Introductio n to Microbiolo gy

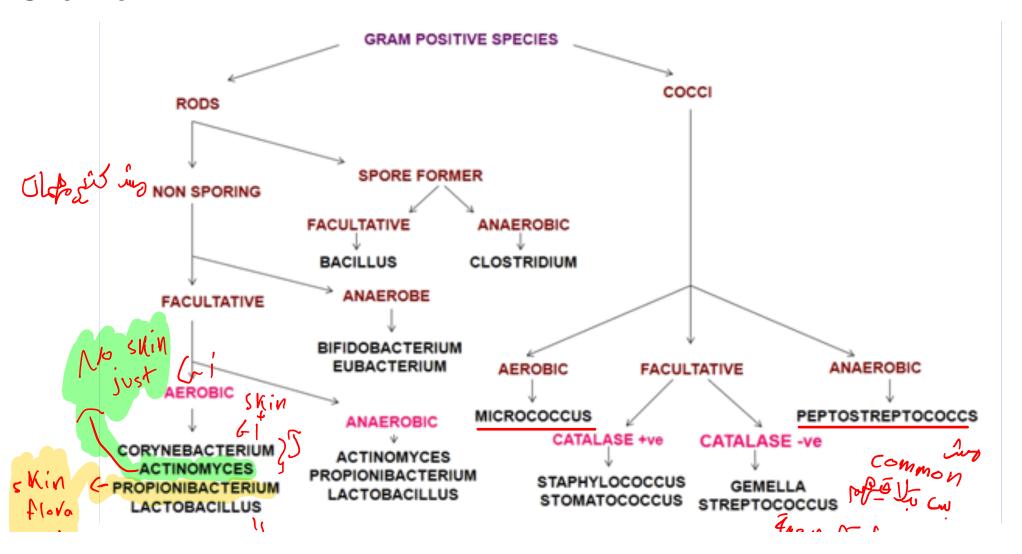
Anas Abu-Humaidan M.D. Ph.D.



