

Practical lesson 12

Introduction to special microbiology. Microbiological diagnosis of staphylococcal and streptococcal infections. Microbiological diagnostics of infections caused by pathogenic *Neisseria* and opportunistic bacteria (genera *Pseudomonas*, *Acinetobacter*, *Proteus*, *Klebsiella*)

Medical microbiology - studies the characteristics of microorganisms that cause disease in humans and the pathological processes that occur in the organism during these diseases. Development of laboratory diagnosis, specific prevention and treatment methods of diseases caused by microorganisms are the main tasks of medical microbiology.

Special microbiology - studies the characteristics of various microorganisms and is divided into the following departments depending on this: - bacteriology (science of bacteria) - virology (science of viruses) - - mycology (science of fungi) - - protozoology (the science that studies protozoas)

Pathogenic cocci

Gram-positive cocci:

- Aerobic genus: Micrococcus, Planococcus vø Deinococcus
- Facultative anaerobic genus: Staphylococcus, Stomatococcus, Streptococcus, Leuconostoc, Pediococcus, Aerococcus and Gemella
- Anaerobic genus: Peptococcus, Peptostreptococcus, Ruminococcus, Coprococcus, Sarcina

There are two medically important genera of gram-positive cocci: Staphylococcus and Streptococcus. Two of the most important human pathogens, Staphylococcus aureus and Streptococcus pyogenes, are described in this chapter. Staphylococci and streptococci are nonmotile and do not form spores. Both staphylococci and streptococci are gram-positive cocci, but they are distinguished by two main criteria: (1) Microscopically, staphylococci appear in grapelike clusters, whereas streptococci are in chains. (2) Biochemically, staphylococci produce catalase (i.e., they degrade hydrogen peroxide), whereas streptococci do not.

Staphylococcaceae family (new taxonomy)

- Domain: Bacteria
- Kingdom: Bacillota
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae
- Genus: Staphylococcus

Staphylococcus and Related Gram-Positive Cocci

STAPHYLOCOCCUS AUREUS

Trigger Words Coagulase, cytotoxins, exfoliative toxins, enterotoxins, toxic shock syndrome toxin, MRSA

Biology and Virulence

☐ Catalase-positive, gram-positive cocci arranged in clusters

☐ Species characterized by the presence of coagulase and protein A

☐ Virulence factors include structural components that facilitate adherence to host tissues and avoid phagocytosis, and a variety of toxins and hydrolytic enzymes

☐ Hospital- and community-acquired infections with MRSA are a significant worldwide Problem Epidemiology

☐ Normal flora on human skin and mucosal surfaces

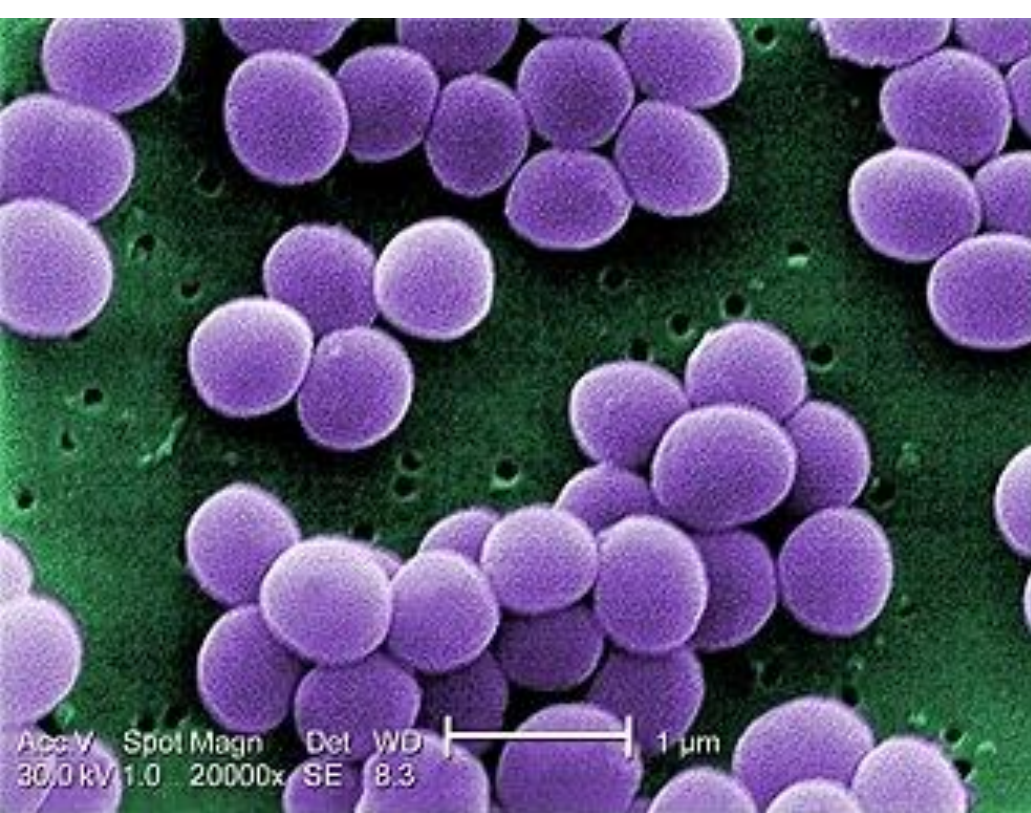
☐ Organisms can survive on dry surfaces for long periods (because of thickened peptidoglycan layer and absence of outer membrane)

☐ Person-to-person spread through direct contact or exposure to contaminated fomites (e.g., bed linens, clothing)

☐ Risk factors include presence of a foreign body (e.g., splinter, suture, prosthesis, catheter), previous surgical procedure, and use of antibiotics that suppress the normal microbial flora

☐ Patients at risk for specific diseases include infants (scalded skin syndrome), young children with poor personal hygiene (impetigo and other cutaneous infections), patients with intravascular catheters (bacteremia and endocarditis) or shunts (meningitis), and patients with compromised pulmonary function or an antecedent viral respiratory infection (pneumonia)

☐ MRSA is now the most common cause of community-acquired skin and soft-tissue infections



Diseases

Diseases include toxin-mediated diseases (food poisoning, toxic shock syndrome, and scalded skin syndrome), pyogenic diseases (impetigo, folliculitis, furuncles, carbuncles, and wound infections), and other systemic diseases

Diagnosis

- Microscopy useful for pyogenic infections but not blood infections or toxin-mediated infections
- Staphylococci grow rapidly when cultured on nonselective media
- Selective media (e.g., chromogenic agar, mannitol-salt agar) can be used to recover *Staphylococcus aureus* in contaminated specimens
- Nucleic acid amplification tests are useful for screening patients for carriage of methicillinsensitive *S. aureus* (MSSA) and MRSA
- S. aureus* is identified by biochemical tests (e.g., coagulase), molecular probes, or mass spectrometry

Treatment, Prevention, and Control

- Localized infections managed by incision and drainage; antibiotic therapy indicated for systemic infections
- Empirical therapy should include antibiotics active against MRSA strains
- Oral therapy can include trimethoprim sulfamethoxazole, doxycycline or minocycline, clindamycin, or linezolid; vancomycin is drug of choice for intravenous therapy, with daptomycin, tigecycline, or linezolid acceptable alternatives
- Treatment is symptomatic for patients with food poisoning (although the source of infection should be identified so that appropriate preventive procedures can be enacted)
- Proper cleansing of wounds and use of disinfectant help prevent infections
- Thorough hand washing and covering of exposed skin helps medical personnel prevent infection or spread to other patients

Treatment, Prevention, and Control

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Common Staphylococcus Species and Their Diseases

Staphylococcus aureus Toxin mediated (food poisoning, scalded skin syndrome, and toxic shock syndrome), cutaneous (carbuncles, folliculitis, furuncles, impetigo, and wound infections), other (bacteremia, endocarditis, pneumonia, empyema, osteomyelitis, and septic arthritis) S. epidermidis *Bacteremia*; endocarditis; surgical wounds; opportunistic infections of catheters, shunts, and prosthetic devices

S. lugdunensis Endocarditis

S. saprophyticus Urinary tract infections

Staphylococcus aureus

Toxin-Mediated Diseases

Scalded skin syndrome: Disseminated desquamation of epithelium in infants; blisters with no organisms or leukocytes

Food poisoning: After consumption of food contaminated with heat-stable enterotoxin, rapid onset of severe vomiting, diarrhea, and abdominal cramping, with resolution within 24 hours

Toxic shock: multisystem intoxication characterized initially by fever, hypotension, and a diffuse, macular, erythematous rash; high mortality without prompt antibiotic therapy and elimination of the focus of infection

Suppurative Infections

Impetigo: localized cutaneous infection characterized by pus-filled vesicle on an erythematous base

Folliculitis: impetigo involving hair follicles

Furuncles or boils: large, painful, pus-filled cutaneous nodules

Carbuncles: Coalescence of furuncles with extension into subcutaneous tissues and evidence of systemic disease (fever, chills, bacteremia)

Bacteremia and endocarditis: Spread of bacteria into the blood from a focus of infection; endocarditis characterized by damage to the endothelial lining of the heart

Pneumonia and empyema: Consolidation and abscess formation in the lungs; seen in the very young and elderly and in patients with underlying or recent pulmonary disease; a severe form of necrotizing pneumonia with septic shock and high mortality is now recognized

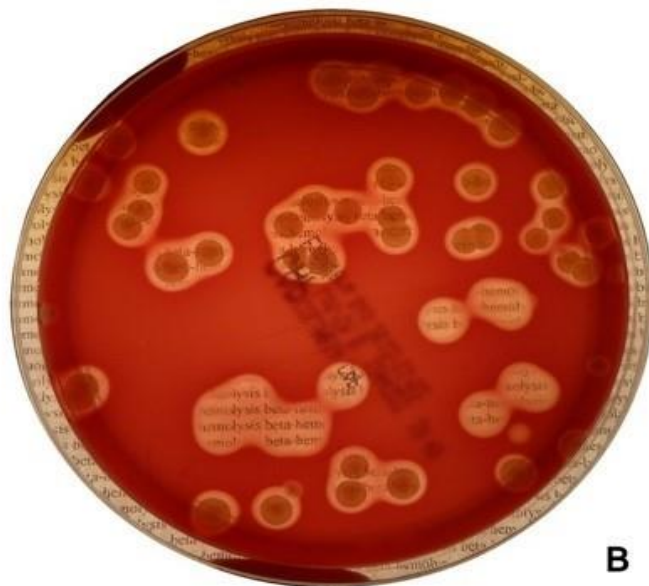
Osteomyelitis: Destruction of bones, particularly the metaphyseal area of long bones

Septic arthritis: Painful erythematous joint with collection of purulent material in the joint space

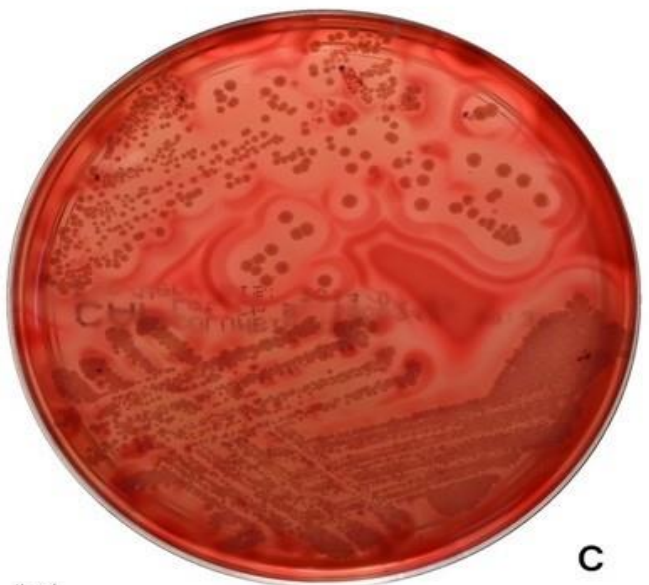




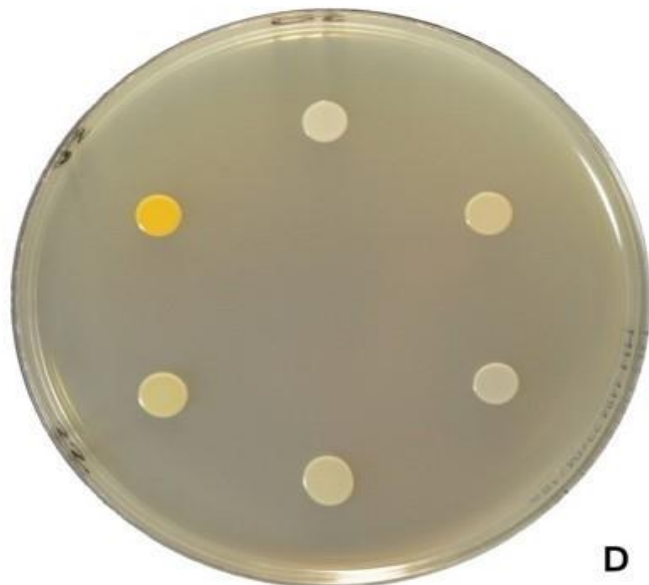
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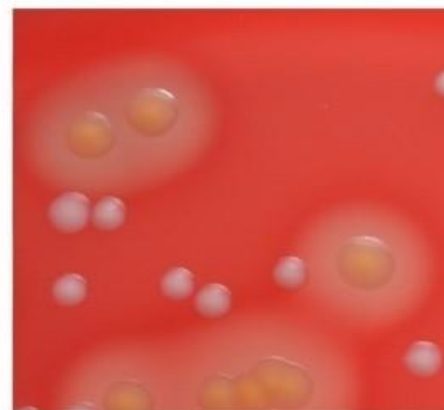
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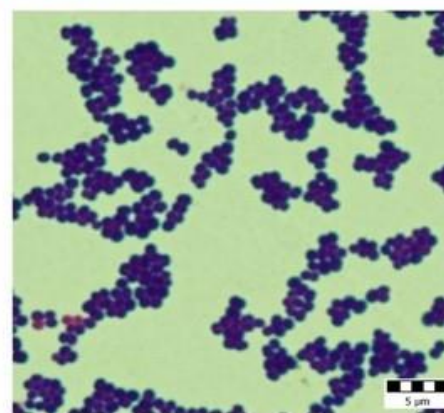
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Streptococcus pyogenes (Group A)

Trigger Words Group A, pharyngitis, pyoderma, rheumatic fever, glomerulonephritis

Biology and Virulence

Rapidly growing gram-positive cocci arranged in chains; group-specific carbohydrate (A antigen) and type-specific proteins (M protein) in cell wall

Virulence determined by ability to avoid phagocytosis (mediated primarily by capsule, M and M- like proteins, and C5a peptidase), adhere to and invade host cells (M protein, lipoteichoic acid, and F protein), and produce toxins (streptococcal pyrogenic exotoxins, streptolysin S, streptolysin O, streptokinase, and DNases)

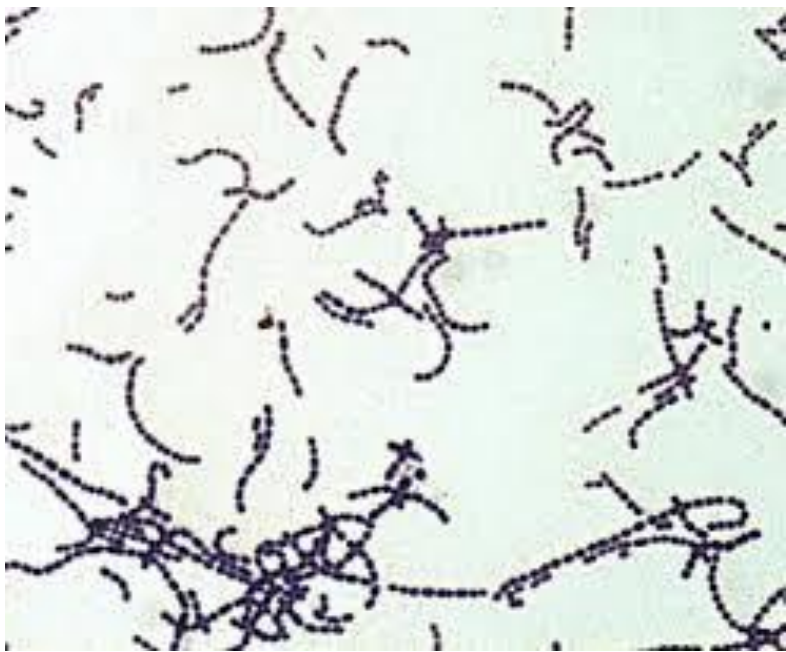
Epidemiology

Transient colonization in upper respiratory tract and skin surface, with disease caused by recently acquired strains (before protective antibodies are produced)

Pharyngitis and soft-tissue infections typically caused by strains with different M proteins

Person-to-person spread by respiratory droplets (pharyngitis) or through breaks in skin after direct contact with infected person, fomite, or arthropod vector

Individuals at higher risk for disease include children 5 to 15 years old (pharyngitis); children 2 to 5 years old with poor personal hygiene (pyoderma); patients with soft-tissue infection (streptococcal toxic shock syndrome); patients with prior streptococcal pharyngitis (rheumatic fever, glomerulonephritis) or soft-tissue infection (glomerulonephritis)



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Diseases

Responsible for suppurative diseases (pharyngitis, soft-tissue infections, streptococcal toxic shock) and nonsuppurative diseases (rheumatic fever, glomerulonephritis)

Diagnosis

Microscopy is useful in soft-tissue infections but not pharyngitis or nonsuppurative complications

Direct tests for the group A antigen are useful for the diagnosis of streptococcal pharyngitis

Isolates identified by catalase (negative), positive L-pyrrolidonyl arylamidase (PYR) reaction, susceptibility to bacitracin, and presence of group-specific antigen (group A antigen)

Antistreptolysin O test is useful for confirming rheumatic fever or glomerulonephritis associated with streptococcal pharyngitis; anti DNase B test should be performed for glomerulonephritis associated with pharyngitis or soft-tissue infections

Treatment, Prevention, and Control

Penicillin V or amoxicillin used to treat pharyngitis; oral cephalosporin or macrolide for penicillin-allergic patients; intravenous penicillin plus clindamycin used for systemic infections

Oropharyngeal carriage occurring after treatment can be re-treated; treatment is not indicated for prolonged asymptomatic carriage because antibiotics disrupt normal protective flora

Starting antibiotic therapy within 10 days in patients with pharyngitis prevents rheumatic fever

For glomerulonephritis, no specific antibiotic treatment or prophylaxis is indicated

For patients with a history of rheumatic fever, antibiotic prophylaxis is required before procedures (e.g., dental) that can induce bacteremias leading to endocarditis

Streptococcus agalactiae (Group B)

Trigger Words Group B, neonatal disease, screening pregnant women

Biology and Virulence

Rapidly growing gram-positive cocci arranged in chains; group-specific carbohydrate (B antigen) and type-specific capsular carbohydrates (Ia, Ib, and II-VIII)

Virulence determined primarily by ability to avoid phagocytosis (mediated by capsule)

Epidemiology

Asymptomatic colonization of the upper respiratory tract and genitourinary tract

Early-onset disease acquired by neonates from mother during pregnancy or at time of birth

Neonates are at higher risk for infection if (1) there is premature rupture of membranes, prolonged labor, preterm birth, or disseminated maternal group B streptococcal disease, and (2) mother is without type-specific antibodies and has low complement levels

Women with genital colonization are at risk for postpartum disease

Men and nonpregnant women with diabetes mellitus, cancer, or alcoholism are at increased risk for disease

No seasonal incidence

Diseases

Responsible for neonatal disease (early-onset and late-onset disease with meningitis, pneumonia, and bacteremia), infections in pregnant women (endometritis, wound infections, and urinary tract infections), and other adults (bacteremia, pneumonia, bone and joint infections, and skin and soft-tissue infections)

Diagnosis

ññ Microscopy useful for meningitis (cerebrospinal fluid), pneumonia (lower respiratory secretions), and wound infections (exudates)

ññ Antigen tests are less sensitive than microscopy and should not be used

ññ Culture most sensitive test; a selective broth (i.e., LIM) is needed for optimal detection of vaginal carriage

ññ Polymerase chain reaction–based assays to detect vaginal carriage in pregnant women are commercially available; currently require use of enrichment broth for optimum sensitivity

ññ Isolates identified by demonstration of group-specific cell wall carbohydrate or positive nucleic acid amplification test

Treatment, Prevention, and Control

Penicillin G is the drug of choice; empirical therapy with broad-spectrum antibiotics (broad-spectrum cephalosporin + aminoglycoside) used until specific pathogen identified; combination of penicillin and aminoglycoside is used in patients with serious infections; a cephalosporin or vancomycin is used for patients allergic to penicillin

STREPTOCOCCUS PNEUMONIAE

Trigger Words Diplococci, capsule, pneumonia, meningitis, vaccine

Biology and Virulence

Elongated gram-positive cocci arranged in pairs (diplococci) and short chains; cell wall includes teichoic acid rich in phosphorylcholine (C polysaccharide), which is required for the activity of an autolytic enzyme, amidase

Virulence determined by ability to colonize oropharynx (surface protein adhesions), spread into normally sterile tissues (pneumolysin, immunoglobulin [Ig]A protease), stimulate local inflammatory response (teichoic acid, peptidoglycan fragments, pneumolysin), and evade phagocytic killing (polysaccharide capsule)

Responsible for pneumonia, sinusitis and otitis media, meningitis, and bacteremia

Epidemiology

Most infections are caused by endogenous spread from the colonized nasopharynx or oropharynx to distal site (e.g., lungs, sinuses, ears, blood, meninges); person-to-person spread through infectious droplets is rare

Colonization is highest in young children and their contacts

Individuals with antecedent viral respiratory tract disease or other conditions that interfere with bacterial clearance from respiratory tract are at increased risk for pulmonary disease

Children and the elderly are at greatest risk for meningitis

People with hematologic disorder (e.g., malignancy, sickle cell disease) or functional asplenia are at risk for fulminant sepsis

Although the organism is ubiquitous, disease is more common in cool months

Diagnosis

👁👁 Microscopy is highly sensitive, as is culture, unless the patient has been treated with antibiotics

👁👁 Antigen tests for pneumococcal C polysaccharide are sensitive with cerebrospinal fluid (meningitis) but not with urine (meningitis, pneumonia, other infections)

👁👁 Nucleic acid–based tests are the tests of choice for the diagnosis of meningitis, particularly in patients who have been treated with an antibiotic

👁👁 Culture requires use of enriched-nutrient media (e.g., sheep blood agar); organism susceptible to many antibiotics, so culture can be negative in partially treated patients

👁👁 Isolates identified by catalase (negative), susceptibility to optochin, and solubility in bile

Treatment, Prevention, and Control

Penicillin is the drug of choice for susceptible strains, although resistance is increasingly common

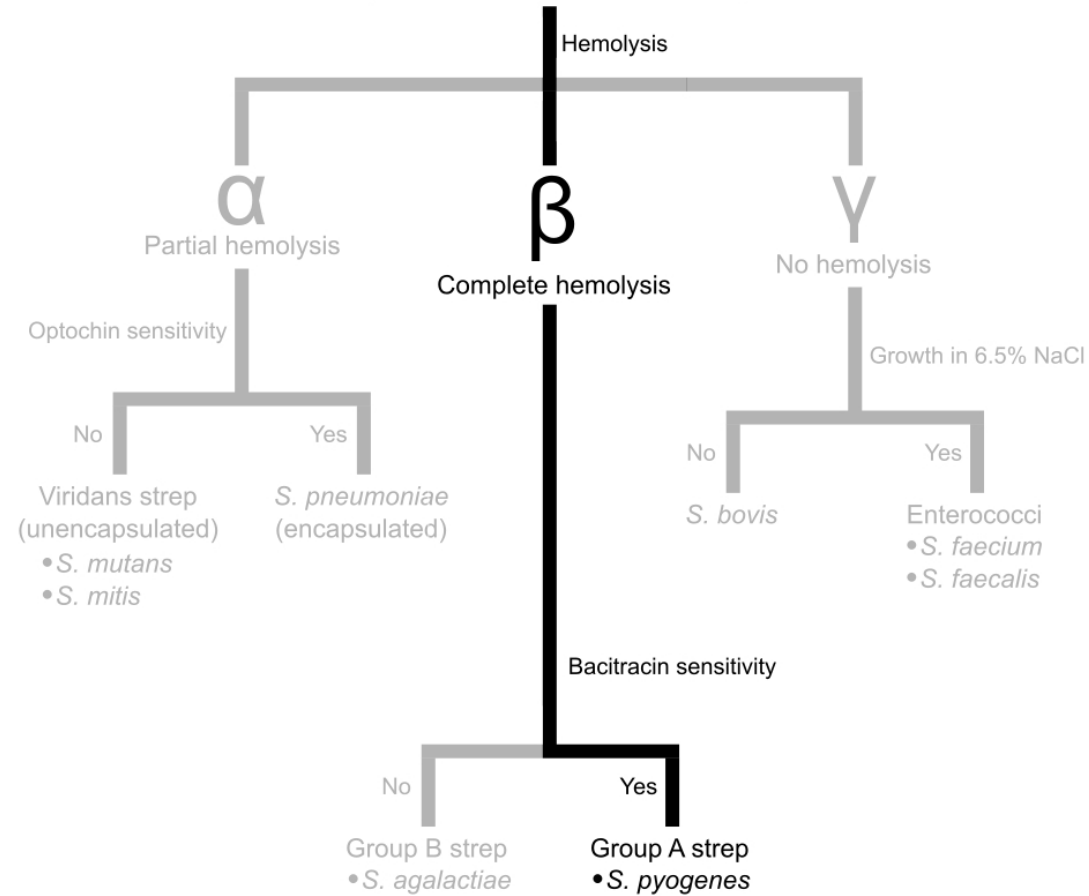
Vancomycin combined with ceftriaxone is used for empirical therapy; monotherapy with a cephalosporin, fluoroquinolone, or vancomycin can be used in patients with susceptible isolates

Immunization with 13-valent conjugated vaccine is recommended for all children younger than 2 years; a 23-valent polysaccharide vaccine is recommended for adults at risk for disease

Streptococcus



Gram positive cocci in chains, catalase negative



NEISSERIA GONORRHOEAE

Trigger Words

Diplococci, gonorrhea, arthritis, ophthalmia

Biology and Virulence

ññ Gram-negative diplococci with fastidious growth requirements

ññ Growth best at 35° C-37° C in a humid atmosphere supplemented with CO₂

ññ Oxidase and catalase positive; acid produced from glucose oxidatively

ññ Outer surface with multiple antigens: pili protein; Por proteins; Opa proteins; Rmp protein; protein receptors for transferrin, lactoferrin, and hemoglobin; lipooligosaccharide; immunoglobulin protease; β-lactamase

ññ Refer to Table 23.2 for summary of virulence factors

Epidemiology

ññ Humans are the only natural hosts

ññ Carriage can be asymptomatic in women

ññ Transmission is primarily by sexual contact

ññ Almost 555,608 cases reported in United States in 2017 (true incidence of disease believed to be at least twice that); estimated 78 million new cases worldwide

ññ Disease most common in blacks, people aged 15-24 years, residents of southeastern United States, people who have multiple sexual encounters

Diagnosis

• Gram stain of urethral specimens is accurate for symptomatic males only

• Culture is sensitive and specific but has been replaced with nucleic acid tests in most laboratories

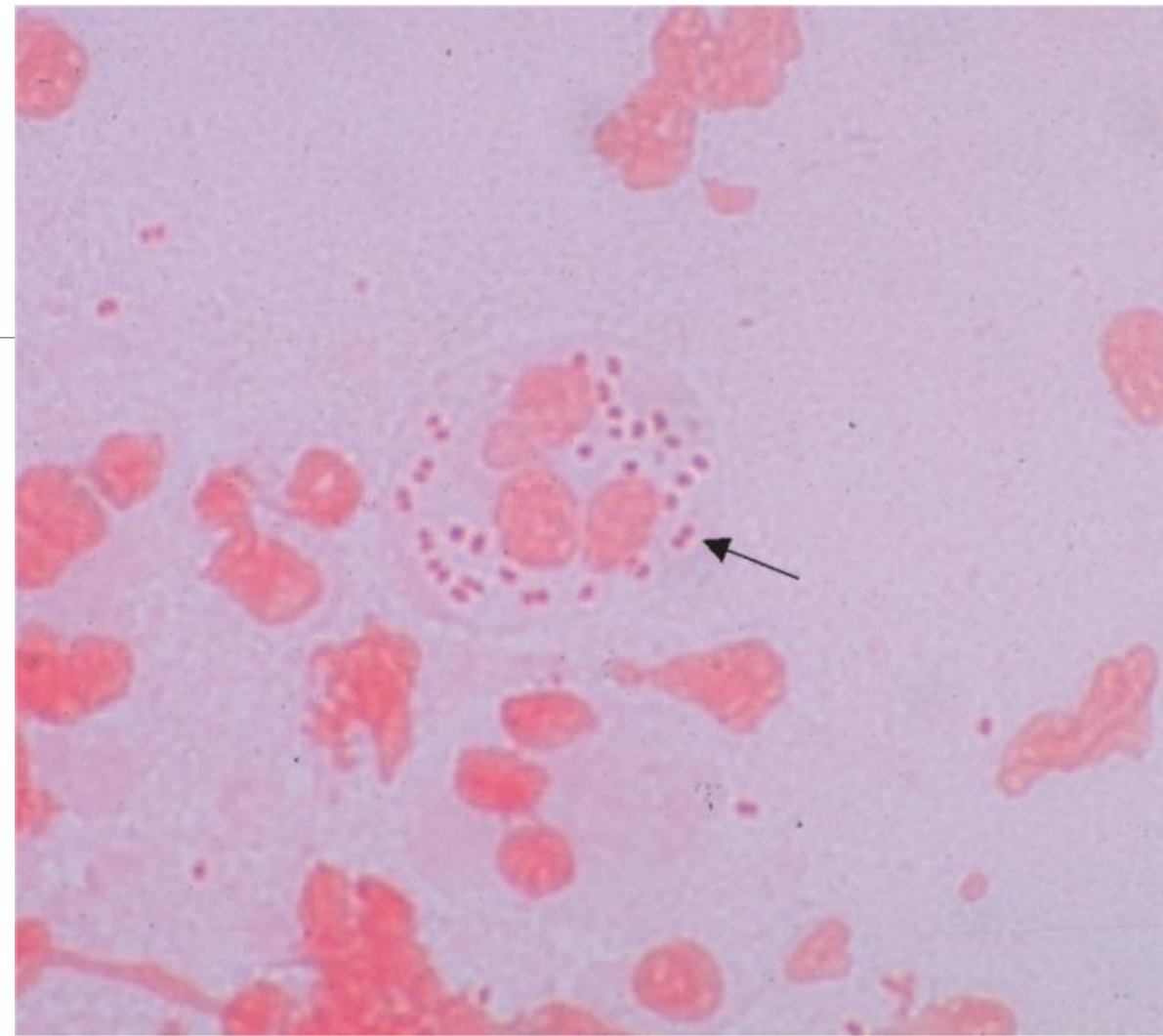
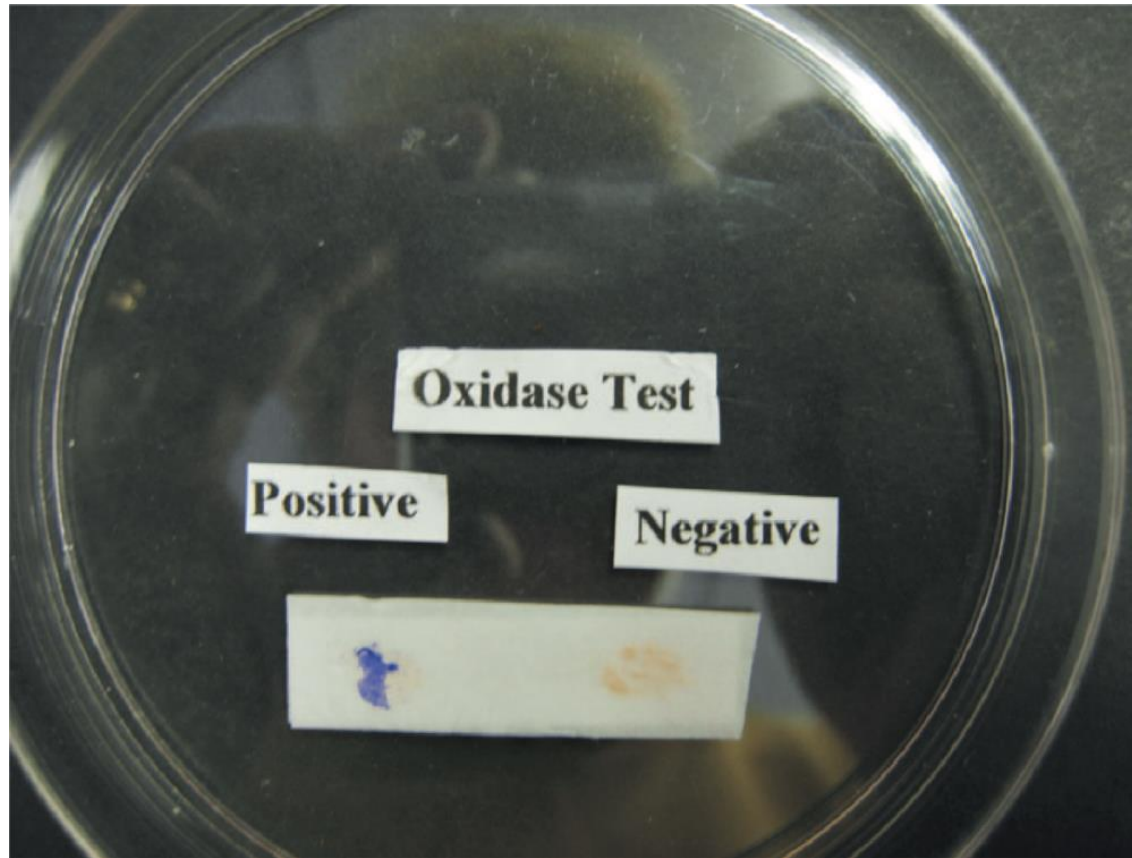
Treatment, Prevention, and Control

• Ceftriaxone with azithromycin is currently the treatment of choice, although high-level resistance to cephalosporins and azithromycin has been observed

• For neonates, prophylaxis with 1% silver nitrate; ophthalmia neonatorum is treated with ceftriaxone

• Prevention consists of patient education, use of condoms or spermicides with nonoxynol-9 (only partially effective), and aggressive follow-up of sexual partners of infected patients

• Effective vaccines are not available



NEISSERIA MENINGITIDIS

Trigger Words

Diplococci, meningitis, meningococemia, pneumonia, vaccine

Biology and Virulence

↻↻ Gram-negative diplococci with fastidious growth requirements

↻↻ Grows best at 35° C-37° C in a humid Atmosphere

↻↻ Oxidase and catalase positive; acid produced from carbohydrates oxidatively

↻↻ Outer surface antigens include polysaccharide capsule, pili, and lipooligosaccharides

↻↻ Capsule protects bacteria from antibodymediated phagocytosis

↻↻ Specific receptors for meningococcal pili allow colonization of nasopharynx and replication; posttranslational modification of the pili enhances host cell penetration and person-to-person spread

↻↻ Bacteria can survive intracellular killing in the absence of humoral immunity

↻↻ Endotoxin mediates most clinical manifestations

Epidemiology

↻↻ Humans are the only natural hosts

↻↻ Person-to-person spread occurs via aerosolization of respiratory tract secretions

↻↻ Highest incidence of disease is in children younger than 1 year old, institutionalized people, and patients with late complement deficiencies

↻↻ Endemic and epidemic disease most commonly caused by serogroups A, B, C, W135, X, and Y; pneumonia most commonly caused by serogroups Y and W135; serogroups A and W135 associated with disease in underdeveloped countries

↻↻ Disease occurs worldwide, most commonly in the dry, cold months of the year

Diagnosis

• Gram stain of cerebrospinal fluid is sensitive and specific but is of limited value for blood specimens (too few organisms are generally present, except in overwhelming sepsis)

• Culture is definitive, but organism is fastidious and dies rapidly when exposed to cold or dry conditions

• Tests to detect meningococcal antigens are insensitive and nonspecific

Treatment, Prevention, and Control

• Breast-feeding infants have passive immunity (first 6 months)

• Empirical treatment of patients with suspected meningitis or bacteremia should be initiated with ceftriaxone; if the isolate is penicillin susceptible, treatment can be changed to penicillin G

• Chemoprophylaxis for contact with persons with the disease is with rifampin, ciprofloxacin, or ceftriaxone

• For immunoprophylaxis, vaccination is an adjunct to chemoprophylaxis; it is used only for serogroups A, C, Y, and W135; no effective vaccine is available for serogroup B; vaccination for serogroup A has been introduced in Africa



Neisseria gonorrhoeae

Gonorrhea: characterized by purulent discharge for involved site (e.g., urethra, cervix, epididymis, prostate, rectum) after a 2- to 5-day incubation period

Disseminated infections: spread of infection from genitourinary tract through blood to skin or joints; characterized by pustular rash with erythematous base and suppurative arthritis in involved joints

Ophthalmia neonatorum: purulent ocular infection acquired by neonate at birth

Neisseria meningitidis

Meningitis: purulent inflammation of meninges associated with headache, meningeal signs, and fever; high mortality rate unless promptly treated with effective antibiotics

Meningococemia: disseminated infection characterized by thrombosis of small blood vessels and multiorgan involvement; small petechial skin lesions coalesce into larger hemorrhagic lesions

Pneumonia: milder form of meningococcal disease characterized by bronchopneumonia in patients with underlying pulmonary disease

PSEUDOMONAS AERUGINOSA

Trigger Words Capsule, exotoxin A, opportunistic, nosocomial infections

Biology and Virulence

Small gram-negative rods typically arranged in pairs

Obligate aerobe; glucose oxidizer; simple nutritional needs

Mucoid polysaccharide capsule

Multiple virulence factors, including adhesins (e.g., flagella, pili, lipopolysaccharide, alginate capsule), secreted toxins and enzymes (e.g., exotoxin A, pyocyanin, pyoverdin, elastases, proteases, phospholipase C, exoenzymes S and T), and antimicrobial resistance (intrinsic, acquired, and adaptive)

Epidemiology

Ubiquitous in nature and moist environmental hospital sites (e.g., flowers, sinks, toilets, mechanical ventilation, and dialysis equipment)

No seasonal incidence of disease


Can transiently colonize the respiratory and gastrointestinal tracts of hospitalized patients, particularly those treated with broad-spectrum antibiotics, exposed to respiratory therapy equipment, or hospitalized for extended periods


Patients at high risk for developing infections include neutropenic or immunocompromised patients, cystic fibrosis patients, and burn patients

Diseases

Diseases include infections of the respiratory tract, urinary tract, skin and soft tissues, ears, and eyes, as well as bacteremia and endocarditis

Diagnosis

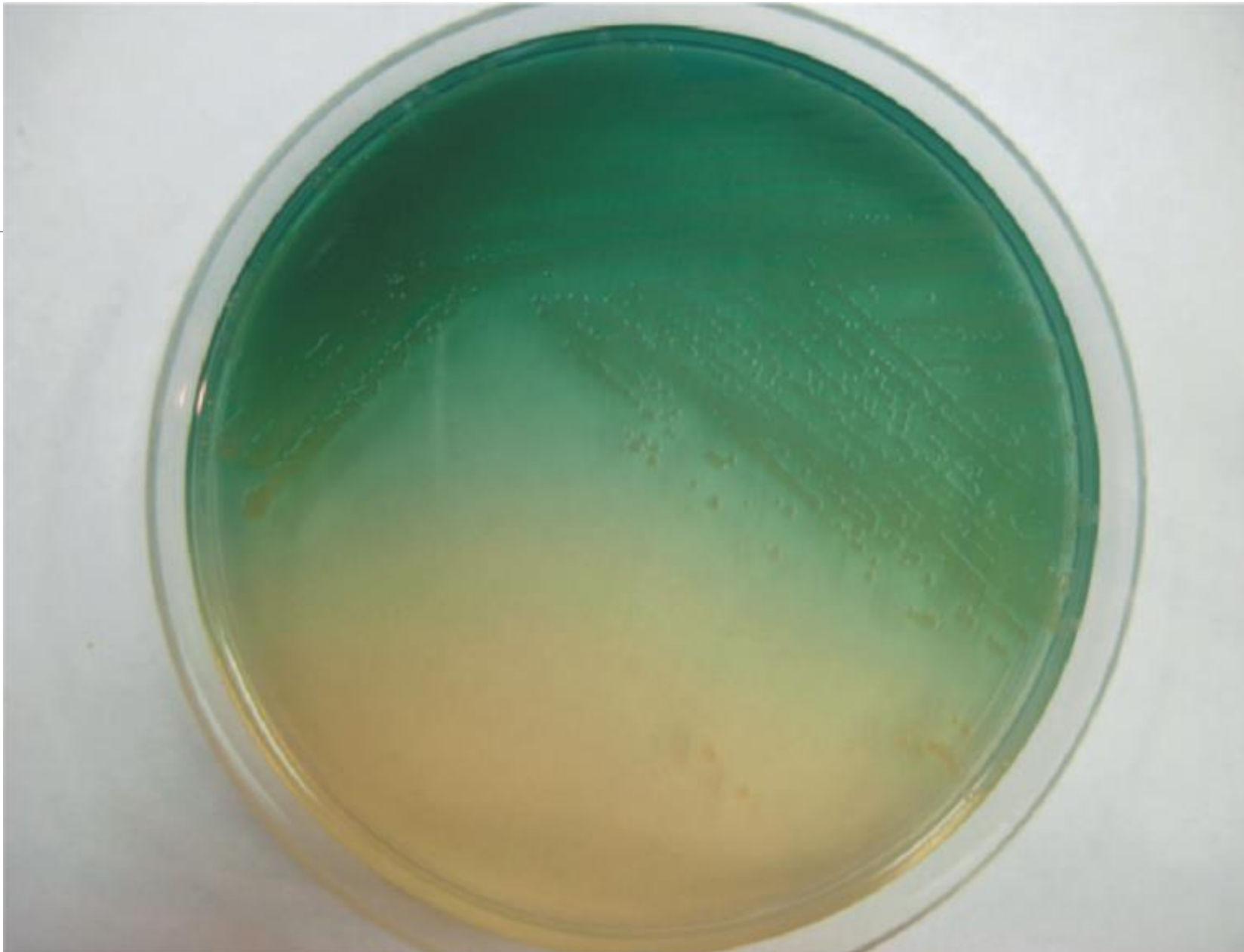
 Grows rapidly on common laboratory media

 Identified by colonial characteristics (e.g., α -hemolysis, green pigment, grapelike odor) and simple biochemical tests (e.g., positive oxidase reaction, oxidative utilization of carbohydrates)

Treatment, Prevention, and Control

Combined use of effective antibiotics (e.g., aminoglycoside and β -lactam antibiotics) frequently required; monotherapy is generally ineffective and can select for resistant strains

Hospital infection-control efforts should concentrate on preventing contamination of sterile medical equipment and nosocomial transmission; unnecessary use of broad-spectrum antibiotics can select for resistant organisms



Clinical Summaries for Nonfermentative Gram-Negative Rods

Pseudomonas aeruginosa

Pulmonary infections: range from mild irritation of the bronchi (tracheobronchitis) to necrosis of the lung parenchyma (necrotizing bronchopneumonia)

Primary skin infections: opportunistic infections of existing wounds (e.g., burns) to localized infections of hair follicles (e.g., associated with immersion in contaminated waters such as hot tubs)

Urinary tract infections: opportunistic infections in patients with indwelling urinary catheters and after exposure to broad-spectrum antibiotics (selects for these antibiotic-resistant bacteria)

Ear infections: can range from mild irritation of external ear (“swimmer’s ear”) to invasive destruction of cranial bones adjacent to the infected ear

Eye infections: opportunistic infections of mildly damaged corneas

Bacteremia: dissemination of bacteria from primary infection (e.g., pulmonary) to other organs and tissues; can be characterized by necrotic skin lesions (ecthyma gangrenosum)

Burkholderia cepacia Complex

Pulmonary infections: most worrisome infections are in patients with chronic granulomatous disease or cystic fibrosis, in whom infections can progress to significant destruction of pulmonary tissue

Opportunistic infections: urinary tract infections in catheterized patients; bacteremia in immunocompromised patients with contaminated intravascular catheters

Burkholderia pseudomallei

Pulmonary infections: can range from asymptomatic colonization to abscess formation (melioidosis)

Stenotrophomonas maltophilia

Opportunistic infections: a variety of infections (most commonly bacteremia and pneumonia) in immunocompromised patients previously exposed to broad-spectrum antimicrobial therapy

Acinetobacter Species

Pulmonary infections: opportunistic pathogen in patients receiving respiratory therapy

Wound infections: traumatic (e.g., resulting from military conflicts) and nosocomial wounds

Moraxella catarrhalis

Pulmonary infections: tracheobronchitis or bronchopneumonia in patients with chronic pulmonary diseases

Klebsiella pneumoniae

klebsiella, named after Klebs; *pneumoniae*, inflammation of the lungs

K. oxytoca - *oxus*, acid; *tokos*, producing; acid producing (refers to biochemical properties)

Proteus *mirabilis* - *proteus*, a god able to change himself into different shapes; *mirabilis*, surprising; refers to pleomorphic colony forms

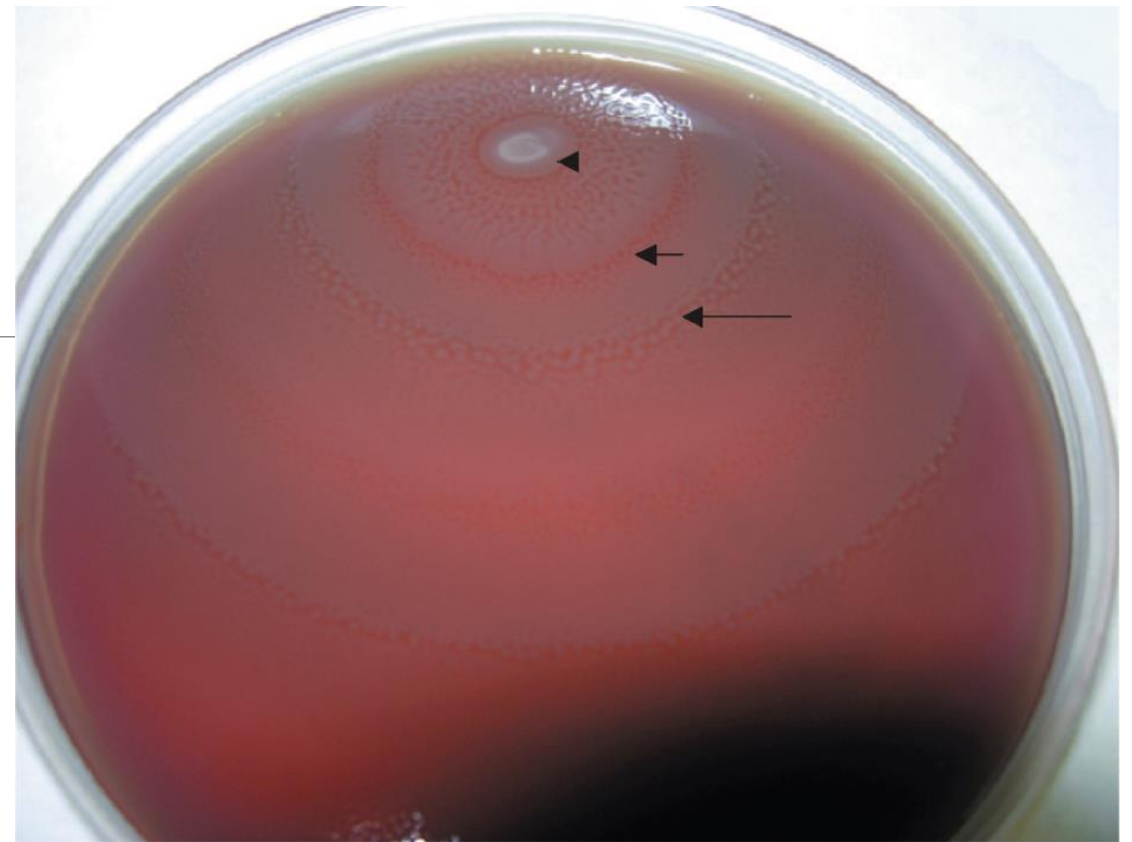
Citrobacter *freundii* - *citrus*, lemon; *bacter*, a rod; citrate-utilizing rod; *freundii*, named after Freund

Citrobacter - *koseri koseri*, named after Koser E.

Enterobacter *cloacae* - enteron, intestine; bacter, a small rod; *cloacae*, of a sewer; originally isolated in sewage

Serratia *marcescens* - *serratia*, named after Serrati; *marcescens*, becoming weak, fading away; originally believed not virulent

-
- **Proteus**
 - *Diseases*
 - These organisms primarily cause urinary tract infections, both community- and hospital-acquired.
 - **Proteus species**—swarming motility on blood agar. Arrowhead points to the site where *Proteus* bacteria were placed on the blood agar. Short arrow points to the edge of the first ring of swarming motility. Long arrow points to the edge of the second ring of swarming motility.





Klebsiella

Diseases

These organisms are usually opportunistic pathogens that

cause nosocomial infections, especially pneumonia and urinary tract infections. *Klebsiella pneumoniae* is an important respiratory tract pathogen outside hospitals as well.

Important Properties

Klebsiella pneumoniae, *Enterobacter cloacae*, and *Serratia marcescens* are the species most often involved in human infections. They are frequently found in the large intestine but are also present in soil and water. These organisms have very similar properties and are usually distinguished on the basis of several biochemical reactions and motility.

Klebsiella pneumoniae has a very large polysaccharide capsule, which gives its colonies a striking mucoid appearance. *Serratia marcescens* produces red-pigmented colonies