MICROBIOLOGY I

Laurie Baeker Hovde
B.S., MT (ASCP)
• Gram stain result provides the first clue for quick, easy, diagnostic tool. It can guide empiric therapy. It divides bacteria into two groups:
  - **positive** = blue or purple
  - **negative** = red or pink

It determines **cell** morphology, i.e., shape, size, and arrangement.
OXYGEN REQUIREMENTS

- anaerobic = intolerant of O2, require proper handling and special conditions for growth
  - specific request required for I.D.
- 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”
Gram positive
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 (H2O2 > H2 + O2)
  - positive = Staphylococcus seen as groups or clusters on Gram stained smear
  - normal flora of surface epithelium
Gram positive

- coagulase test (an enzyme that binds fibrinogen)
  - positive = S. aureus
    a virulent pathogen
    - skin infections, pneumonia, osteomyelitis, food poisoning, toxic shock syndrome
  - negative = “SCN”
    - S. epidermidis - SBE
    - S. saprophyticus - UTI

30% nationwide are “MRSA”
Gram positive

• cocci
• aerobic
• catalase test (H₂O₂ > H₂ + O₂)
  • negative = Streptococcus
    seen scattered, in pairs, and as chains on Gram stained smear
    hemolytic properties on blood agar
  • beta, alpha, gamma
Gram positive
Gram positive
Gram positive

- cocci
- aerobic
- gamma or alpha 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 (to see if the organism has the enzyme that hydrolyzes the PYR substrate)
  - positive = Enterococcus E. faecalis (80-90%), E. faecium
- UTIs, wound infections, intraabdominal abscesses, nosocomial infections
  “VRE”
**Gram positive**

Strep, group D
- growth in 6.5 % NaCl
- PYR test
  - negative = Strep, group D, not Enterococcus (S. bovis)
Gram positive

- cocci
- aerobic
- beta hemolytic
- PYR test
  - positive = Strep, Group A (S. pyogenes)
    - pharyngitis, scarlet fever, skin and soft tissue infections ("the flesh eating strep")
    - autoimmune sequelae = glomerulonephritis, rheumatic fever
Gram positive

- PYR test
  - negative = Strep, group B, C, F, G, S. milleri
- CAMP test (synergism between Group B Strep and S. aureus produces an enhanced arrow-shaped zone of hemolysis at the intersection)
- Hippurate hydrolysis (a positive test results in a colored end product after addition of ninhydrin reagent)
Gram positive

• CAMP test
• Hippurate hydrolysis
  • positive = Strep, Group B (S. agalactiae)
    common (~33%) cause of neonatal sepsis
    and meningitis
    postpartum fever and sepsis
    joint infection, osteomyelitis, UTI, wound infection
Gram positive

- CAMP test and Hippurate hydrolysis
  - negative = Strep, Group C, F, G, S. milleri
- Commercial latex agglutination tests (classifies beta-hemolytic Streptococcus into Lancefield groups in about an hour)
  - S. milleri and Strep, Group F: urogenital tract infections, abscesses
  - Strep, Group C an G: pharyngitis, postpartum sepsis, rash, bacteremia
Gram positive

- cocci
- aerobic
- 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 stained smears
  - community-acquired pneumonia, otitis media, sinusitis, meningitis
  - ~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 GI mucosa
    - opportunistic pathogen, low virulence
    - major etiological agent of endocarditis in the U.S.
Gram positive

- bacilli (rectangular shape)
- anaerobic
  - Clostridium species
    - spore-formers
    - 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
Gram positive

- C. difficile
  - antibiotic associated diarrhea and pseudomembranous colitis; most common agents are beta-lactams and clindamycin
  - nosocomial infection
  - carried asymptomatically as part of GI flora in up to 50% of kids < 1 year old
  - tissue culture = gold standard; also EIA, latex agglutination, bacterial culture for ID
Gram positive

- C. perfringens
  - gas gangrene
  - third most common cause of food poisoning in the U.S. (behind Salmonella and S. aureus)
    - characteristic double zone of hemolysis around colonies
Gram positive

- C. tetani
  - tetanus

  often associated with puncture wounds
  autolysis, neurotoxin release, binding to cells in CNS, blockage of inhibitory impulses = prolonged muscle spasms
  - “T” in DPT vaccine
Gram positive

- C. botulinum
  - botulism

rare, but often fatal
the mechanism of action of the toxin is
similar to C. tetani, binding site differs
the three manifestations of the disease
are food, wound, and infant botulism
trademark is acute flaccid paralysis
**Gram positive**

- **Propionibacterium, Eubacterium, Bifidobacterium, Actinomyces**
  - normal flora, opportunistic pathogens
- **Lactobacillus**
  - prevents yeast infection by competing for nutrients
  - found in yogurt and other foods
- **Mobiluncus**
  - not part of normal flora
  - associated with bacterial vaginosis
Gram positive

- bacilli
- aerobic
  - *Listeria monocytogenes*
    - primary habitat is soil and decaying vegetable matter
    - will multiply at refrigerator temperatures
    - contaminated foods are the primary vehicles of transmission
    - listeriosis
Gram positive

- Listeria monocytogenes
  - seen almost exclusively in neonates, pregnant women and immunocompromised individuals
  - untreated/transplacental infection can lead to premature labor, septic abortion, neonatal meningitis

  colony morphology closely resembles Strep, group B - must differentiate
Gram positive

- bacilli
- aerobic
- Erysipelothrix - veterinary pathogen, human infection subsequent to animal exposure is rare
- Corynebacterium
  - C. diphtheriae
diphtheria
  - “D” in DPT vaccine
Gram positive

• Bacillus species
  • troublesome contaminant
  • anthrax - rare
  • food poisoning
    spore-formers
• Nocardia
  • inhabit soil, vegetation, water
  • infection is subsequent to inhalation or inoculation through breaks in the skin
Gram negative
Gram negative

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

- cocci or diplococci
- aerobic
- oxidase test (organisms that produce this enzyme oxidize a substrate and form a purple end product)
  - positive = Neisseria species and Moraxella catarrhalis

adjacent sides are flattened, characteristic kidney or coffee bean shape
Gram negative

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

- Neisseria gonorrhoeae (gonococci)
  - many strains are penicillin-resistant (PPNG)
  - gonorrhea
    - the most frequently reported communicable disease in the U.S.
      - intracellular G (-) diplococci = diagnostic
  - neonatal gonococcal conjunctivitis
    - antibiotic eye drops at birth have almost eliminated the disease in developed countries
Gram negative

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

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

- bacilli
- anaerobic
- Prevotella, Porphyromonas, Fusobacterium
  - part of normal flora of oropharynx, GI tract, female genital tract
  - some species are important pathogens in oral, dental, and bite infections
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
  - broad spectrum of disease
    - superficial skin infections to fulminant sepsis
    - major cause of nosocomial infection
  - multidrug resistance is a problem
Gram negative

- bacilli
- aerobic (or facultative)
- glucose fermentation
  - non-fermenters
  - oxidase negative
    - *Stenotrophomonas maltophilia*
    - *Acinetobacter* species
Gram negative

- Stenotrophomonas maltophilia
  - recently reclassified (Xanthomonas)
  - ubiquitous in nature, often found in hospital environments
  - may be colonizers or infectious agents
  - septicemia, pneumonia, wound infections
  - often resistant to antibiotics; Trimethoprim/sulpha is typically useful
**Gram negative**

- Acinetobacter species
  - second most commonly isolated nonfermenters
  - found in nature and in hospital environments
  - more often colonizers than infectious agents
Gram negative

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

- Pasturella
  - often isolated from infected animal bite wounds
- Vibrio
  - inhabits brackish and salt water worldwide
  - disease is associated with ingestion of contaminated water or seafood
  - cholera; 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
- Plesiomononas
  - 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 recognized species
Gram negative

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

- Enterobacteriaceae
  - enteric pathogens
    Salmonella: etiological agent of most foodborne gastroenteritis in U.S.,
    typhoid fever
    Shigella: dysentary-like syndrome
    Yersinia: the agent of human 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
- aerobic (or facultative)
- special growth requirements
  - Haemophilus
    - TINY Gram (-) rods
    - require hemin and nicotine adenine dinucleotide (NAD) for growth
    - (Chocolate agar)
    - can be normal respiratory flora
Gram negative

• H. influenzae
  • 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 under 5 y
    incidence of invasive infection has dropped sharply
Gram negative

- bacilli
- aerobic (or facultative)
- 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.; usually self-limiting
Gram negative

- bacilli
- aerobic (or facultative)
- special growth requirements
  - Legionella
    - widespread in the environment, no known animal reservoir
    - Legionnaires’ disease and Pontiac fever transmitted via infected aerosols
    - diagnosis = DFA + culture
Gram negative

- bacilli
- aerobic (or facultative)
- special growth requirements
  - *Bordetella pertussis*
    - reside on mucous membranes of respiratory tract of animals and humans
    - can cause “whooping cough”
    - “P” in DPT vaccine
    - lab diagnosis is difficult; lacks sensitivity
Gram negative

- bacilli
- aerobic (or facultative)
- special growth requirements
  - Brucella

  zoonotic disease, domestic animal reservoir
  humans acquire via contaminated milk or
  through occupational exposure
  brucellosis; a chronic and relapsing febrile
disease
  about 100 cases per year in U.S.
**Gram negative**

- bacilli
- microaerobic
- special growth requirements
  - *Helicobacter pylori*
    - major habitat is human gastric mucosa
    - etiological agent of gastritis and gastric ulcer
  - lab diagnosis = serology, stains, culture
References

• Diagnostic Microbiology
  • Bailey and Scott
  • 9th edition
• Manual of Clinical Microbiology
  • Murray, Baron, Pfaller, Tenover, Yolken
  • 6th edition
**BACTERIAL GROWTH CURVE**

*Fig. 5-2.* Phases of bacterial growth, starting with an inoculum of stationary-phase cells. Note that the classic phases, defined in terms of cell number, do not precisely coincide with the phases of changing growth in terms of protoplastic mass.
Susceptibility Testing

The attempt to predict, using *in vitro* methods, the likelihood of successfully treating an infection with a particular antimicrobial agent.

Testing is based on either the principle of diffusion or dilution.
Disk diffusion Sensitivity Testing (Kirby-Bauer)

- A “standardized” suspension of the organism is used to inoculate agar surface.
- Filter paper disks containing abx are placed onto surface of agar.
- Abx diffuses into agar and establishes a concentration gradient (rate of diffusion differs among abx).
- Plates are incubated 16-20 hours.
Disk **diffusion** Sensitivity Testing (Kirby-Bauer)

- Zones of growth inhibition are measured (mm)
- Zone size is referenced to NCCLS interpretive chart
- Qualitative results = S, I, R
- Advantages
  - firmly established method
  - relatively inexpensive
  - flexible drug selection
Disc Diffusion Sensitivity Testing
(Kirby-Bauer)
Etest diffusion sensitivity testing

- Variation of disk diffusion method
- Etest strips replace filter paper disks
- MIC is read where “ellipse” (growth inhibition) intersects the strip
- Allows quantitative determination of MIC on agar
- More expensive
Ettest

- Antibiotic code
- Inert carrier
- Reading scale (MIC μg/ml)
- Exponential antibiotic gradient

- PDM Epsilometer test principle
- Inhibitory Concentration (IC)
- Lawn of bacterial growth
- "Inhibition" (i.e., no growth)

- ME
MIC/MBC dilution testing

- A range of abx concentrations is established in agar or broth
- The abx concentration is reduced by half with each successive dilution
- Each plate, tube, or well is inoculated with ~ the same amount of bacteria (10⁵ - 10⁶ CFU/mL) and incubated
MIC/MBC dilution testing
MIC/MBC dilution testing

• Examine all plates or tubes in the series for growth (colonies or turbidity)
• The MIC is the lowest concentration of abx that inhibits the growth of the bacteria (mcg/mL)
• Reliable, standardized reference method
• Quantitative results
MIC/MBC dilution testing

- MBC testing may be performed with broth dilution method
  - Remove an aliquot from “clear” tubes or wells and transfer to an agar plate; incubate
  - Each colony represents one viable bacterium
  - MBC is the concentration of abx that kills 99.9% or 3-logs of the original inoculum
MIC/MBC Testing

Antibiotic Concentration (mg/L)

128  64  32  16  8  4  2  1  0.5  0.25  0.125  0.06
SIT/SBT testing (Schlichter)

- Measures the activity of the patient's own serum (containing one or more antibiotics) against his/her specific pathogen
- Typically tests peak (and sometimes trough) sera
- Can be used to detect antibiotic failure
- Labor intensive, many confounding variables, poorly standardized, difficult to reproduce
SIT/SBT Testing
Serum Titer

1:2 1:4 1:8 1:16 1:32 1:64 1:128 1:256

1:2 1:4 1:8 1:16
Automation in the microbiology

- BACTEC (Becton-Dickinson) - an automated method for blood cultures
  - measures CO₂ production by metabolizing bacteria
  - An increase in CO₂ above baseline signals a positive culture
- Others = BacT/Alert, ESP
Automation in the microbiology
Automation in the microbiology
Automation in the microbiology

- Sensitivity testing
  - Utilize robotics, micro-processors, micro-computers to provide results
  - Vitek, Microscan, Sensititre, Pasco, Sceptor, Esteem
- Rapid results (3.5 - 8 hours)
Automation in the microbiology

- Vitek (1966)
  - Uses “test cards”
  - Micro-wells contain either substrate (ID) or antimicrobials (sensitivity)
  - Wells are filled with bug suspension
  - Photometer measures color (ID) or turbidity (sensitivity) changes over time
  - Computer compiles data and IDs the organism and/or calculates MIC
Automation in the microbiology
Automation in the microbiology
Synergy, Indifference, and Antagonism

Indifference (Additive)—the activity of two drugs in combination is equivalent to the activity of the most active of the two drugs.

Antagonism—the activity of two drugs in combination is less than the activity of the most active of the two drugs.

Synergy—the activity of two drugs in combination is greater than the activity of either alone.
Hypothetical Situation

A pt is recovering from hip surgery. The incision becomes infected. A culture of the wound is positive and the organism is identified as P. aeruginosa. The reported MIC of Ciprofloxacin is 1 ug/mL.

1. Is the organism sensitive to Cipro?
2. Should the pt be treated with Cipro?
**MIC Breakpoints**

- Interpretive categories attempt to correlate *in vitro* susceptibility data with clinical outcome.
- Based on two premises:
  - If bug is inhibited by a concentration of the drug that is readily achievable in patients blood, then susceptible.
  - If bug is resistant *in vitro* to achievable concentrations of drug, then patient will not respond to therapy.
**MIC Breakpoints**

- Only apply if:
  - **Standard** recommended dose of drug is administered by **normal** routes of delivery to **adults** with **normal** renal function

- General rule:
  - The achievable level of antibiotic at the site of infection should be 2-4 times the MIC if the drug is to be effective
NCCLS Interpretive Standards

Quinolones

Ciprofloxacin

Susceptible ≤ 1
Intermediate 2
Resistant ≥ 4

(Answer #1 is yes!)
Other important considerations

- Level of “active” drug at site of infection
  - protein binding, route of administration, concentration, renal/hepatic function, $T_{1/2}$
- Host factors
  - disease, immune status, compliance
- Bug
  - virulence, CFU/mL, mixed infection, resistance factors
- Inoculum effect
  - MIC $\sim 10^5$, active infection $>10^8$