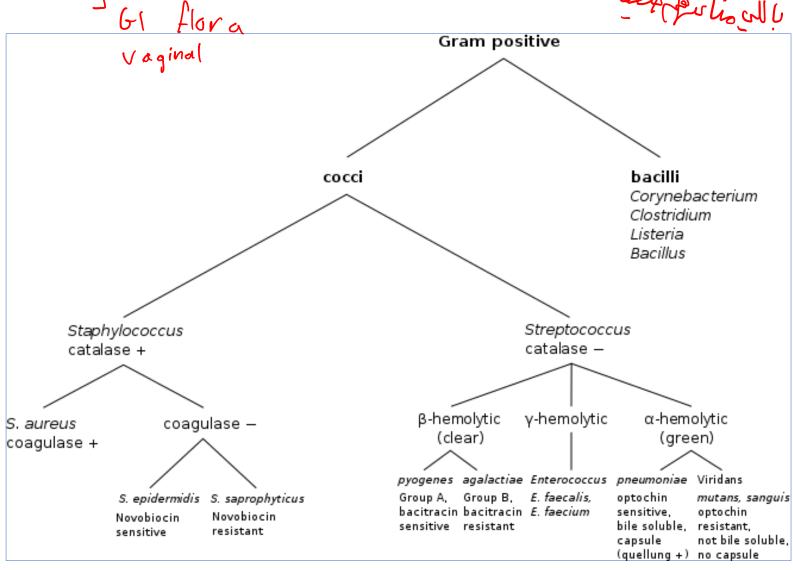
Lecture 14

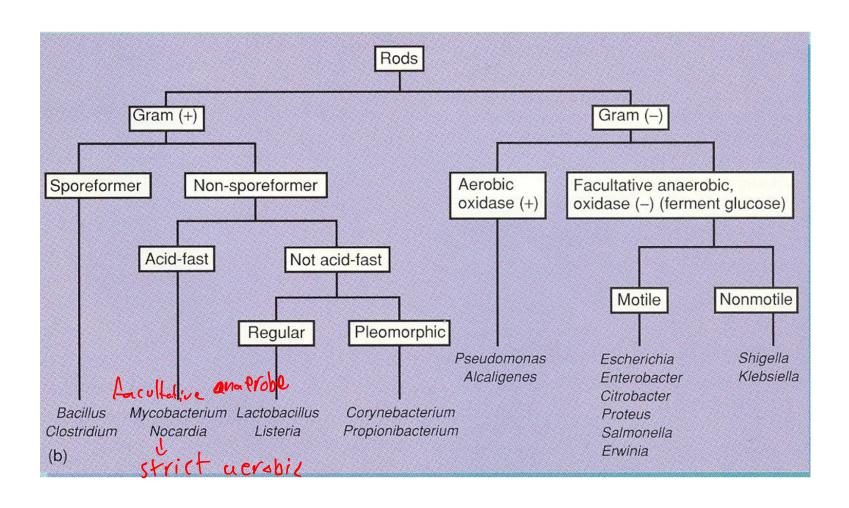
Pathogens that will be discussed this lecture are

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





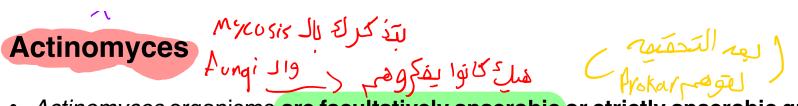




NON-SPORE-FORMING gram-positive rods

- 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.

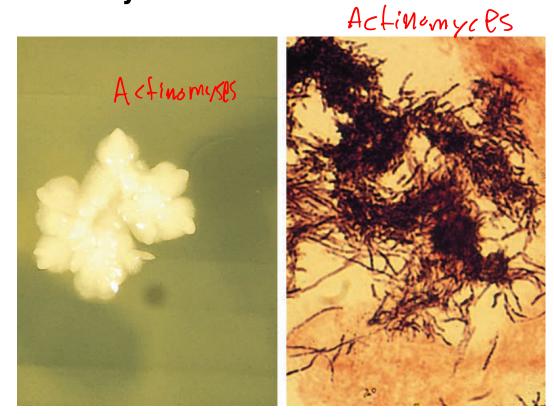




- Actinomyces organisms are facultatively anaerobic or strictly anaerobic grampositive rods

 Normal Plantico Co
- 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).

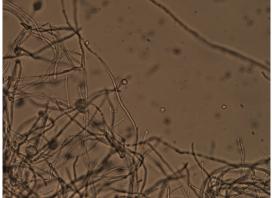
Actinomyces



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

م یا کاک میل





Fungal colonies and hyphae

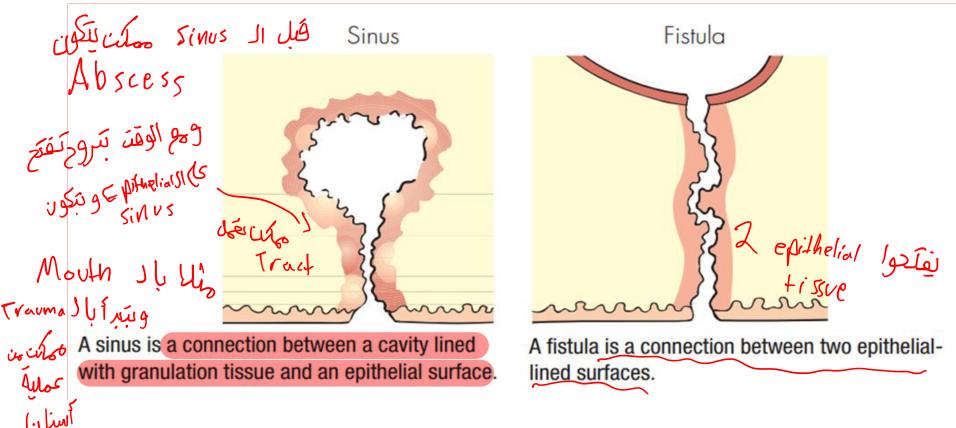
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Actinomyces

- 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). le sions
- 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) Andominal

الك الكوال UT الكوال UT

Treatment for actinomycosis involves the combination of drainage of a localized abscess or surgical debridement of the involved tissues, and prolonged administration of antibiotics. بالك تشيل العطام



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.

Livides ly Ugs pjy

Acid fast: mycobacterium tubercolosis

Chronic + slow growing

Nocardia (added here for similarity to actinomyces)

• Nocardiae are **strict aerobic rods** that form branched filaments in tissues and culture. بوندان و بالمان عليما كالمان المان المان عليما كالمان المان عليما كالمان المان عليما كالمان المان المان عليما كالمان المان ا

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.

 \[
 \lambda \text{Vormal}
 \]

 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.

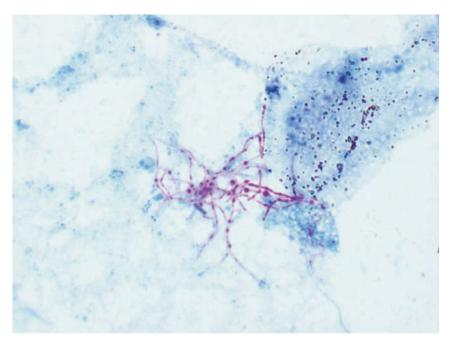


FIGURE 22-10 Acid-fast stain of *Nocardia* species in expectorated sputum. In contrast with the mycobacteria, members of the genus *Nocardia* do not uniformly retain the stain ("partially acid-fast").

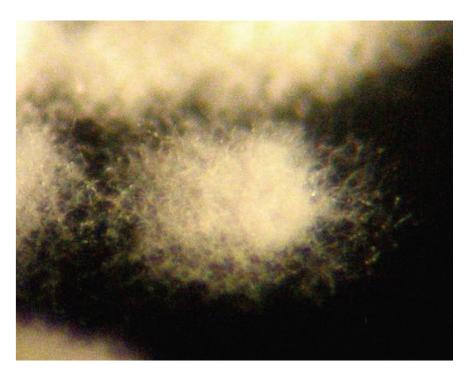


FIGURE 22-12 Aerial hyphae of Nocardia.

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 ,	وبمبر لمنعافي المنائة
infection"	وبمسر لمتعنفي المنائة

Cord factor shilled in

 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. Tumor, tistula, grain

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 ولأن يُخْتَلُونُ الْعِبَةِ الْعُلِيِّةِ الْعُلِيّةِ الْعُلِيقِيّةِ الْعُلِيّةِ الْعُلْمِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعُلِيّةِ الْعِلْمِيّةِ الْعِلْمِيّةِ الْعِلْمِيّةِ الْعِلْمِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيّةِ الْعِلْمِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ لِلْعِلْمِي الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِي الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِي الْعِلْمِيْعِيلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيّةِ الْعِلْمِيْعِلِيلِيّةِ الْعِلْمِي عِلْمِيْعِلِي الْعِلْمِيْعِي

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

Chithelial J 961s sinus raing Abscess in raths

2 sinus
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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

Jest Julia

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• 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**.

