**BACTERIOLOGY I**

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**OXYGEN REQUIREMENTS**

- **anaerobic** = intolerant of O2, require proper handling and special conditions for growth  
- **aerobic** = utilizes O2 and grows well in an atmosphere of room air  
- **facultatively anaerobic** = will grow aerobically or anaerobically and includes most clinically significant “aerobes”

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**GRAM STAIN**

- Gram stain result provides the first clue  
  - quick, easy, diagnostic tool  
  - can guide empiric therapy  
  - divides bacteria into two groups  
  - positive = blue or purple  
  - negative = red or pink  
- determines cell morphology  
  - i.e. shape, size, and arrangement  

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**Gram positive**

- cocci (round or spherical shape)  
- anaerobic  
- Peptostreptococcus species  
  - part of indigenous or normal flora  
  - “opportunistic pathogens” = will only cause infection if the integrity or immunity of the host is compromised
**Gram positive**

- cocci
- aerobic
- catalase test \((H_2O_2 > H_2 + O_2)\)
  - catalase positive = Staphylococcus
    - seen as groups or clusters on Gram stained smear
    - normal flora of surface epithelium

**Catalase test**

- Catalase positive = Staphylococcus

**Gram positive**

- coagulase test (an enzyme that binds fibrinogen)
  - coagulase positive = S. aureus
    - a virulent pathogen that can cause skin infections, pneumonia, osteomyelitis, food poisoning, toxic shock syndrome
    - MRSA (50% nationwide), VISA, VRSA
  - coagulase negative
    - S. epidermidis – opportunistic; nosocomial infections, endocarditis
    - S. saprophyticus – urinary tract infections

**Gram positive**

- cocci
- aerobic
- catalase test \((H_2O_2 > H_2 + O_2)\)
  - catalase negative = Streptococcus
    - seen scattered, in pairs, and as chains on Gram stained smear
    - hemolytic properties on blood agar are important for species identification
    - beta, alpha, gamma

**Beta Hemolysis**
Gram positive

• beta hemolytic
• PYR test (determines if the strep can enzymatically hydrolyze L-pyrrolidonyl-β-naphthylamide [PYR]; the end product plus reagent forms red/pink color)
  • positive = Strep, Group A (GAS, S. pyogenes)
    ❖ can cause pharyngitis, scarlet fever, skin and soft tissue infections (“the flesh eating strep”)
    ❖ autoimmune sequelae = glomerulonephritis, rheumatic fever

• PYR test
  • negative = β-hemolytic strep, not group A

• CAMP test (synergism between Group B Strep and S. aureus produces an enhanced arrow-shaped zone of hemolysis at the intersection)

• Commercial latex agglutination test
  • positive = Strep, Group B (GBS or S. agalactiae)

• Strep, Group B
  • most common cause of neonatal sepsis and meningitis
    ❖ CDC - screen pg women 35-37 wks gestation
      (10-30% are asymptomatic carriers)
    ❖ intrapartum abx if screen is pos
  • can also cause sepsis, skin/soft tissue infection, pneumonia in adults

• CAMP test
  • negative = Strep, Group C, F, G, S. milleri
  ❖ Commercial latex agglutination tests can be used to further classify beta-hemolytic Streptococcus into Lancefield groups in < 1 hour

• Alpha Hemolysis
Gram positive

- alpha hemolytic
- Optochin test (disk susceptibility test)
- Bile solubility test (positive colonies autocatalyze or dissolve in the presence of bile salts)

Gram positive

- Optochin test and Bile solubility test
  - sensitive/soluble = S. pneumoniae (pneumococci)
    - seen as lancet-shaped pairs on Gram stain
    - can cause community-acquired pneumonia, otitis media, sinusitis, meningitis=primarily in children < 5 yo
    - Vaccine introduced in 2000
    - ~45 % are Penicillin I or R in U.S.

Gram positive

- Optochin test and Bile solubility test
  - resistant/insoluble = S. viridans
    - a miscellaneous group of Strep that are part of the normal flora of oral, respiratory, and G.I. mucosa
    - opportunistic pathogen, low virulence
    - major etiological agent of endocarditis in the U.S.

Gram positive

- gamma hemolytic
- bile-esculin test (esculin hydrolysis in the presence of bile)
  - positive = Strep, group D

Gram positive

- Strep, group D
  - growth in 6.5 % NaCl
  - PYR test
    - positive = Enterococcus
    - E. faecalis (80-90%), E. faecium
      - can cause UTIs, wound infections, intraabdominal abscesses, nosocomial infections
      - “VRE” (Europe, 1986)

Gram positive

- Strep, group D
  - growth in 6.5 % NaCl
  - PYR test
    - negative = Strep, group D, not Enterococcus
    - S. bovis – main human pathogen in this group
Gram positive

- bacilli (rectangular shape)
- anaerobic
- sporeformers
  - Clostridium species
    - found in soil, water, dust, sewage, and in the intestinal tracts of animals and humans
    - produce nasty toxins that are often responsible for the symptoms

- C. difficile
  - overgrowth of toxigenic strains following abx treatment causes abx-associated diarrhea and pseudomembraneous colitis
  - colonization is common in hospitals and nursing homes
  - carried asymptomatically as part of G.I. flora in ~ 50% of kids < 2 years old
  - tissue culture = “gold standard” method to detect toxigenic strains in stool

- C. perfringens
  - most common cause of gas gangrene - exotoxin causes tissue death
  - common cause of bacterial food poisoning (~10,000 cases/yr in U.S.) – enterotoxin causes illness
  - characteristic colony morphology includes a double zone of hemolysis around colonies

- C. tetani
  - causative agent of tetanus
    - often associated with puncture wounds
    - a toxigenic disease causing prolonged muscle spasms
    - “T” in DPT vaccine

- C. botulinum
  - causative agent of botulism
    - rare, but often fatal, food poisoning
    - the three manifestations of the disease are food, infant, and wound (rare)
    - trademark is acute flaccid (weak, limp) paralysis

- bacilli (rectangular shape)
- anaerobic
- non-sporeformers
  - Propionibacterium, Eubacterium, Bifidobacterium, Actinomyces
  - Mobiluncus
    - not part of normal flora
    - associated with bacterial vaginosis
**Gram positive**

- Bacilli
- Aerobic
- Non-sporeformers
  - Listeria monocytogenes
    - Primary habitat is soil and decaying vegetable matter, contaminated foods are the primary vehicles of transmission
    - Will multiply at refrigerator temperatures
    - Colony morphology closely resembles Strep, group B – differentiate using Gram stain and catalase reaction

- Listeria monocytogenes
  - Causes Listeriosis, ~ 2500 cases/yr in U.S.
  - Seen almost exclusively in neonates, pg women and immunocompromised individuals
  - Untreated/transplacental infection can lead to premature labor, septic abortion, neonatal meningitis

**Gram positive**

- Other non-sporeformers
  - Corynebacterium - most species are harmless saprophytes (diphtheroids)
    - C. diphtheriae - causes diphtheria, the “D” in DPT vaccine
  - Lactobacillus
    - “Active cultures” in yogurt
  - Nocardia

**Gram positive**

- Bacilli
- Aerobic
- Sporeformers
  - Bacillus species
    - Most are not pathogenic
    - Can contaminate OR surfaces, surgical dressings, Rx products, and foods
  - B. anthracis - causes anthrax; cutaneous, pulmonary, intestinal
  - B. cereus - causes food poisoning

**Gram negative**

- cocci
- Bacilli

**Gram negative**

- Enterobacteriaceae (family)
- Other Gram (-) Bacilli (special growth requirements)

- Haemophilus species
- Campylobacter species
- Legionella pneumophilia
- Bordetella pertussis
- Brucella species
- Francisella species
- Helicobacter pylori

- Coagulase-negative staphylococci
- Staphylococcus epidermidis

- Staphylococcus aureus
- Enterococcus species
- Staphylococcus hominis
- Micrococcus species
- Propionibacterium species
- Bacillus species
- Clostridium species
- Brevibacterium species
- Micrococcus species

- Staphylococcus species
- Micrococcus species
- Propionibacterium species
- Bacillus species
- Clostridium species
- Brevibacterium species
- Micrococcus species
- Propionibacterium species
- Bacillus species
- Clostridium species
- Brevibacterium species
- Micrococcus species
Gram negative

- cocci
- anaerobic
- Veillonella species
  - part of the normal flora of the upper respiratory tract
  - seldom a significant pathogen

Gram negative

- cocci
- diplococci
  - adjacent sides are flattened, characteristic kidney or coffee bean shape
- aerobic
  - oxidase test (enzyme oxidizes a substrate and forms a purple end product)
  - positive = Neisseria species and Moraxella catarrhalis

Gram negative

- Oxidase test

Gram negative

- Neisseria meningitidis (meningococci)
  - can colonize naso-pharynges
  - can disseminate and cause meningitis
  - highest incidence = school age (5-25 y)
  - can progress rapidly and result in sudden death within a few hours after the onset of symptoms
  - rapid diagnosis (latex agglutination) and aggressive treatment are imperative

Gram negative

- Neisseria gonorrhoeae (gonococci)
  - causes gonorrhea
  - the most frequently reported communicable disease in the U.S.
  - intracellular Gram - diplococci = diagnostic
  - some strains are penicillin-resistant (PPNG)
  - causes neonatal gonococcal conjunctivitis
  - abx eye drops at birth nearly eliminated the disease in developed countries

Gram negative

- Moraxella (Branhamella) catarrhalis
  - normal flora of upper respiratory tract???
  - acute localized infections include otitis media, sinusitis, conjunctivitis, bronchopneumonia
  - systemic diseases - endocarditis, meningitis: most cases are in elderly patients
  - >90% beta-lactamase positive (penicillin-resistant)
Gram negative

- bacilli
- aerobic (or facultative)
- glucose fermentation
  - non-fermenters
    - oxidase positive
      - Pseudomonas species

Gram negative

- Pseudomonas aeruginosa
  - not part of the normal flora in healthy people
  - environmental organism (water, soil, plants)
  - well adapted to survival in harsh environments
  - causes a broad spectrum of disease
    - superficial skin infections > sudden, severe sepsis
    - major cause of nosocomial infection
      - multidrug resistance is a problem

Gram negative

- glucose fermentation
  - non-fermenters
    - oxidase negative
      - Acinetobacter species
      - Stenotrophomonas maltophilia; recently reclassified, may be referred to as Xanthomonas maltophilia

Gram negative

- Stenotrophomonas maltophilia
  - ubiquitous in nature, often found in hospital environments
  - may be colonizers or infectious agents
  - may cause septicemia, pneumonia, wound infections
  - often multi-drug resistant

Gram negative

- Acinetobacter species
  - found in nature and in hospital environments
  - more often colonizers than infectious agents

Gram negative

- bacilli
- aerobic (or facultative)
- glucose fermentation
  - fermenters
    - oxidase positive
      - Pasteurella
      - Vibrio
      - Aeromonas
      - Plesiomonas
Gram negative

- *Pasturella multocida*
  - often isolated from infected animal bite wounds
- *Vibrio*
  - inhabits brackish and salt water worldwide
  - disease is associated with ingestion of contaminated water or seafood
  - can cause gastroenteritis or cholera with rapid dehydration

Gram negative

- *Aeromonas*
  - ubiquitous inhabitants of fresh and salt water
  - associated with infected wounds acquired near or in water or with diarrheal disease
- *Plesiomonas*
  - also maintains a water habitat
  - primarily associated with gastroenteritis after eating raw shellfish or foreign travel

Gram negative

- bacilli
- aerobic (or facultative)
- glucose fermentation
- fermenters
  - oxidase negative
  - *Enterobacteriaceae* (family) - more than 100 species

Gram negative

- *Enterobacteriaceae*
  - many are normal G.I. flora in humans and animals, also found in soil and water and on plants
  - common nosocomial pathogens
  - account for 50% of all clinically significant isolates, 50% of septicemia cases, 70% UTIs
  - most microbiology labs use an automated system for ID and susceptibility testing

Gram negative

- *Enterobacteriaceae*
  - enteric pathogens
    - *Salmonella enteritidis*: gastroenteritis due to ingestion of undercooked eggs
    - *S. typhi*: typhoid fever
    - *Shigella*: shigellosis (pediatric diarrhea) and dysentery
    - *Yersinia pestis*: the agent of bubonic plague
    - *Escherichia coli*: most common bacterium isolated in clinical labs

Gram negative

- *Enterobacteriaceae*
  - others commonly isolated
    - *Proteus*
    - *Klebsiella*
    - *Providencia*
    - *Enterobacter*
    - *Serratia*
    - *Citrobacter*
    - *Morganella*
Gram negative

• bacilli
• anaerobic
• Bacteroides species (B. fragilis group)
  • most commonly recovered anaerobe in clinical specimens
  • predominant flora of the colon
  • can cause intra-abdominal infections

Gram negative

• bacilli
• anaerobic
• Prevotella, Porphyromonas, Fusobacterium
  • part of normal flora of oropharynx, G.I. tract, female genital tract
  • some species are important pathogens in oral, dental, and bite infections

Gram negative

• bacilli
• special growth requirements
• Haemophilus
  • TINY Gram (-) coccobacilli
  • will only grow on media containing necessary growth factors, i.e. chocolate agar
  • can be normal respiratory flora

Gram negative

• H. influenzae
• can cause meningitis, conjunctivitis, otitis media
• Hib vaccine (1985) for protection against the particularly virulent encapsulated strain belonging to serotype B
  • historically a leading cause of disease in children < 5 years old
  • incidence of invasive infection has dropped sharply

Gram negative

• special growth requirements
• Campylobacter
  • inhabit the G.I. tract of animals
  • transmitted via contaminated food, milk, and water
  • common cause of gastroenteritis in the U.S. (2M/yr); usually self-limiting

Gram negative

• special growth requirements
• Legionella
  • widespread in the environment, no known animal reservoir
  • transmitted via infected aerosols
  • causes Legionnaires’ disease and Pontiac fever
  • lab diagnosis = DFA + culture
**Gram negative**

- special growth requirements
- *Bordetella pertussis*
  - resides on mucous membranes in respiratory tract of animals and humans
  - “P” in DPT vaccine (late 1940s)
  - can cause “whooping cough” (~ 8000 cases/yr in U.S.)
  - lab diagnosis is difficult; lacks sensitivity

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**Gram negative**

- special growth requirements
- *Brucella*
  - zoonotic; domestic animal reservoir
  - can cause Brucellosis; a chronic and relapsing febrile disease, ~ 100 cases/yr in U.S.
- *Francisella tularensis*
  - zoonotic; rodents, rabbits, beaver, muskrats
  - intracellular parasites
  - one of few infectious agents that can penetrate intact skin

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**Gram negative**

- microaerobic
- special growth requirements
- *Helicobacter pylori*
  - major habitat is human gastric mucosa
  - etiological agent of gastritis and gastric ulcer
  - diagnosis: serology, breath test, gastric biopsy

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**Too much information?**

14 V.I.B. (very important bacteria)

**Gram positive – top seven**
- *Staphylococcus aureus*
- *Staph epidermidis*
- *Streptococcus, group A*
- *Strep pneumoniae*
- *Enterococcus*
- *Clostridium species*
- *Listeria monocytogenes*

**Gram negative – top seven**
- *Neisseria meningitidis*
- *Neisseria gonorrhoeae*
- *Moraxella catarrhalis*
- *Enterobacteriaceae*
- *Pseudomonas aeruginosa*
- *Bacteroides fragilis*
- *Haemophilus influenzae*