Basic Microbiology

Jim Gauthier, Providence Care
A Webber Training Teleclass

Basic Microbiology

Welcome to the Bug Man's World!
And yes, it's a Small World!

Jim Gauthier

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Objectives

- Be at ease with the terminology
- Understand normal vs. abnormal flora
- Demystify all the Latin and Greek (ya, right)!
- See some of the wonders of the microbial world

The Basics

- The bugs are small – 2-5 microns (10^{-6} meters)
  - Viruses are even smaller – nanometers (10^{-9})
- Classification based on three things:
  - Shape
  - Gram Reaction
  - Growth Requirements

The Basics

- Microscopes give a phenotype view
  - Phenotype: what you can see
- Growth and playing gives the genotype view
  - What it can do because of genetics
- Staff generally wants the results yesterday!

The Basics

- Most human pathogenic bacteria take 18-24 hours to grow enough on the laboratory media to be visible and to be able to distinguish single colonies with the naked eye.
- Sensitivity testing from a pure culture can be anywhere from 4 – 24 hours later.
- Full identification can also take up to 24 – 48 hours.

Identification

- Oxygen requirements
- Able to ferment or oxidize sugars to produce acid end products
- Temperature ranges
- Salt tolerance
- Chemical tolerance
- Enzymes
- Motile

Identification

- PCR, Gene probes
  - In use more and more
  - Chlamydia, GC, Tuberculosis, MRSA, VRE
  - Norovirus
- ELISA
  - Organism is an antigen and reacts with labelled antibody
    - Influenza, RSV, Rotovirus

The Basics- Terms

- Bacteria can either grow or not grow in the presence of oxygen
- Oxygen: Aerobic (Pseudomonas, Bacillus)
- No Oxygen: Anaerobic (Clostridium, Bacteroides)
- Either: Facultative Anaerobe (*E. coli*)

The Basics - Terms

- Hemolysis
  - Beta: complete destruction of the red blood cells in the (sheep) blood agar plate
  - Alpha: partial destruction of the cells, leaving a greenish hue to the blood
  - Gamma: old term, no hemolysis

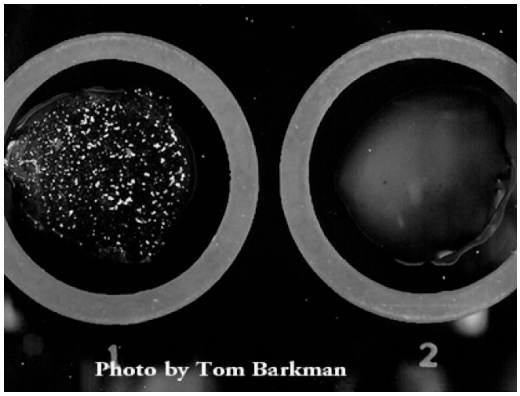
http://www.bellarmine.edu/faculty/mlassiter/images/Figure20014ABG_000.jpg

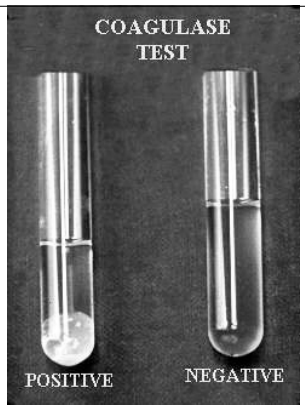
The Basics - Terms

- Catalase
  - Tests the organism's ability to liberate oxygen from hydrogen peroxide
  - Main distinguishing feature between Staphylococci and Streptococci / Enterococci
  - Pure organism placed into H_2O_2 – observe!

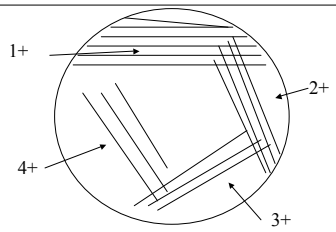
The Basics - Terms

- Coagulase
  - The ability of the organism under study to clump, clot, or coagulate rabbit plasma, turning a solution from liquid to semi-solid
    - Can use plasma or latex particles
  - Used as main identification of *Staphylococcus aureus*, distinguishing it from other *Staph.* species.



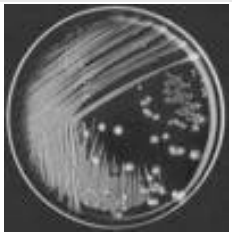


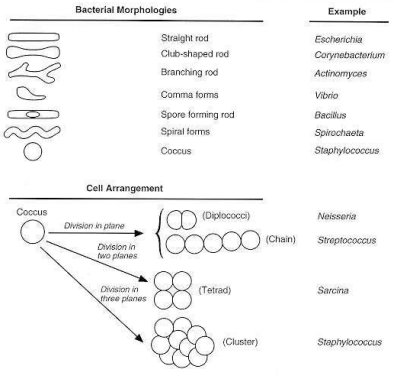
http://www.telmeds.org/AVIM/Abacterio/cocos%20gram%20positivos/images/coagulase.jpg



-Quantitation of growth based on how many organisms are present in a certain inoculum
- Growth medium first used by Robert Koch

Agar Plates



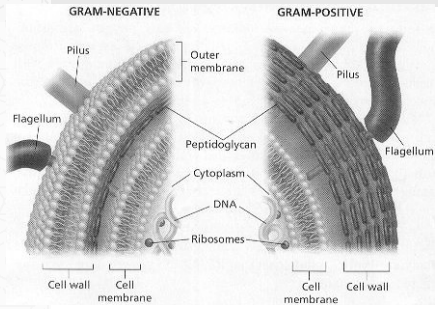


http://www.gsbs.utmb.edu/microbook/ch002.htm

The Gram Stain

- Developed in the late 1800's by Dr. Gram, a pathologist
- Originally noted while staining lung (more trivia)
- Gram positive organisms are purple
- Gram negative organisms are red
- Based on cell wall composition

Cell Wall Composition – Simple!



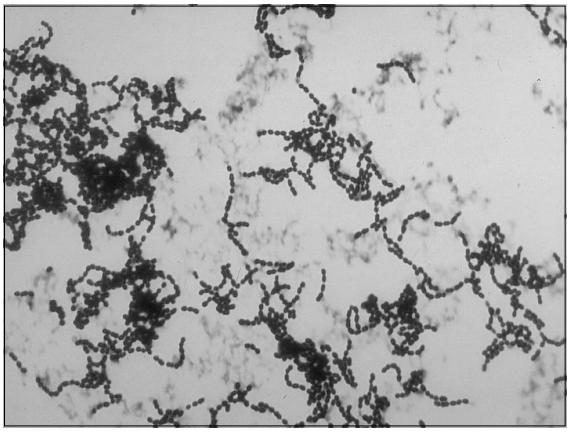
http://sps.k12.ar.us/massengale/bacteria_notes_b1.htm

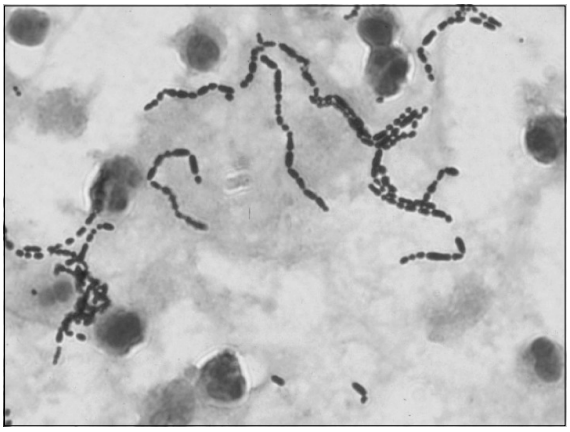
Gram Stain

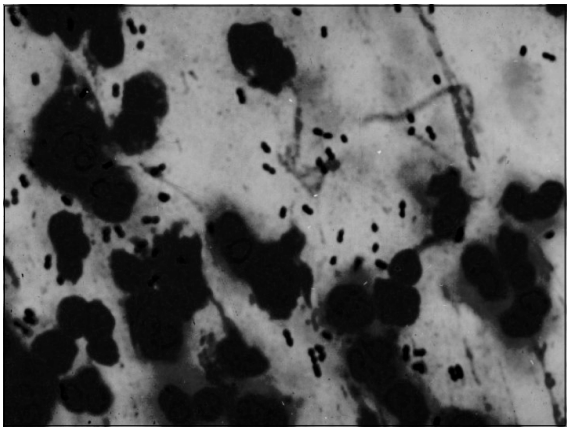
- Gives a quick look at the specimen
  - Presumptive identification
- Can interpret quality of specimen
  - Number of "pus" (polymorphonuclear) cells present
    - Infection
  - Number of epithelial cells present
    - Surface
  - Number of bacteria present (and likely Genus)
    - Normal vs. abnormal

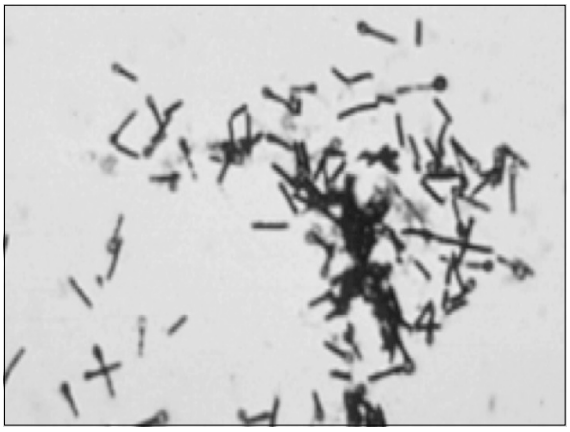
Gram Stain

- Can help direct antibiotic therapy
  - Based on cell wall composition
- Not so helpful if lots of normal flora present
  - throats, stool, decubital ulcers
- Quite significant on sterile body sites
  - CSF and other fluids
  - Aspiration from petechiae
  - Assists in the interpretation of culture results

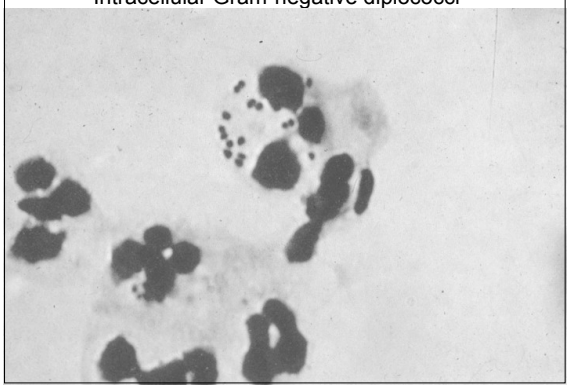






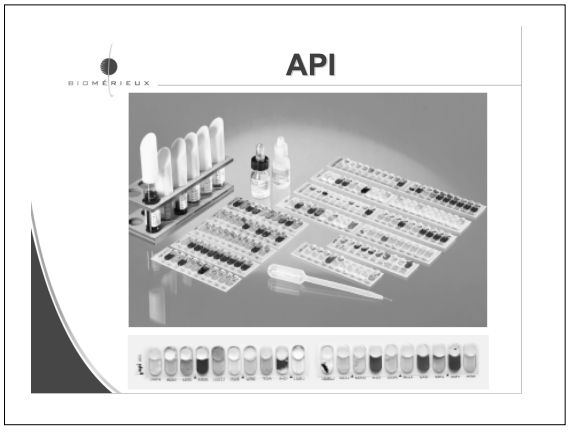


Intracellular Gram-negative diplococci



Biochemical Identification

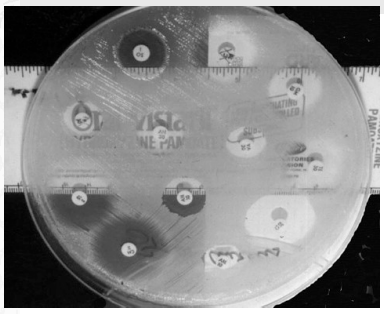
- Use various sugars and substrates to detect ability to ferment, oxidize or use an enzyme.
- Most of this is now automated.



Sensitivity Testing

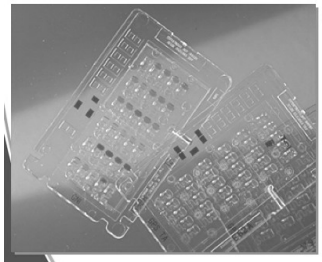
- Basically expose organism to antibiotic and see if it kills the bug!
- Antibiotic impregnated discs
- Microwells to which an organism suspension is added
- 4 - 24 hours

Kirby-Bauer



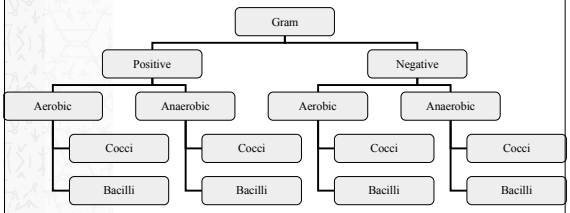
http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/AntibioticDisk.htm

VITEK Card Convenience



- Compact
  - easy to handle
  - space saving
  - low waste volume
- Sealed
  - spill resistant
  - safe disposal
- No reagents to add

Naming Scheme



Gram Positive

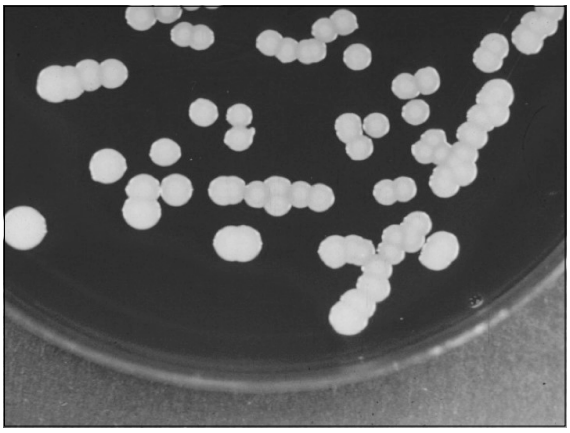
- Aerobic Cocci
  - Staphylococcus, Streptococcus*, Enterococcus* spp.
- Anaerobic Cocci
  - Peptostreptococcus, Peptococcus spp.
- Aerobic Bacilli
  - Bacillus, Listeria, Corynebacterium, Erysipelothrix spp.
- Anaerobic Bacilli
  - Clostridium, Proprionibacterium spp.

Gram Negative

- Aerobic Cocci
  - Neisseria, Moraxella (Branhamella) spp.
- Aerobic Bacilli
  - Haemophilus*, Pseudomonas, Stenotrophomonas spp.
- Facultative Anaerobic
  - Escherichia, Klebsiella, Enterobacter spp.
- Anaerobic
  - Prevotella, Bacteroides spp.

Staphylococci

- Catalase Positive
- Coagulase divides group into *Staph. aureus*, and coagulase negative Staph.
  - Allows *Staph. aureus* to be a great pathogen, as it can cover itself in a coagulated shield of plasma, evading treatment
- All are potential pathogens

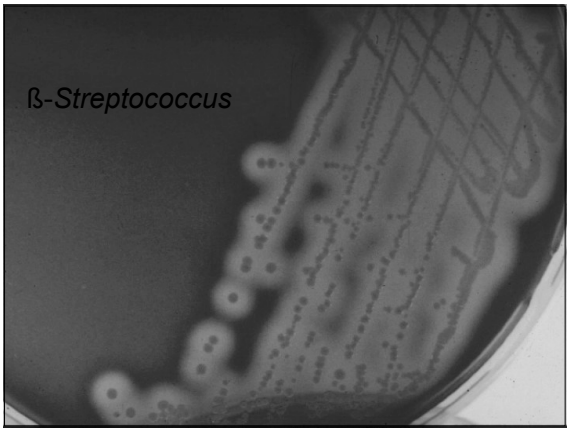


Staphylococci

- *Staph. aureus* can be normal flora
  - Nose, skin, vagina, rectum, feces, mouth
- All CNS are considered skin flora
  - Presence in blood or sterile body fluid needs to be interpreted carefully
  - Collection is very important
    - antiseptics

Streptococci, Enterococci

- Catalase negative
- Streptococci
  - Facultative anaerobic
  - Normal flora – alpha haemolytic
    - Oral flora, viridans streptococci, *Str. pneumoniae**
  - Pathogenic – beta haemolytic
    - Groups A – G potential pathogens



Enterococci

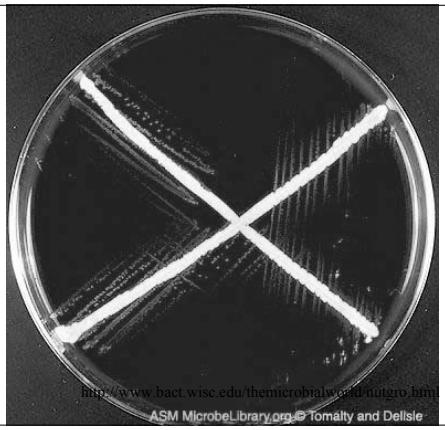
- Gut flora
  - Over half of the bacteria in feces can be Enterococci
- Not very virulent
  - Third leading cause of urinary tract infections
  - Fecally contaminated abscess
  - Resistance
    - VRE

Gram Negatives

- Neisseria
  - N. gonorrhoea, N. meningitidis
    - Pathogenic
  - N. lactamica, N. sicca
    - normal respiratory flora
- Moraxella catarrhalis
  - Many name changes, potential pathogen
    - Neisseria, Branhamella

Haemophilus

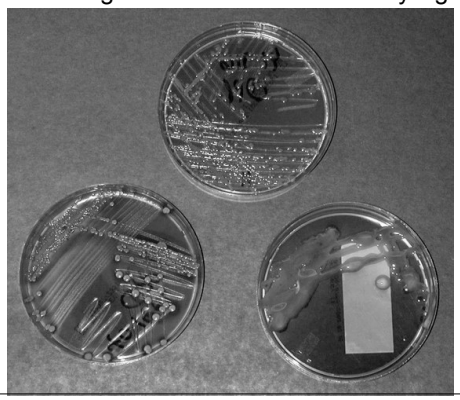
- Coccobacilli
- Normal flora of throat, nose
- "Satellites" around Staph. aureus
- Finicky growth requirements
- Was leading cause of meningitis in children until HIB vaccine developed



Enterobacteriaceae

- Gram negative, facultative AnO_2, rods
- All ferment glucose
- Catalase positive
- Many are gut flora
- Many cause nosocomial infections
- Many are referred to as "coliforms"
  - From the gut
- Grow on MacConkey Agar – selective-differential

Gram-negative bacilli on MacConkey Agar



Enterobacteriaceae

- E. coli, Klebsiella, Citrobacter, Enterobacter, Proteus, Morganella, Providencia, Serratia*,
- Shigella, Salmonella, Yersinia
- Numerous species of each
- Various pathogenic mechanisms
  - Toxins, invasive
- Infection Control – think feces

Other Gram Negatives

- Pseudomonas species
  - Environmental bugs
  - Think "water"
- *Stenotrophomonas maltophilia*
  - Opportunistic
  - Think "sink drain"

Other Gram Negatives

- *Acinetobacter calcoaceticus*
  - anitratus, lwoffii
  - Think oral contamination
  - Many are very resistant to antibiotics

Clostridia

- Anaerobic Gram positive bacilli
- Spore bearing
- *C. perfringens*
  - Gas gangrene
- *C. difficile*
  - Antibiotic associated diarrhea
- *C. tetani*
  - tetanus

Yeasts

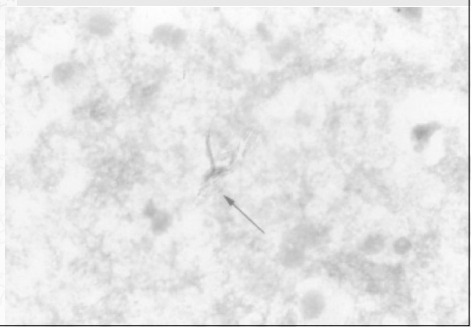
- Single cell organisms
- Numerous species
  - *Candida albicans*
    - Germ tube test
- Opportunistic
  - Normal respiratory flora
- Urinary, vaginal, systemic

Germ Tubes

Mycobacteria

- Do not stain with Gram's stain
- Use carbol fuchsin, heated, then decolorize with HCl and alcohol for 5 minutes
  - Acid fast (AFB)
  - Retain red color
- M. tuberculosis (MTb) [human pathogen]
- M. avium-intracellularae (MAI) [HIV]

AFB



Mycobacteria

- Divide once every 24 hours
  - 2-8 weeks for visible colonies
- Some environmental species
  - M. gordonae, M. marinum
- MOTT: Mycobacterium other than TB

Unusual Organisms?

- "Atypical" respiratory and genital pathogens
- Mycoplasma
  - No cell wall, just cell membrane
  - Very fastidious to grow and stain
    - Not Gram!
- Ureoplasma ureolyticum
- Chlamydia
  - pneumonia, trachomatis

What is a virus?

- Viruses are NOT like bacteria!
- Viruses are NOT little bacteria
- Viruses DO NOT "grow" or divide
- Viruses make copies of themselves using:
  - Tools (enzymes, proteins) they code for
  - Cell machinery

What is a Virus?

- Obligate intracellular parasite
  - "Pirate of the cell"
- NOT a cellular organism
  - No organelles or ribosomes, energy-less
- NOT FREE-LIVING
  - Completely dependent on host cells

Viruses

- Enveloped
  - Easier to kill, less hardy
- Non-enveloped
  - Hardy, resistant to lower concentrations of alcohol
- Both DNA and RNA viruses

Normal Flora

- Positive culture doesn't necessarily mean infection or clinical significance
- Many organisms are part of the "normal flora" of that site
- Most surface and mucosal surfaces are not "sterile" and are loaded with bacteria

Vaginal Flora of Normal Women

Microorganism	%
S. aureus	5 - 10
S. epidermidis	50
Group B Strep	20 - 30
Group A Strep	3
Enterococcus	15
Enterobacteriaceae	15 - 20
Gardnerella	> 50
Lactobacillus	>50
Peptococcus	80
Peptostreptococcus	30
Bacteroides	15 - 35
Fusobacteria	10
Clostridia	5 - 10
Yeast	15 – 30 (30-40 if pregnant)

Normal Respiratory Flora

- Oral anaerobes
  - *Fusobacterium, Bacteroides, Peptostreptococcus*
- *Streptococci* esp. viridans group
- *Neisseria* spp. (incl. meningococcus)
- *Corynebacterium* spp.
- *Haemophilus* spp.

Normal Respiratory Flora

- *S. pneumoniae*
- *H. influenzae*
- *S. pyogenes* (Group A)
- *M. catarrhalis*
- *Enterobacteriaceae*
- Yeast

But these are also important & recognized causes of pneumonia

Never Normal Flora

- *Mycobacterium tuberculosis*
- *Legionella* spp.
- *Brucella* spp.
- etc.

Not Normal But May Still Be Asymptomatic

- *Neisseria gonorrhoeae*
- *Salmonella* spp.
- *Bacillus anthracis*
- etc.

What we Won't Get To!

- Other Anaerobes
- Actinomycetes
  - Norcardia, Rhodococcus, Streptomyces
- Gardnerella
- Brucella, Francisella, Bordatella
- Parasites
- Fungus

Summary

- The names may change but the bugs stay the same
  - Please don't get mad at the lab!
- Not as rapid a science as we would like
- Take a good swab to get good results!

Thanks!

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