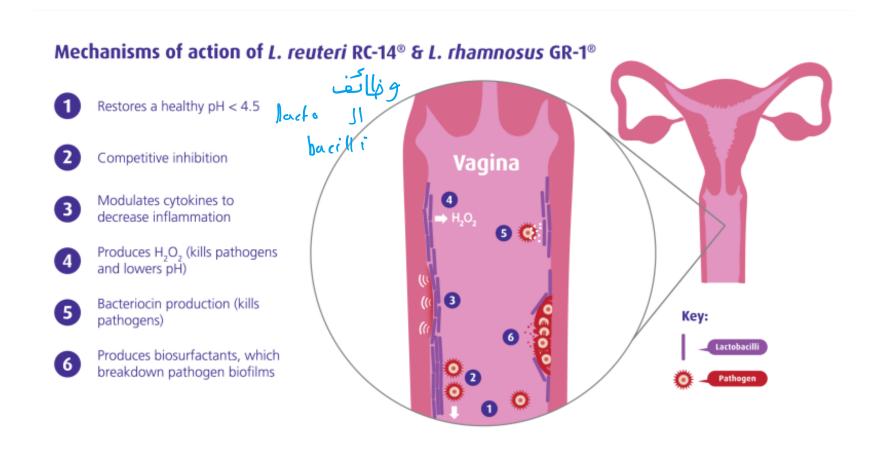
- Rarely cause infections.
- Commonly found in probiotics.

• 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.

fermentation = lacto bucillus

Te histics 2) sportunistic



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

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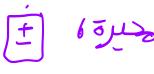
rods often arranged nd on the skin (in iva, and external ear,



Propionibacterium



Other non-spore-forming anaerobic gram-positive rods Mohiluncus curtisii



• *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. -> fathogen ino

• Bifidobacterium and Eubacterium: commonly found in the oropharynx, large intestine, and vagina. Usually represent clinically insignificant contaminants

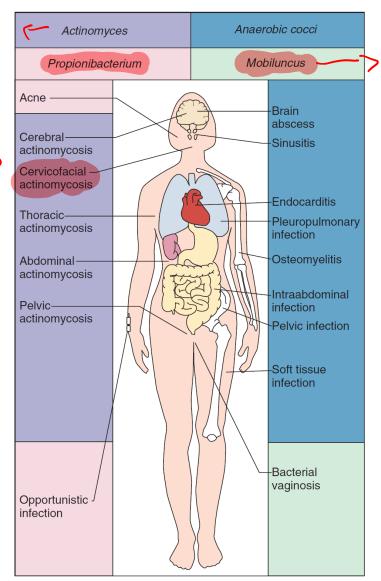
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colistin Il Jermin Lail 9

Sinuses

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Cenactin
Cervi



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facultative gt

Non-spore forming Aerobic Gram-Positive Rods Gram Positive

Gramposifive 1 aerobic

• 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)

جانيكم يا منعاف

Actinomyces موقد Actinomyces مولا ملل المال أفناها Actinomyces مورد مع المالك أفناها معرد المسلام المالك أفناها المسلام المسلا

ガデビー

Listeria monocytogenes intra cellular mainly

L. monocytogenes is a short (0.4 to 0.5 \times 0.5 to 2 μ m), 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 B **-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 welldefined populations: neonates, the elderly, pregnant women, and patients with defective cellular immunity Intracellular 1901



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.

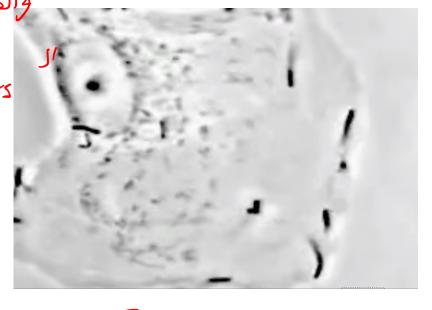
Listeria monocytogenes المكامام intracellular المكامام والمنافع المعامل intracellular المكامام المعامل المكامام المكام المكامام المكامام المكامام المكامام المكامام المكامام المكامام

• 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

Pktra cellular



Internalin
with
cod novin

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.

 مالدولمع المعالمة المعالم
- 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 Catalase Pasitive

• *C. diphtheriae* is an irregularly staining, pleomorphic rod (0.3 to 0.8×1.0 to $8.0 \ \mu m$).

- 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.

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

- 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.

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.



- 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 . کان مین و بنشر السم تبعل
- 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**.

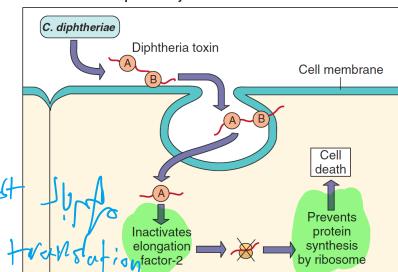
A Inhibition of protein synthesis

• 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



Corynebacterium diphtheriae

my sis deadly

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.

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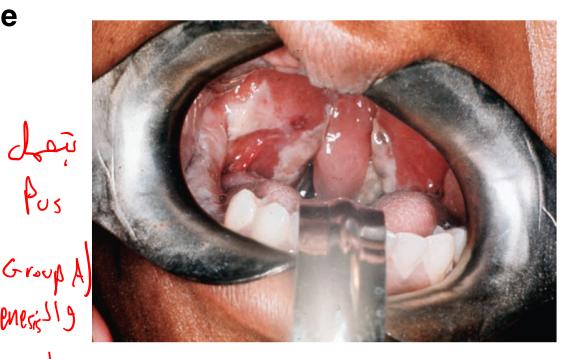


FIGURE 21-5 Pharynx of a 39-year-old woman with bacteriologically confirmed diphtheria. The photograph was taken 4 days after the onset of fever, malaise, and sore throat. Hemorrhage caused by removal of the membrane by swabbing appears as a dark area on the left. (From Mandell G, Bennett J, Dolin R: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

Vaccinest

مستأكل بالضرو بدهم كالإج سريع

- 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.



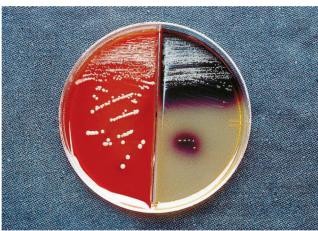
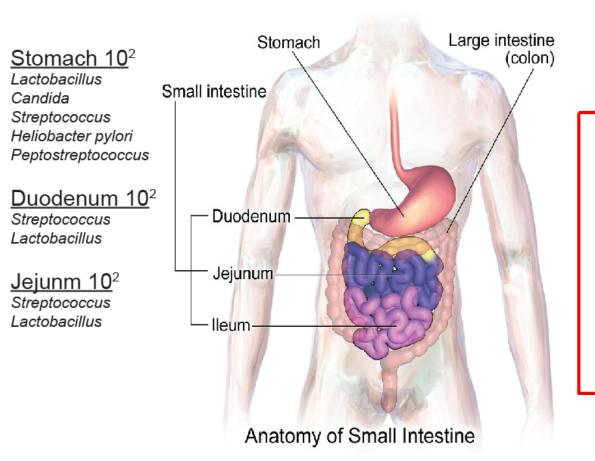


FIGURE 31-13 Growth of *Bacteroides fragilis* on *Bacteroides* bile-esculin agar. Most aerobic and anaerobic bacteria are inhibited by bile and gentamicin in this medium, whereas the *B. fragilis* group of organisms is stimulated by bile, resistant to gentamicin, and able to hydrolyze esculin, producing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



Proximal ileum 10²

Streptococcus Lactobacillus

Distal ileum 10⁸

Clostridium Streptococcus Bacteroides Actinomycinae Corneybacteria

Colon 10¹²

Bacteroides Clostridium Bifidobacterium Enterobacteriacae

- 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.



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

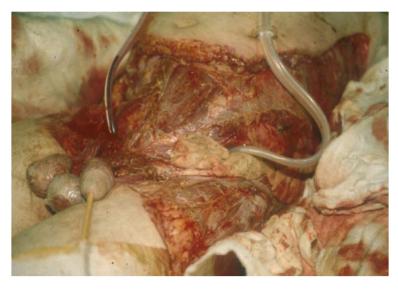


FIGURE 31-12 Synergistic polymicrobial infection involving *Bacteroides fragilis* and other anaerobes. The infection started at the scrotum and rapidly spread up the trunk and down the thighs, with extensive myonecrosis.

- 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: