGURE 31-13** Growth of *Bacteroides fragilis* on *Bacteroides* bile-esculin agar. Most aerobic and anaerobic bacteria are inhibited by bile and gentamicin in this medium, whereas the *B. fragilis* group of organisms is stimulated by bile, resistant to gentamicin, and able to hydrolyze esculin, producing a black precipitate.

# Anaerobic Gram-Negative Rods

## Stomach $10^2$

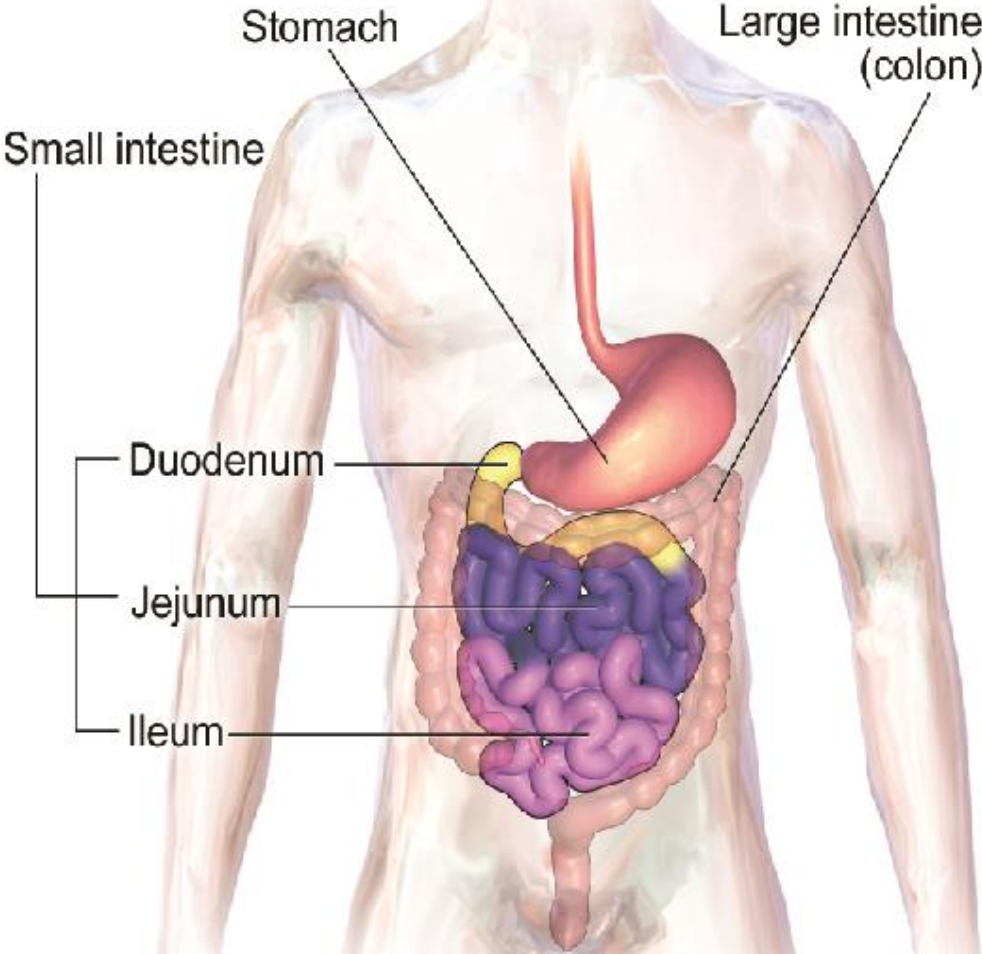
*Lactobacillus*  
*Candida*  
*Streptococcus*  
*Helicobacter pylori*  
*Peptostreptococcus*

## Duodenum $10^2$

*Streptococcus*  
*Lactobacillus*

## Jejunum $10^2$

*Streptococcus*  
*Lactobacillus*



## Proximal ileum $10^2$

*Streptococcus*  
*Lactobacillus*

## Distal ileum $10^8$

*Clostridium*  
*Streptococcus*  
*Bacteroides*  
*Actinomycinae*  
*Corneibacteria*

## Colon $10^{12}$

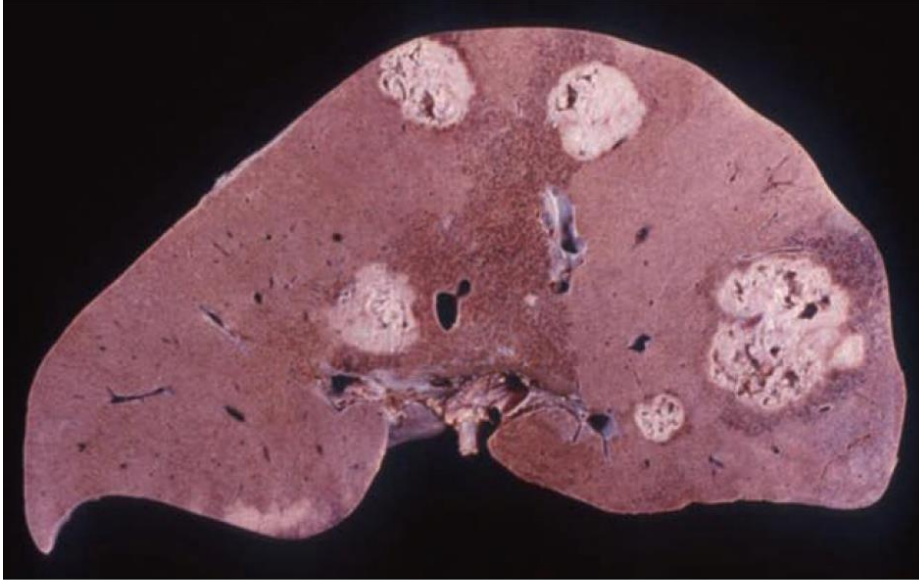
*Bacteroides*  
*Clostridium*  
*Bifidobacterium*  
*Enterobacteriaceae*

Anatomy of Small Intestine

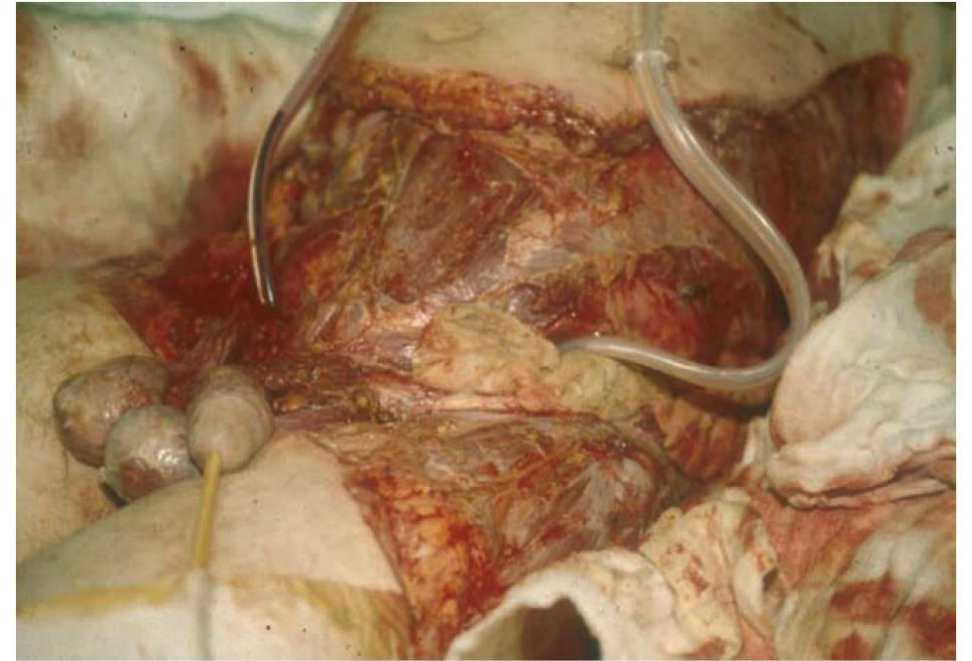
# Anaerobic Gram-Negative Rods

- To cause disease, *Bacteroides fragilis* in the resident flora are able to **spread by trauma or disease from the normally colonized mucosal surfaces to sterile tissues or fluids**
- Infections are **usually polymicrobial**.
- **Respiratory Tract Infections** , Nearly half of the **chronic infections of the sinuses and ears**, and virtually all periodontal infections, involve **mixtures of gram-negative anaerobes**, with *Prevotella*, *Porphyromonas*, *Fusobacterium*, and **non-fragilis *Bacteroides*** the most commonly isolated.
- Strains of enterotoxigenic *B. fragilis* that cause diarrheal disease produce a **heat-labile zinc metalloprotease toxin (*B. fragilis* toxin)**. This toxin causes morphologic changes of the intestinal epithelium via F-actin rearrangement, with the resultant stimulation of chloride secretion and fluid loss. *B. fragilis* can produce a **self-limited watery diarrhea**.
- **Bacteremia**, Anaerobes were at one time responsible for more than 20% of all clinically significant cases of bacteremia; however, these organisms now cause 3% to 10% of such infections.

# Anaerobic Gram-Negative Rods



**FIGURE 31-11** Liver abscesses caused by *Bacteroides fragilis*.



**FIGURE 31-12** Synergistic polymicrobial infection involving *Bacteroides fragilis* and other anaerobes. The infection started at the scrotum and rapidly spread up the trunk and down the thighs, with extensive myonecrosis.

- **Intraabdominal Infections,** Anaerobes are recovered in **virtually all** of these infections, with ***B. fragilis*** the most common organism.
- **Skin and Soft-Tissue Infections,** *B. fragilis* is the organism most commonly associated with significant disease.

# Anaerobic Gram-Positive Cocci

- **Anaerobic Gram-Positive Cocci:** The anaerobic gram-positive cocci normally colonize the oral cavity, gastrointestinal (GI) tract, genitourinary tract, and skin. They produce infections when they spread from these sites to normally sterile sites.
- Although anaerobic cocci can be isolated from infections at all body sites, a predisposition for certain sites has been observed.
- *Peptostreptococcus* species have been recovered more often **from subcutaneous and soft tissue abscesses and diabetes-related foot ulcers** than from intra-abdominal infections. *Peptostreptococcus* infections occur more often in **chronic infections**. Many infections caused by *peptostreptococcus* bacteria are **synergistic**.

## Further reading:

- Murray - Medical Microbiology 8th Edition  
Section 4: Bacteriology  
Chapter 21:  
Chapter 31: