**MICROBIOLOGY I**

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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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**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

- 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

- coagulate test (an enzyme that binds fibrinogen)
  - positive = S. aureus
    - a virulent pathogen
      - skin infections, pneumonia, osteomyelitis,
        food poisoning, toxic shock syndrome
      - MRSA (50% nationwide), VISA, VRSA
  - negative = “SCN”
    - S. epidermidis - SBE
    - S. saprophyticus - UTI

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**

- 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” (1988)

**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**

- 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 = other 
- CAMP test (synergism between Group B Strep and S. aureus produces an enhanced arrow-shaped zone of hemolysis at the intersection) …OR
- 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
  - GBS or S. agalactiae
    - most common cause of neonatal sepsis and meningitis
    - amnionitis, endometritis in pg women
    - blood, skin/soft tissue infection, pneumonia
    - can be carried asymptomatically

**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 < 1 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)

- Optochin test and Bile solubility test
  - sensitive/soluble = S. pneumoniae (pneumococci)
    - seen as lancet-shaped pairs on Gram stain
    - community-acquired pneumonia, otitis media, sinusitis, meningitis – primarily in children < 5 years old
    - 75% are Penicillin I or R in U.S.
    - Prevnar™ vaccine (2000)

- 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, chromatographic assay

Gram positive

• C. perfringens
  • gas gangrene – toxin breaks down muscle
  • third most common cause of bacterial 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
  - disease course: 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
- Lactobacillus
  - prevents yeast infection, competes 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 refrigeration temperatures
  - contaminated foods are the primary vehicles of transmission
  - listeriosis ~ 2500 cases/year in U.S.

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 – differentiate using Gram stain and catalase

Gram positive

- bacilli
- aerobic
- Erysipelothrix - veterinary pathogen, human infection subsequent to animal exposure is rare
- Corynebacterium - most species are harmless saprophytes (diphtheroids)
  - C. diphtheriae – causes diphtheria
    - “D” in DPT vaccine
**Gram positive**

- Bacillus species = spore-formers
  - most are troublesome contaminants
- B. anthrasis: anthrax - rare
- B. cereus: food poisoning
- Nocardiophiles
  - inhabit soil, vegetation, water
- infection (rare) 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
- adjacent sides are flattened, characteristic kidney or coffee bean shape
- aerobic
- oxidase test (organisms that produce this enzyme oxidize a substrate and form a purple end product)
  - positive = Neisseria species and Moraxella catarrhalis

**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 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)
  - causes 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

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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)

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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

Gram negative

- Acinetobacter species
  - second most commonly isolated nonfermenter
  - 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 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 cholera and 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 recognized species

Gram negative

- Enterobacteriaceae
  - many are normal GI 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 I.D. and susceptibility testing

Gram negative

- Enterobacteriaceae
  - enteric pathogens
    - Salmonella: etiological agent of most foodborne gastroenteritis in U.S., typhoid fever
    - Shigella: shigellosis and dysentery
    - Yersinia: the agent of human plague
    - Escherichia coli: most common bacterium isolated in clinical labs
Gram negative

- Enterobacteriaciae
  - 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 < 5 years old
  - 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. (2M/yr); 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” (8000/yr)
    - “P” in DPT vaccine (late 1940s)
    - 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.

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**Gram negative**

- bacilli
- 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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**Know your bugs!**

- Viruses
- Bacteria
- Fungus
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
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

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 ($10^5 - 10^6$ CFU/mL) and incubated
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

**SIT/SBT testing (Schlichter)**

- Measures the activity of the patients own serum (containing one or more abx) against his/her specific pathogen
- Typically test peak sera
- Can be used to detect abx failure
- Labor intensive, many confounding variables, poorly standardized, difficult to reproduce
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

• 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
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 ≤ 10^5, active infection >10^8