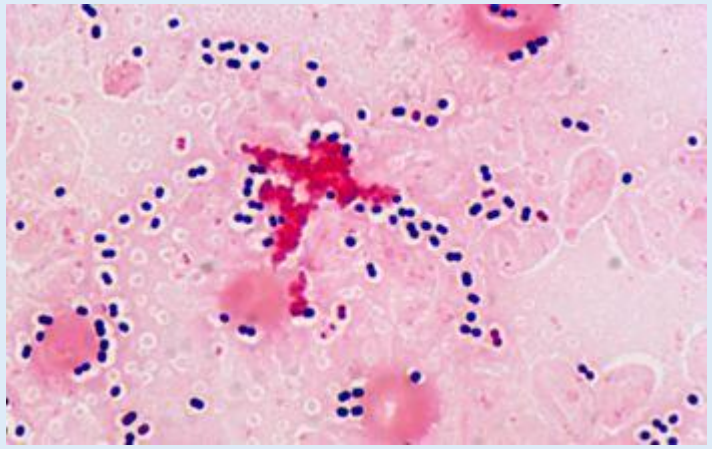
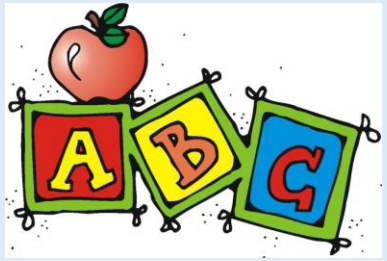


Bacteriology Update 2020

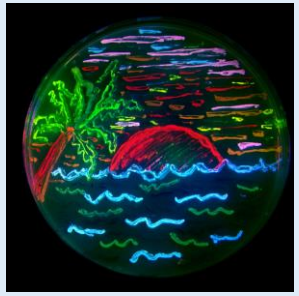
Margie Morgan, PhD, D(ABMM)

Objectives

- Bacteriology is a massive area of study, and it is impossible to review everything!
- This lecture reviews important organisms and laboratory tests, and relevant antimicrobial therapy
- There has been many taxonomy changes over the last few years. The updated classification is noted in parenthesis, such as Micrococcus (Kochuria) species.



Definitions



- **Obligate Aerobe** – require high level of oxygen (20%) to grow
- **Microaerophilic** – grow better with reduced oxygen and elevated carbon dioxide %
- **Obligate Anaerobe** – >30 min of oxygen exposure can be deadly
- **Facultative anaerobes** – grow in both aerobic and anaerobic conditions, most so-named “aerobic” bacteria are actually facultative (ie. E. coli)
- **Aerotolerant** anaerobes– anaerobe is not killed by prolonged exposure to oxygen, but grow best anaerobically, example: *Clostridium tertium*
- **Lag Phase** - >24 hrs old on agar plates, growth is slowing, not appropriate for biochemical or susceptibility testing
- **Stationary phase** – Organisms alive but not replicating, appropriate for transporting specimens

Specimen Collection - Aerobic

Throat / Wound / Abscess

1. Swabs should be polyester fiber or flocked (prickly sponge)
2. Cotton fibers are not optimal, trap bacteria in fibers and potentially toxic
3. Specimen is collected with swab then placed in Stuart's or Amie's transport media (buffered solution with peptones) for transport and storage
4. Transport media preserves viability of the bacteria but does not promote growth of bacteria, provides stasis of numbers prior to plating onto solid media
5. Each transport media has stability limits / usually up to 72 hours

Urine collection (2 methods)

1. Boric acid container / induce organisms into stationary phase for transport
2. Refrigerate urine at 4*C within one hour after collection

   Both methods maintain original colony count and viability of organisms

   Must plate onto agar plates within 24 hours of collection

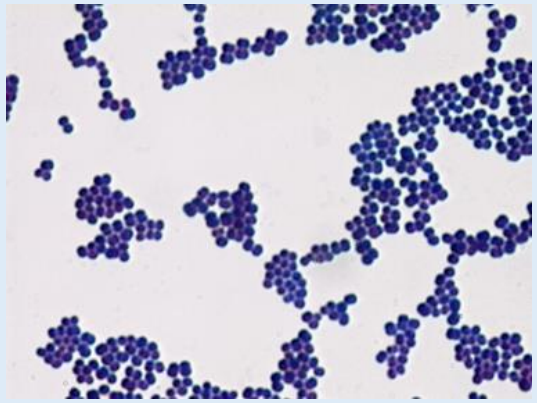
Tissues/Sterile body fluid collection –

1. Adequate volume transported in sterile container

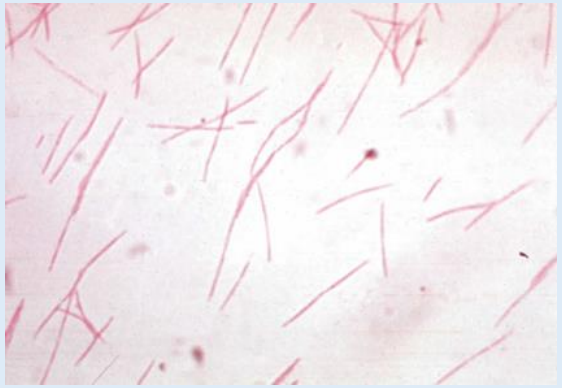
Blood Cultures

- Two most important collection issues
  - **Prevent contamination**: Cleanse collection site using Chlorhexidine
    - National Benchmark: Blood culture contamination rate should be <=3%
  - **Adequate volume** of blood per blood culture bottle
    - Adult blood culture should approach 8-10 ml of blood per bottle

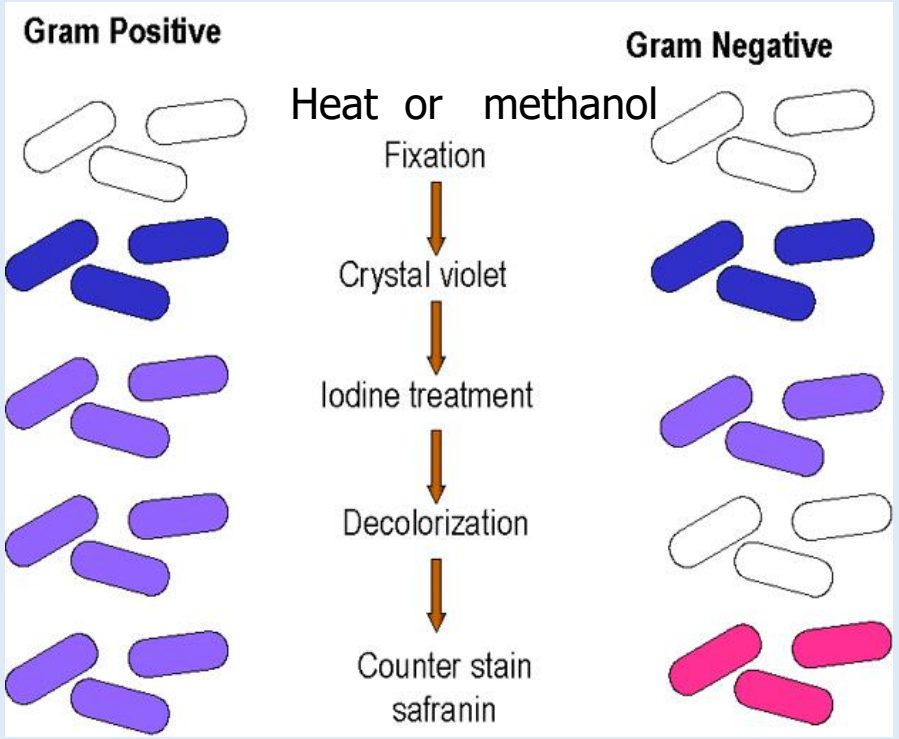
- One Blood culture set consists of two bottles:
  - One aerobe / one anaerobe
  - Incubated at 35*C for 5 days
  - Automated instruments to detect positive cultures is the standard of practice
  - Growth is detected by increasing number of bacteria causing an increase in the amount of C02 in the bottle air space. This triggers a fluorescent indicator to cause an instrument alarm indicating a positive bottle.



Gram stain Procedure



Prepare thin film of specimen on glass microscope slide



Primary stain — 1 minute Rinse

Mordant — 1 minute Rinse

Decolorization — 5-10 seconds Rinse

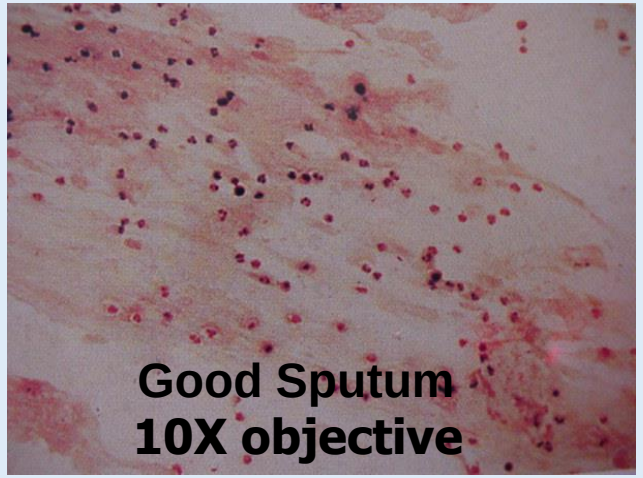
Counter stain — 1 minute Rinse

Gram positive organisms have a high amount of peptidoglycan in the cell wall. Peptidoglycan traps the crystal violet in the cell wall which gives Gram positive organisms a blue color.
Gram stain should document the color (red/blue) and shape of the stained organism.

Gram Stain to Assess Quality of Sputum for Performance of Bacterial Culture

- Expectorated sputum is examined for presence of epithelial cells and neutrophils (WBCs)
  - If <10 epithelial cells/low power field (LPF) is observed and >25 WBCs/LPF (except in leukopenia)
  - Sputum acceptable for bacterial culture

  - If >10 epithelial cells / LPF
    - Sputum is judged to be spit
    - Bacterial culture should not be performed
    - Request made for a “deep cough” specimen



Commonly used agar plated media

Blood agar- 5% sheep's blood agar

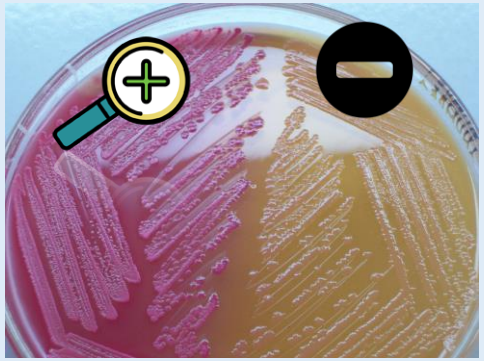
- Used to gauge hemolytic reactions of bacteria (alpha, beta, gamma)
- Culture numerous species of non-fastidious bacteria and yeast

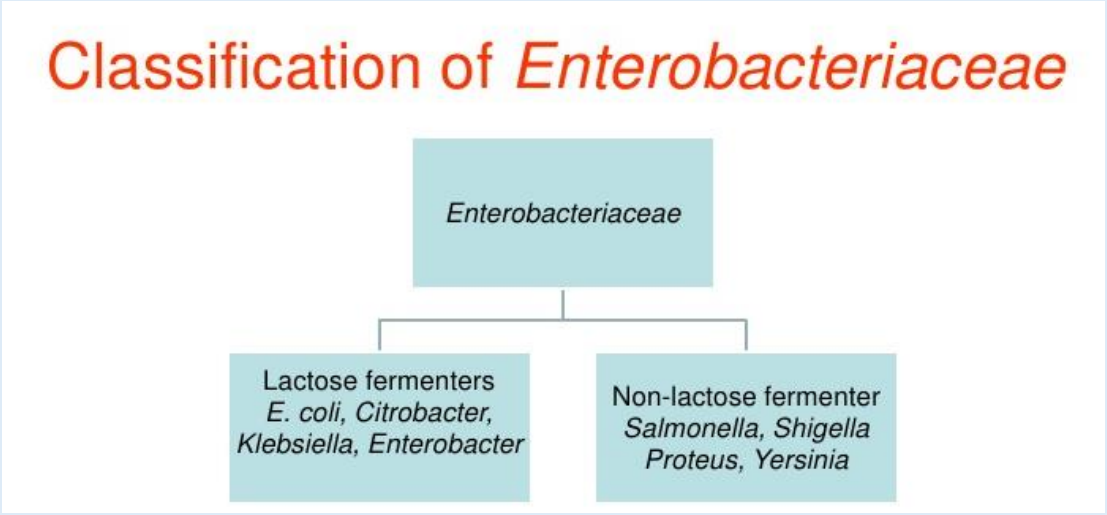
Chocolate agar

- "Caramelized" blood agar – with additional growth enrichment
- Supports the growth of the same organisms as 5% sheep's blood agar plus fastidious bacteria – such as *Haemophilus influenzae and Neisseria gonorrhoeae*
- Incubation in a 5-8% CO2 incubator to nurture for fastidious species

Commonly used agar plated media

- **MacConkey agar** – Selective and differential medium
- Selective for Gram negative rods (GNRs) - supports growth of GNRs, crystal violet in this medium inhibits growth of Gram positive organisms
- Differential for lactose fermentation - fermenting organisms produce a pink colony, neutral red indicator turns colony pink from acid production
    - Lactose fermentation positive = pink
    - Non-lactose fermentation negative = no color
    - Major branchpoint in enteric Gram negative rod identification



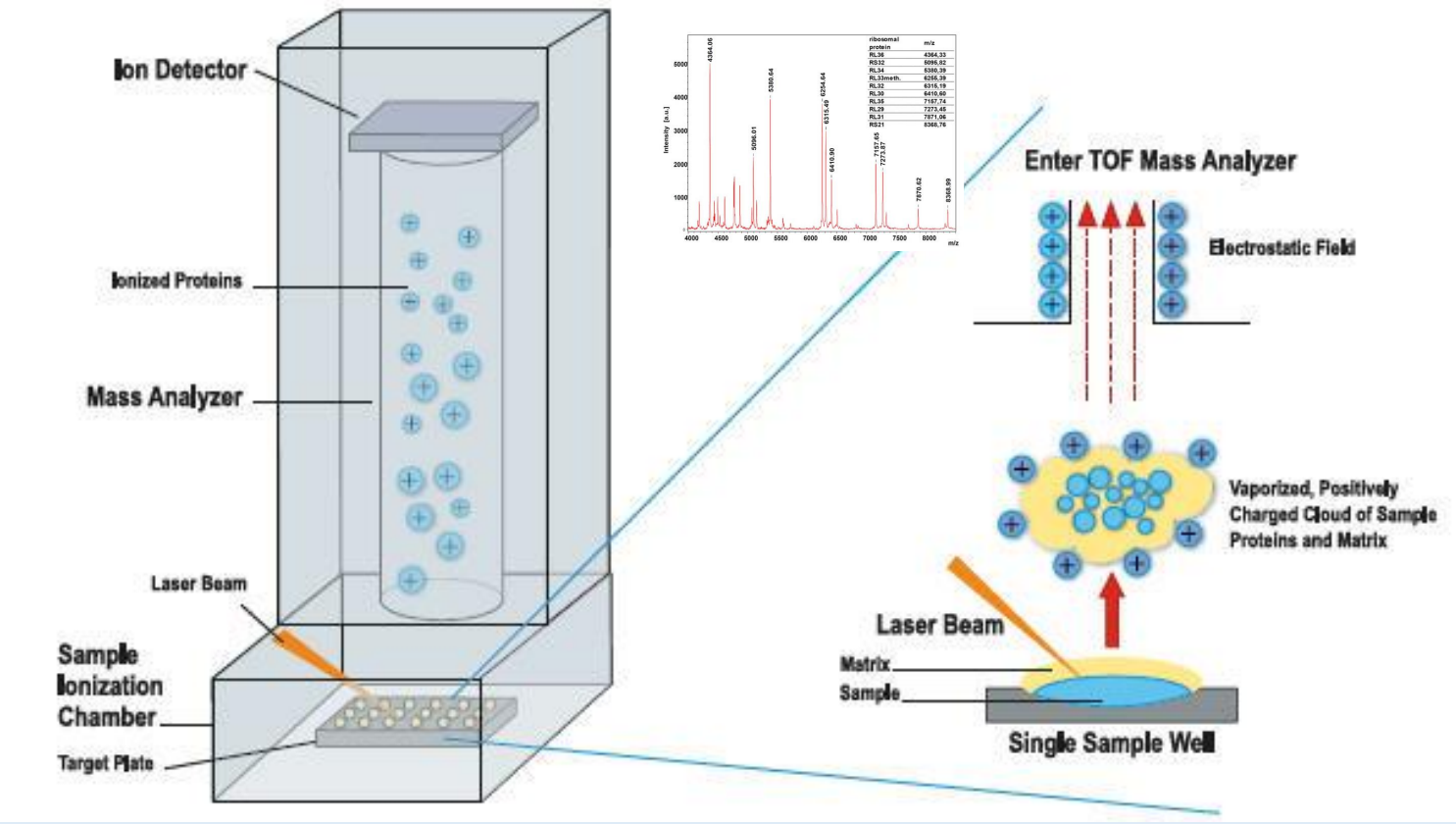


MALDI-TOF Mass Spectrometry / advancement in the identification of bacteria

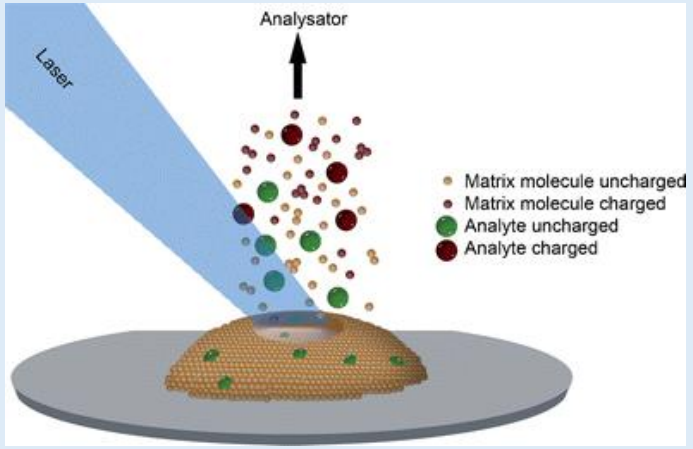
Matrix-Assisted Laser Desorption/Ionization – Time of flight

– Identification by analyzing protein fingerprints of organisms

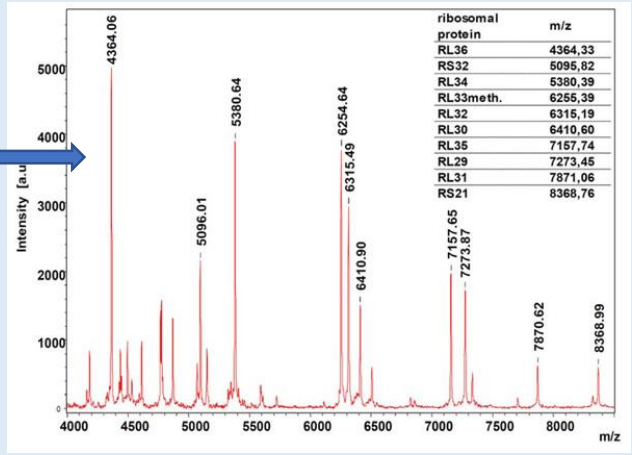
– Replaced many/most biochemical tests for identification of bacteria

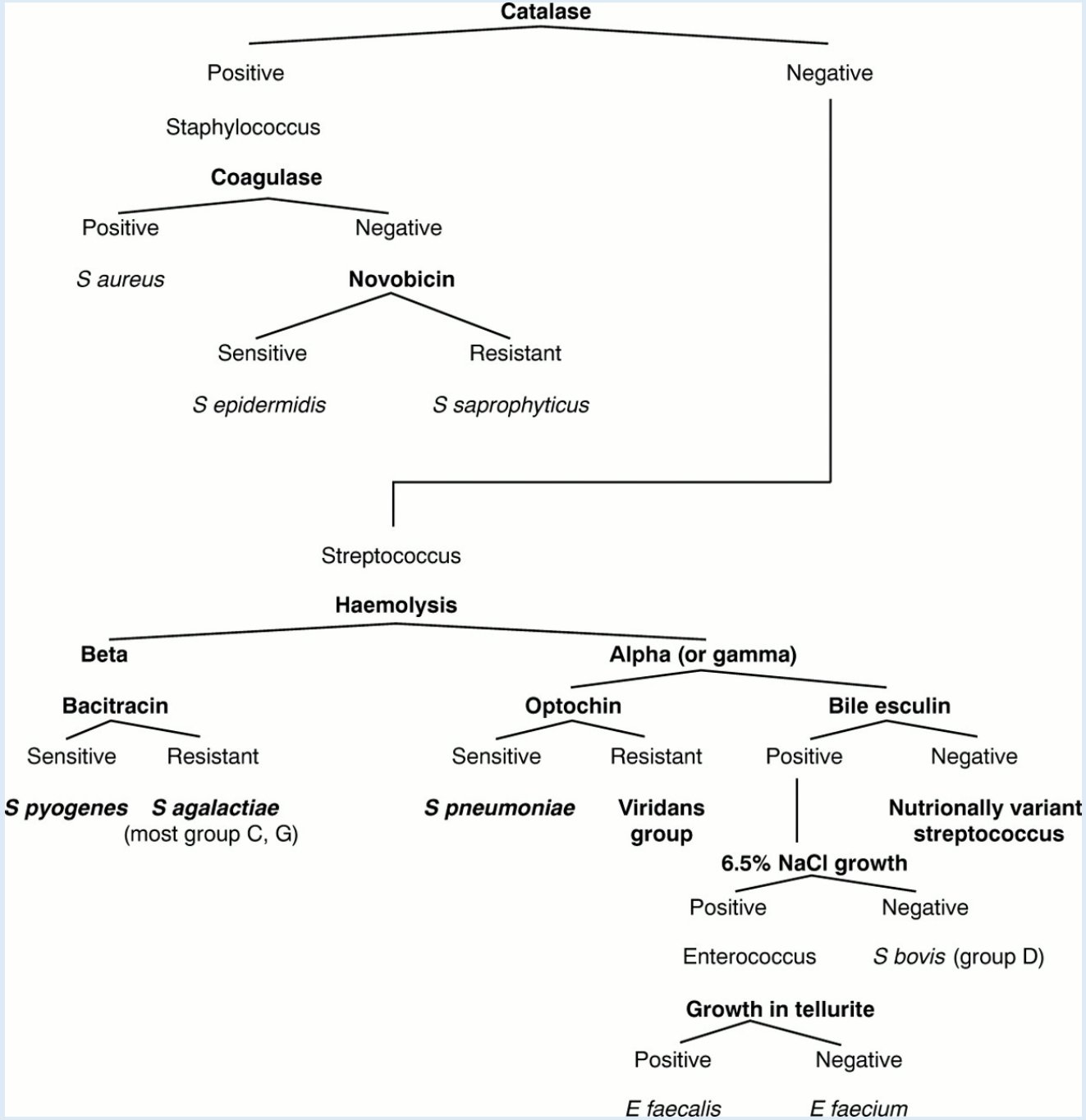


MALDI-TOF Theory



- Laser is fired at target containing matrix and sample
- Laser energy is absorbed by the matrix and converted to heat energy and ionizes the sample.
- Positive ions (proteins) are accelerated through a vacuum tube by an applied electrical field
- The time taken for the proteins to travel through the vacuum tube and reach the detector depends on their mass/charge ratio (m/z) and creates spectrograph.
- Each organism species has a different protein composition, thus giving rise to a specific mass spectrograph.
- The mass spectrograph produced by a sample is then compared with many thousands stored in a spectrograph database to see which one it most closely matches. Thus an identification is achieved.

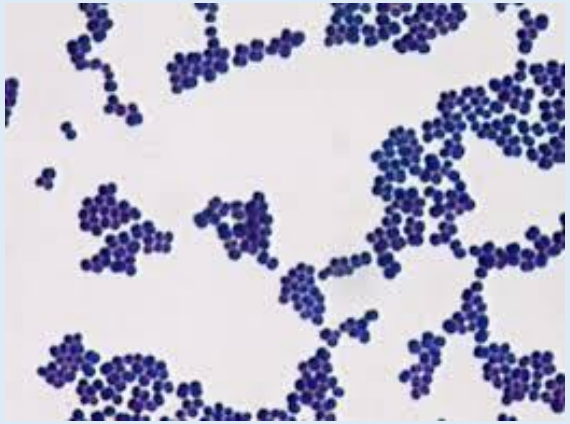




Gram Positive Cocci

Staphylococcus
Streptococcus
Enterococcus

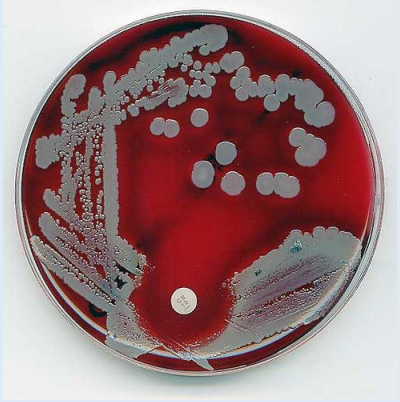
Staphylococcus



Gram positive cocci in clusters – clusters formed due to bound coagulase or “clumping factor”

Catalase enzyme test = **Positive**

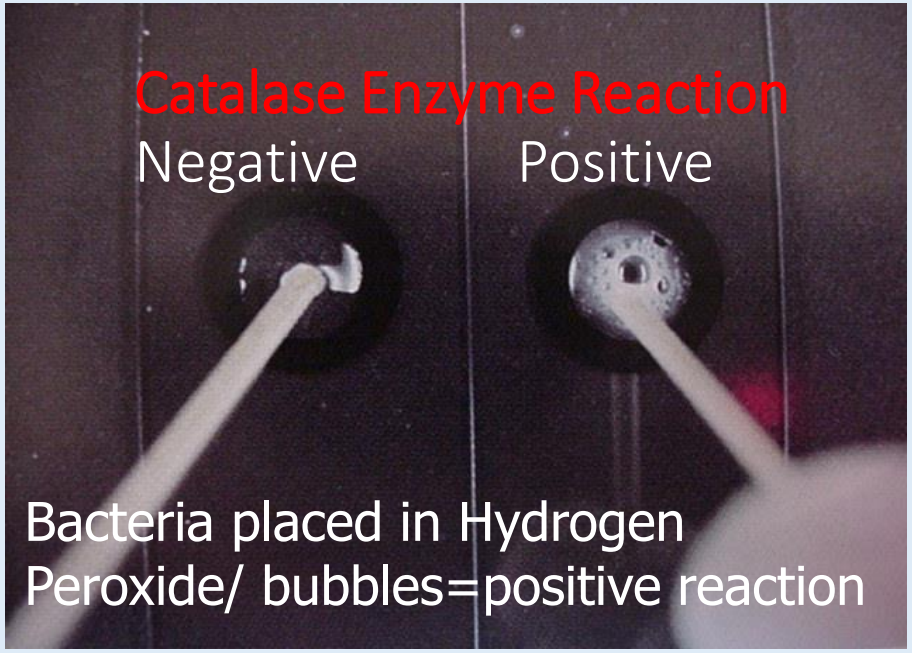
Staphylococcus aureus
Coagulase Enzyme **Positive**

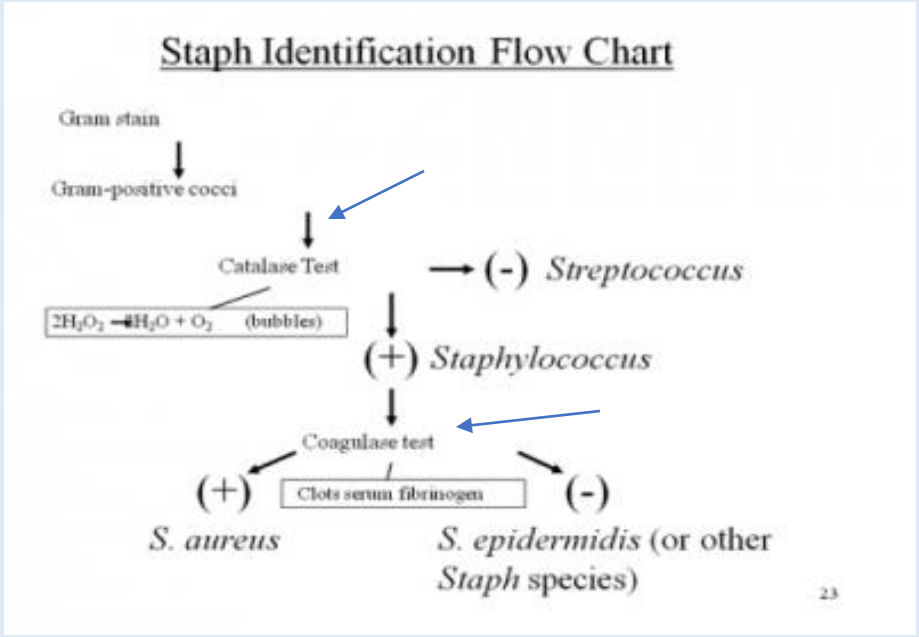


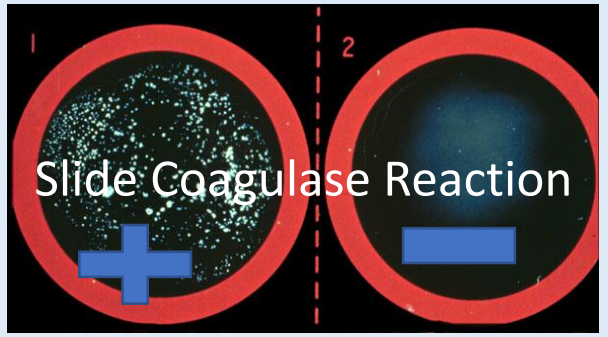
+/- Yellow colony
Beta hemolysis on
Sheep’s blood agar plate

Coagulase Negative Staph
Coagulase Enzyme **Negative**

White colony
Most not hemolytic
on Sheep’s blood agar
plate







Slide Coagulase reaction

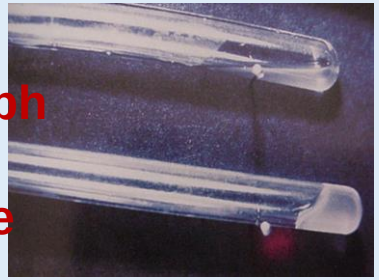
Staphylococcus organism emulsified in rabbit plasma/ mix well/ agglutination is positive reaction
Positive Coagulase enzyme = Staph aureus

Tube Coagulase Reaction

Rabbit plasma inoculated with organism / Incubate at 35˚C / observe for clot at 4 hours and if negative read again at 24 hours

Negative tube coagulase

No clot formed/liquid
Coagulase negative Staph



Positive Tube Coagulase
Clot formed
Staph aureus

Staphylococcus aureus

- Virulence mechanisms:
  - **Protein A – Primary virulence factor,** surface protein, ability to bind immunoglobulin and combat the immune response
  - **Toxins** - act as super antigens, recruit host defense cells that liberate cytokines with systemic effects

- Diseases:
  - Toxic shock syndrome (TSST-1 toxin)
  - Scalded skin syndrome (Exfoliatin (SSS) toxin)
  - Soft tissue infection (Panton valentine leukocidin toxin – PVL)
  - Food poisoning / Enterotoxins – Produce toxins stable to heating at 100*C for 30 minutes

  - Bacteremia
  - Endocarditis
  - Primary cause of adult septic arthritis

Methicillin Resistant Staph aureus (MRSA)

- Methicillin resistance occurs due the presence of altered penicillin binding proteins (PBP2a) from the **mecA** gene.
- Codes for resistance to oxacillin/methicillin /nafcillin resistance (the semisynthetic penicillin antibiotics)
- Cephalosporin antibiotics should be reported as resistant
- Vancomycin becomes an antibiotic of choice.
- Methods to detect MRSA
  - Molecular tests to detect the PBP2a (mecA)
  - Cefoxitin susceptibility testing

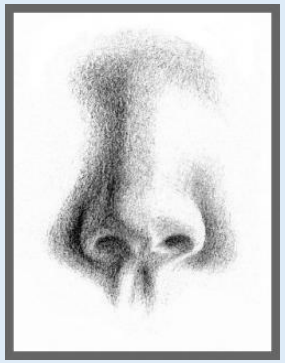
Of note: Emergence of mecC producing MRSA, these are not detected using a mecA based test and best detected using cefoxitin susceptibility test

The “D” Test for Inducible Clindamycin Resistance

- Is *S aureus* susceptible to Clindamycin?
- *S aureus* isolates resistant to Erythromycin possess enzymes capable of inducing Clindamycin resistance in the organism
- Not detected by routine susceptibility testing
- D test – the inhibitory zone around Clindamycin KB disk will be blunted to form a “D” shape, meaning Clindamycin was induced by Erythromycin disk to be resistant – “**INDUCIBLE RESISTANCE”**

D test **positive**
Inducible resistance to clindamycin
Do not use for therapy

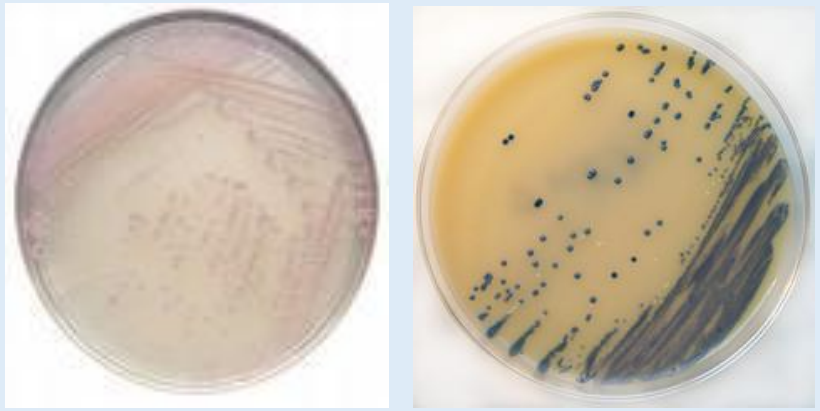
D test **negative**
Susceptible to clindamycin
Clindamycin used for therapy



Methicillin Resistant *Staphylococcus aureus* (MRSA) Surveillance cultures to assist with Hospital Epidemiology

Nares is primary colonization site used for surveillance cultures

Methods for surveillance cultures:



Chromogenic Agar for MRSA

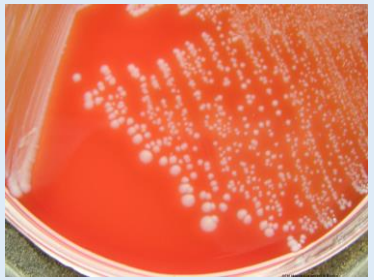
Chromogenic media - selective for MRSA due to the addition of cefoxitin. Differential due to chromogenic substrates that turn a specific color to identify *Staph aureus*

Molecular assays (MA) can also be used to screen nares for MRSA presence. MA increase the sensitivity of detection over culture methods by 5-10% but greatly increase laboratory costs.

Mupirocin therapy for short term elimination of MRSA carriage
Chlorhexidine bathing to decolonize skin

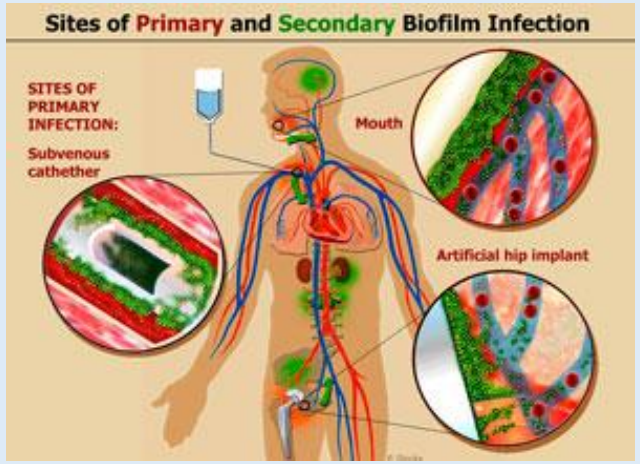
Coagulase Negative Staph (CNS)

@ 15 species infect humans

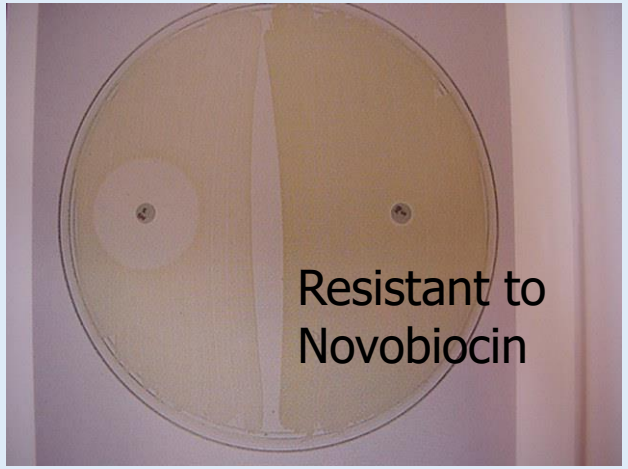


White non-hemolytic colony

- ***Staph epidermidis*** – most common species in humans
  - Common component of normal skin flora
  - Pathogen of opportunity
  - Common cause of catheter related bacteremia, endocarditis, and prosthetic joint infection
  - Pathogenicity from cell adhesion factors that form biofilm on biologics and plastics

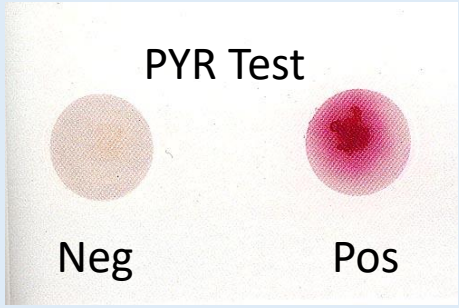


- ***Staph saprophyticus*** –
  - Urinary tract infection in the child-bearing age female
  - This CNS adheres efficiently to epithelial cells
  - Only CNS resistant to Novobiocin (KB disk test)
    - Used as a test of identification

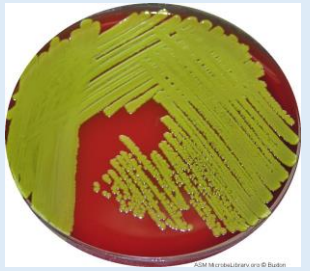


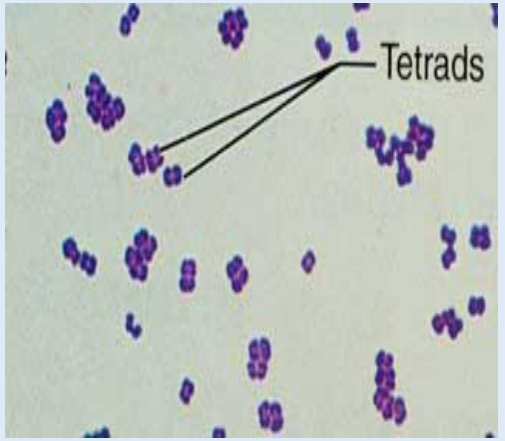
Coagulase Negative Staph (CNS)

- **Staphylococcus lugdunensis**
  - Normal skin flora in humans
  - Pathogen in variety of infections particularly skin and soft tissue infection
  - Biochemical test of note: PYR test is positive = pink
    - PYR = pyrolidonyl arylamidase

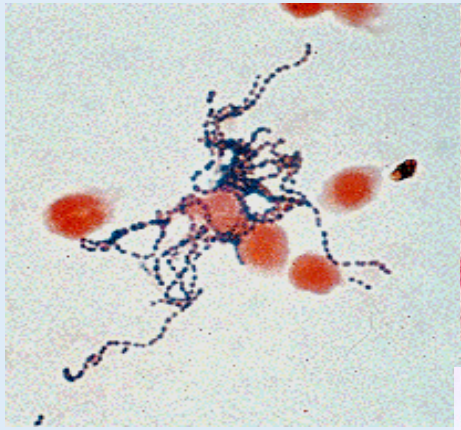


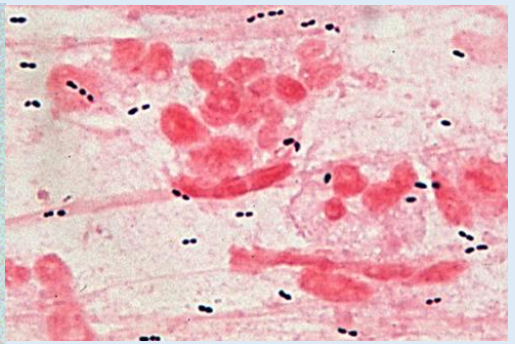
- **Micrococcus (Kocuria) species/ close relative of Staph**
  - Gram positive cocci in tetrads
  - Environmental contaminate/ seldom if ever a pathogen
  - Mustard yellow colony
  - Catalase = positive
  - Coagulase enzyme = negative

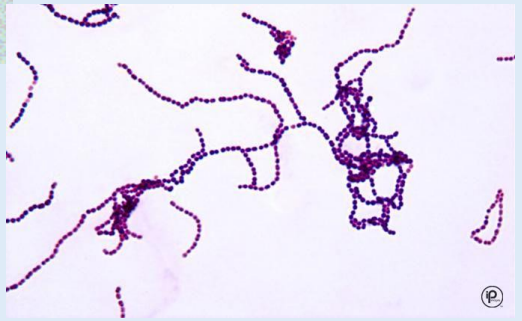




Streptococcus



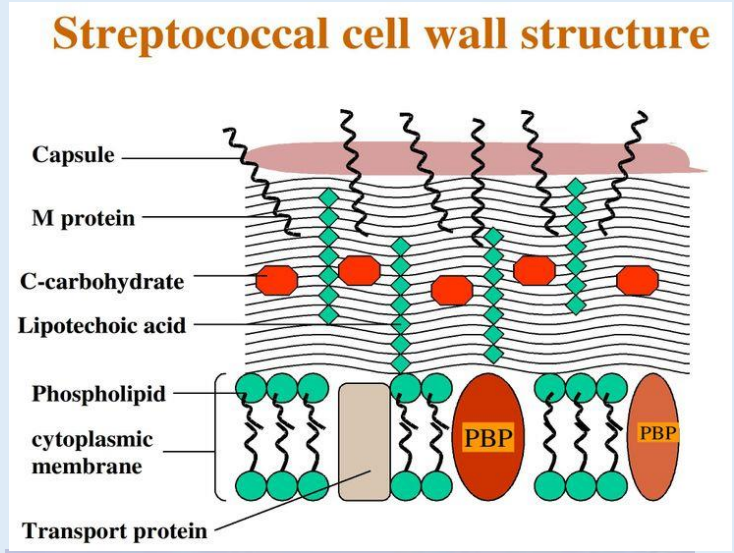


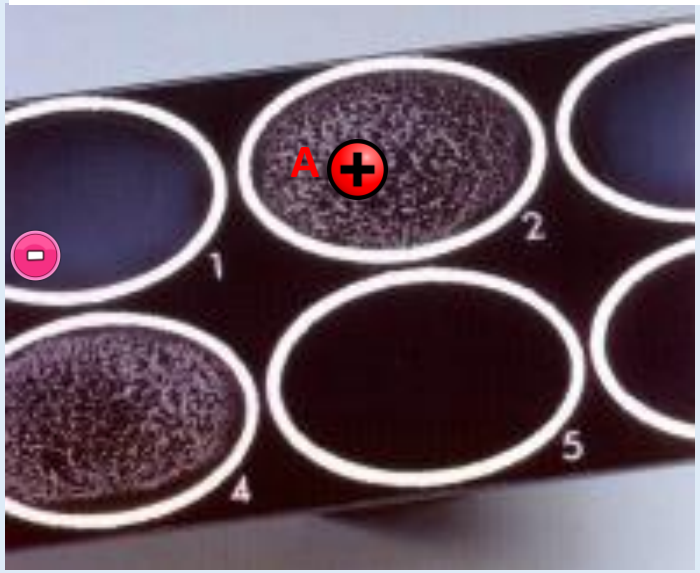


- Gram positive cocci in chains and pairs
- Catalase enzyme = negative
- Three groups based on hemolytic reaction produced when grown on 5% sheep's blood agar
  - **Alpha** – greening of agar, partial hemolysis of RBCs
    - Viridans Streptococcus, *Streptococcus pneumoniae*, *Granulicatella and Abiotrophia*
  - **Beta –** clearing of agar, complete hemolysis of RBCs
    - Beta hemolytic Streptococcus, ie. *Streptococcus pyogenes and Streptococcus agalactiae*
  - **Gamma –** no clearing of agar, intact RBCs
    - *Streptococcus bovis (gallolyticus)*

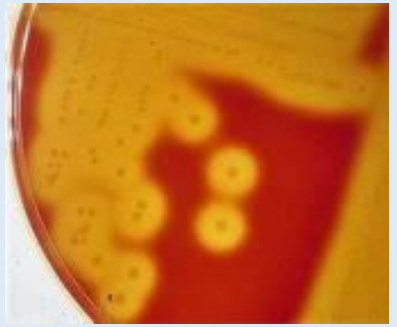
Beta Hemolytic Streptococcus Typing

- Lancefield typing system: Beta hemolytic Streptococcus are grouped (typed) by identifying the “C” carbohydrate (CHO) present in the bacteria cell wall.
- Classifies Beta Streptococcus into separate groups, ie. A, B, C, F, and G, the groups most commonly associated with human infections
- The “C” CHO in the cell wall can be used the in the Lancefield slide agglutination test. It bonds with specific monoclonal antibody for each individual Streptococcus group.
- Shown in picture is a positive test, with monoclonal antibody coated latex beads for group A (Strep pyogenes)

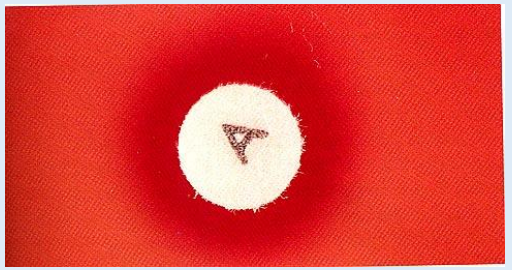


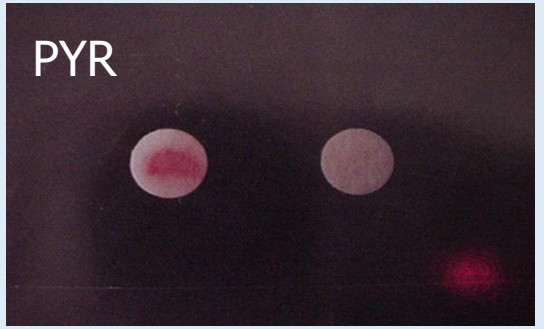


Streptococcus pyogenes



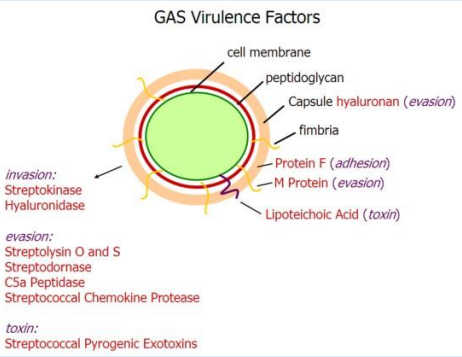
- Group A Streptococcus [GAS] intense beta hemolysis on blood agar
- Biochemical tests used for identification:
  - **Bacitracin** KB sensitivity test – GAS is inhibited by antibiotic Bacitracin (A)
    - Not specific for GAS, inhibition also occurs with Beta hemolytic Streptococcus group C
  - **PYR** (pyrrolidonyl arylmidase) reaction
    - Organism spotted onto moist PYR disk
    - 2 min – room temperature incubation
    - Add cinnamaldehyde reagent
    - **Pink = positive = Streptococcus pyogenes**
      - This test is not exclusive for *Strep pyogenes* – Enterococcus and *Staph lugdunensis* also test positive



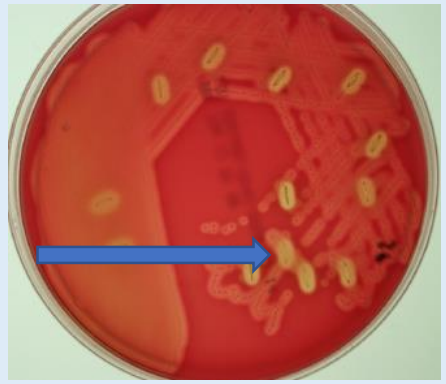


  - **Therapy :** Penicillin, Amoxicillin or Cephalosporin antibiotics
    No resistance reported to these agents

Streptococcus pyogenes primary virulence factors:

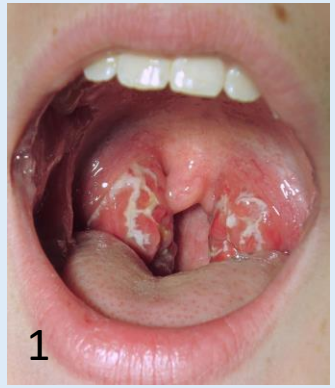


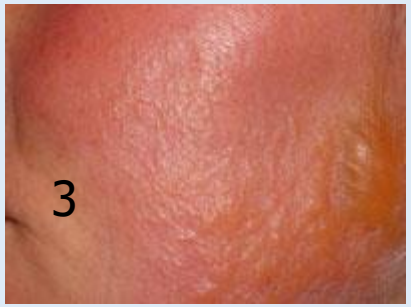
- **M Protein** – prevents phagocytosis
- **Capsule** – hyaluronic capsule prevents phagocytosis
- **Streptolysin O and Streptolysin S toxins**
  - Comprise the ASO titer assay that assists in the diagnosis of Strep pyogenes sequelae of rheumatic fever and GAS glomerulonephritis
  - These toxins lead to evasion from the immune system
  - Toxin activity can be demonstrated on 5% Sheep's blood agar media
    - Streptolysin O toxin is oxygen labile
    - Streptolysin S toxin is oxygen stable
    - When both toxins are present, the stabbed area of the media will demonstrate increased beta hemolysis.

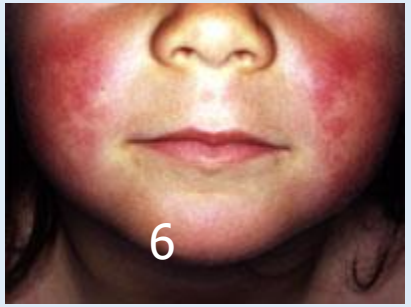


Streptococcus pyogenes / most common **Infections**

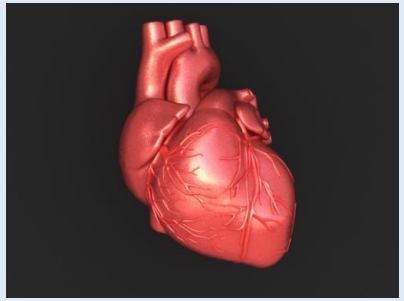
- Pharyngitis (1)
- Impetigo (2)
- Erysipelas (3)
- Cellulitis (4)
- Necrotizing fasciitis (5)
- Puerperal sepsis
- Toxic Shock
- Scarlet fever (6)





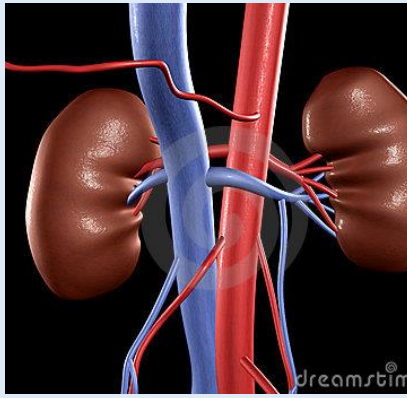


Sequelae of *Strep pyogenes* Infection



Rheumatic fever

- Inadequate treatment of GAS skin or pharyngitis infection
  - Family history, strain of GAS and multiple exposures can more likely evolve into sequelae, occurs 10-30 days post infection
- Usually occurs in children 5 – 15 years
- Pathogenicity due to molecular mimicry: similarity between the proteins of Strep A and human muscle tissue that causes an autoimmune mechanism that leads to confusion. The immune system is then armed to attack heart (heart valves, muscle), joint, and bones
- Usually leads to need for valve replacement surgery

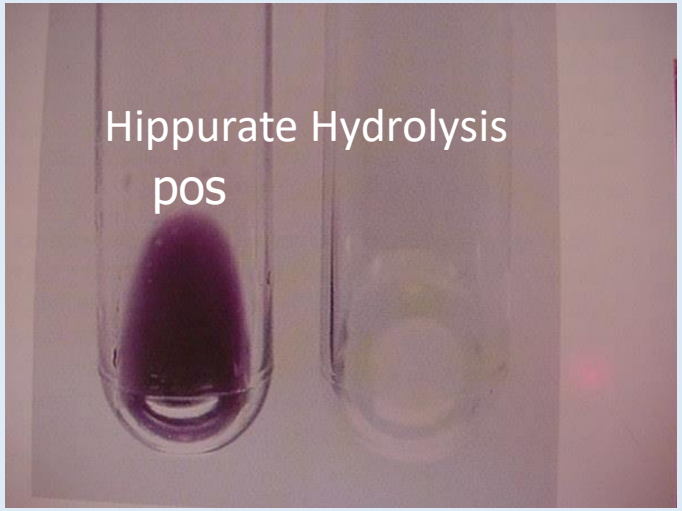


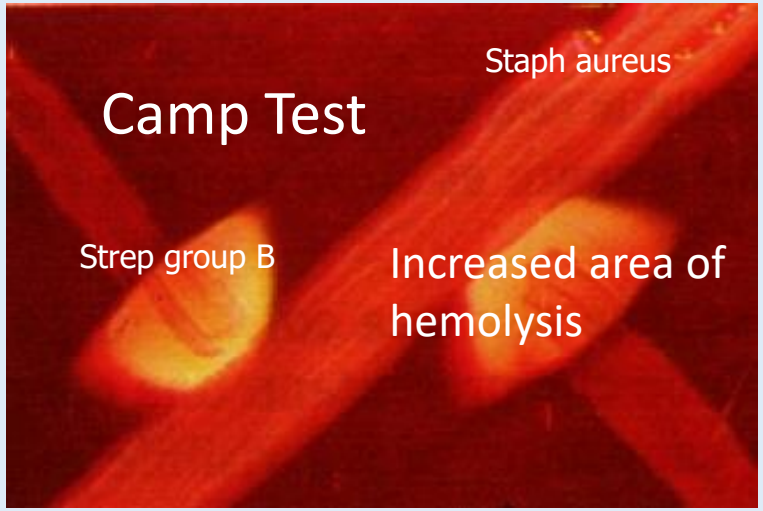
Glomerulonephritis

- Post infection with Nephritogenic strain of *GAS*
- Leads to immune mediated destruction of the renal glomeruli
- Usually resolves without therapy

Streptococcus agalactiae (GBS)

- Biochemical tests:
  - **Camp test** – *Staph aureus* strain that contains Camp factor streaked perpendicular to group B Strep on a 5% sheep's blood agar plate, Incubate 24 hr. and view for intensified arrow shaped hemolysis. Positive test = GBS (see pix)
  - **Hippurate hydrolysis –** used to detect the ability of GBS to hydrolyze the chemical hippurate into glycine and benzoic acid by action of the hippuricase enzyme – 4 hour incubation. Positive test = purple





Streptococcus agalactiae [GBS]

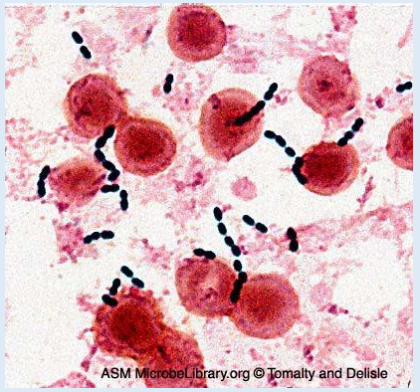
- Pathogen of the **elderly**
  - Bacteremia and urinary tract infection,
  - Acquisition most likely from the intestine

- Pathogen of **neonate**
  - Bacteremia or central nervous system infection
  - In utero or perinatal organism acquisition during birthing process,
    - infection in @ 1/2000 births
  - Early onset infection within 7 days of birth
  - Late onset infection within 8 – 28 days of birth
- Treatment: Penicillin or Cephalosporin (3rd generation)

Streptococcus agalactiae (GBS)

- Pregnant women colonized (>=25%) in the cervix and/or rectal area with GBS
- All pregnant should be screened at 35 – 37 weeks of pregnancy for GBS (Regulation/standard of practice)
  - **Enrichment** methods for GBS screening are standard of practice
    - Cervix and rectal swab incubated in an enrichment broth for 18 hours at 35 ˚C then cultured onto 5% sheep's blood agar.
    - Enrichment broth can also be used to increase sensitivity in molecular testing methods
  - Ampicillin drug of choice for prophylaxis of pregnant women testing positive for GBS
  - Susceptibility testing for alternative therapies for GBS must be performed in the penicillin allergic patient

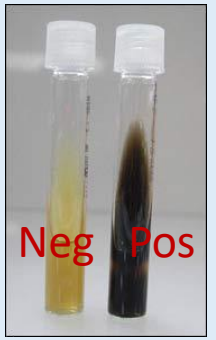
Enterococcus

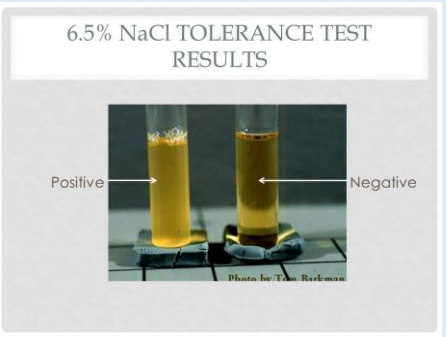


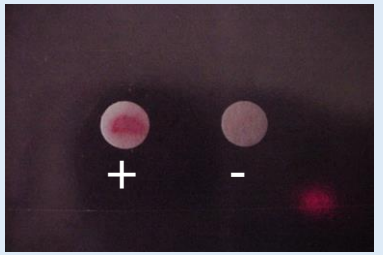
- Most common species
  - *E. faecium* and *E. faecalis*
- No defined virulence factors
- Gamma hemolytic
- Gram positive cocci in pairs and short chains

- Biochemical tests:
  - Bile esculin agar = grows in presence of bile & reduces esculin to esculetin to produce black color
  - 6.5% NaCl tolerance = grows in presence of NaCl
  - PYR = positive

  - *E. faecium* = arabinose fermentation positive
  - *E. faecalis* = arabinose fermentation negative







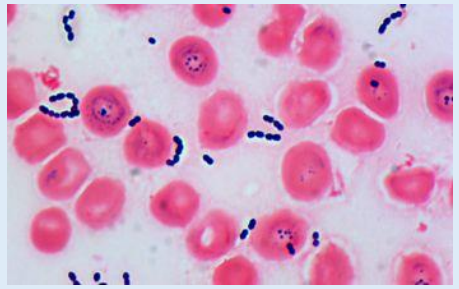
PYR

Enterococcus

- Pathogen of opportunity
- Normal human intestinal normal flora
- Infections include UTI, bacteremia, and abdominal abscess
- Antimicrobial therapy:
  - Natural resistance to cephalosporin antibiotics
  - Ampicillin plus Aminoglycoside can be synergistic for therapy in cases of endocarditis
  - Vancomycin is an antibiotic of choice

- **Unique susceptibility issues**
  - Acquired resistance to vancomycin known as “vancomycin resistant enterococcus” or VRE. Resistance is due to acquisition of genetic material:
    - Van A resistance gene = *E. faecium*
    - Van B resistance gene = *E. faecalis*

Streptococcus bovis (gallolyticus)



- *Streptococcus gallolyticus* ssp. gallolyticus (S. bovis biotype 1)

  Isolation from blood culture is associated with colon cancer (73%)

- *Streptococcus gallolyticus* ssp. pasteurianus (S. bovis biotype 2)

  Isolate from CSF in neonatal meningitis

- Gamma hemolytic, Gram positive cocci in pairs and short chains
- Biochemical reactions:

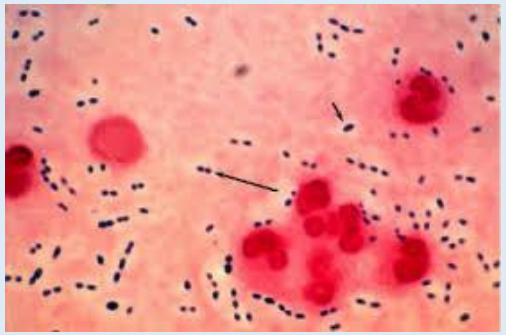
  Bile esculin slant positive
  6.5% NaCl no growth
  PYR reaction negative

  Susceptible to Penicillin

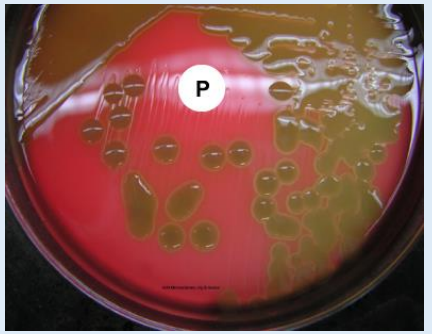
Bile Esculin Positive

6.5% No Growth	6.5% Growth
PYR Negative	PYR Positive
Strep gallolyticus	Enterococcus

Streptococcus pneumoniae



- Alpha hemolytic
- Gram positive bullet (lancet) shaped cocci in pairs
- **Polysaccharide capsule** = virulence factor / antiphagocytic
- Mucoid colony due to increasing amount of capsule
- Identification:
- **Bile soluble** – colonies dissolve in sodium deoxycholate (bile)



Inhibited by **Optochin –** ethyl ethyl hydrocupreine hydrochloride

Zone of inhibition must be >=14 mm

Streptococcus pneumoniae

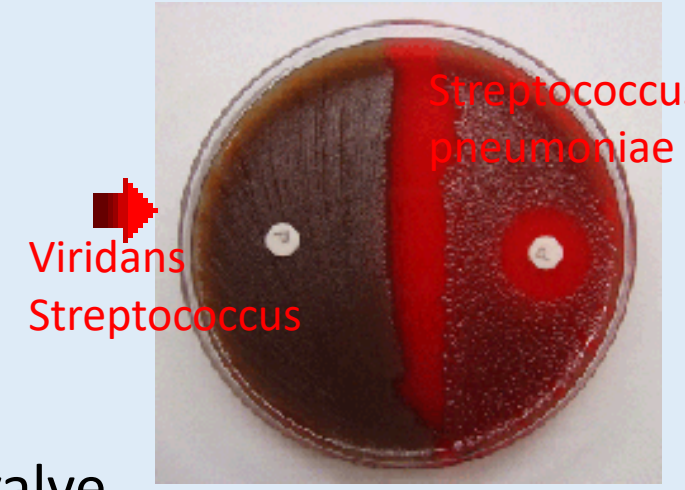
- Normal inhabitant of the upper respiratory tract
- Infections: Upper and Lower respiratory tract infection (Lobar pneumonia), Sepsis, Meningitis, middle ear, ocular, sinus
- Asplenic and immune suppressed patients particularly at risk
- 13 valent pneumococcal conjugate vaccine aids in preventing invasive infections – those at risk need vaccination
- Susceptibility issues:
  - Acquired Resistance to Penicillin due to Penicillin binding proteins
  - If susceptible,1st line therapies include Penicillin or 3rd generation Cephalosporin (Ceftriaxone)

Viridans Streptococcus

- Several species of viridans group Streptococcus are NF in mouth and upper respiratory tract. Most common species:

  S. mutans *S. salivarius* *S. sanguis* *S. mitis*

- Bile esculin negative
- Bile solubility negative
- Optochin resistant (zone size <=13 mm)
- Cause 30 – 40% cases of sub acute endocarditis on native valve usually due to bad dentition
- Cause abscess and various infections throughout the body in the immune suppressed host
- Variable susceptibility patterns can have elevated MICs to Penicillin so usual therapy is 3rd generation cephalosporin.

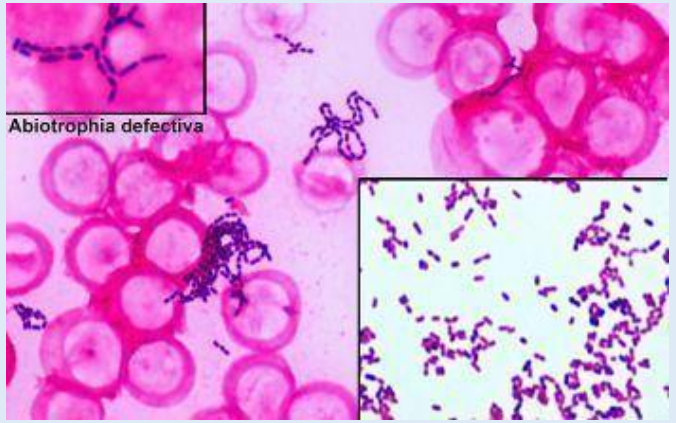


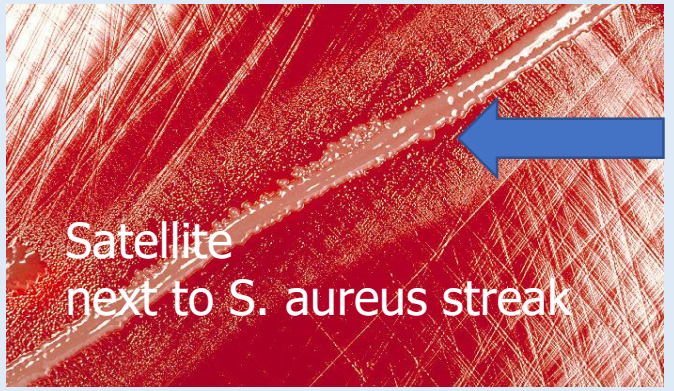
Viridans Streptococcus unique species

- **Streptococcus anginosis group:**
  - *S. anginosus S. constellatus S. intermedius*
- Normal flora in human mouth
- More virulent than “normal” viridans Streptococcus, due to capsule
- Grows best when incubated in 5 – 10% CO^2 incubation (microaerophilic)
  - Butterscotch odor to colony
- Cause deep tissue abscess, bacteremia, endocarditis, and intra abdominal infection
- Variable susceptibilities – so best to do susceptibility testing, always susceptible to vancomycin

Nutritionally Variant Streptococcus

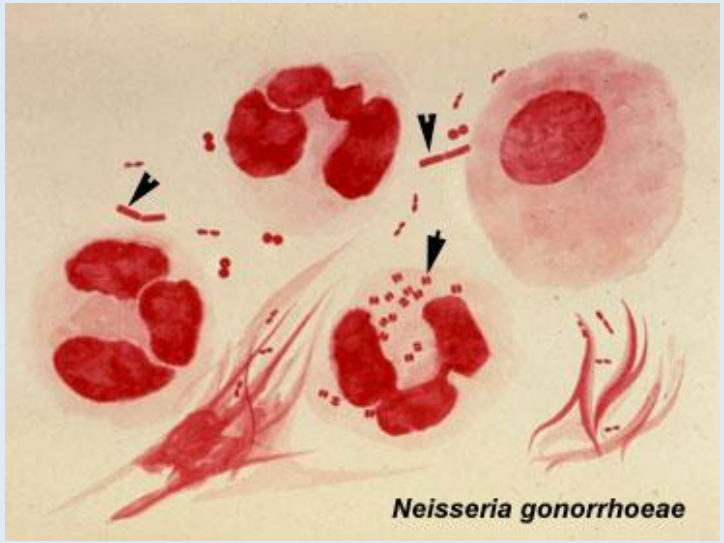
- **Vitamin B6 (pyridoxal) deficient** –
  - Will not grow on agar medium without B6 supplementation
- Will grow in blood culture bottle due to vitamin B6 in patient's blood
  - Will not grow on 5% Sheep's blood agar plate
  - Will grow with Staph aureus streak that supplies vitamin B6
  - MALDI-TOF can supply definitive identifcation
- Two genera:
  - *Abiotrophia defectiva*
  - *Granulicatella adiacens*
- Bacteremia and Endocarditis –
  - More destructive to valve than "regular" viridans Streptococcus
  - Higher MIC's to Penicillin, susceptible to 3rd generation Cephalosporins.
  - Combination therapy: Penicillin and Gentamicin





Opportunistic Gram positive cocci

- ***Aerococcus ureae*** – Gram positive cocci in pairs and clusters
  - Alpha hemolytic on blood agar , difficult to identify, often confused with viridans Streptococcus
  - Urinary tract pathogen
- ***Rothia mucilaginosa*** – Gram positive cocco-baccilli
  - Neutropenia and gut problems predispose to infection
  - Normal flora in the oral cavity and upper respiratory tract
  - Pathogen in dental caries and periodontal disease
  - Bacteremia with endocarditis
- ***Gemella morbillorum–***
  - Easily over decolorized, Gram positive in pairs – requires CO2 to grow
  - Normal flora in oral cavity
  - Bacteremia with endocarditis
- ***Leuconostoc mesenteroides–*** Gram positive cocci in chains
  - Intrinsic resistance to vancomycin
  - Bile esculin = negative
  - Bacteremia in immune suppressed
  - Watch out! Do not confuse with vancomycin resistant enterococcus (VRE)

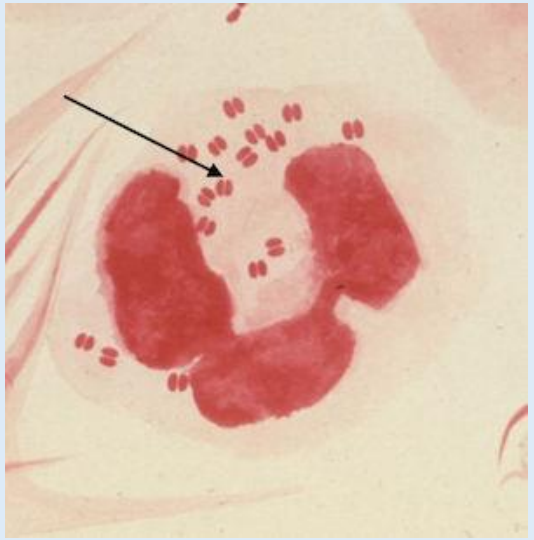


Gram Negative Cocci

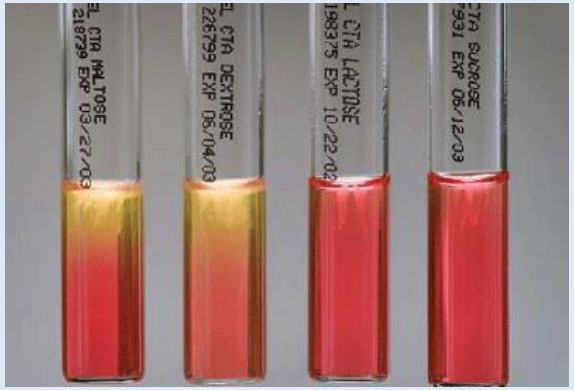
Neisseria species

Moraxella catarrhalis

Gram Negative Cocci

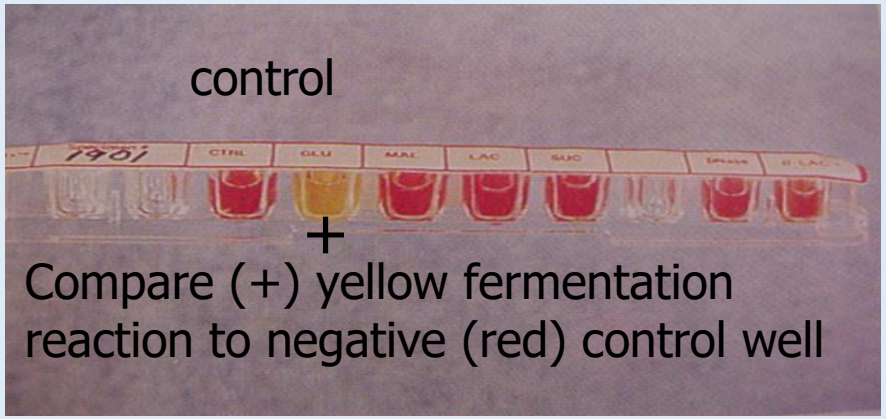


- *Neisseria species and Moraxella catarrhalis*
  - Small kidney bean shaped cocci in pairs
- Oxidase enzyme positive
- CTA (Cysteine Trypticase Agar) carbohydrate fermentation tests are an older method to identify cultured organisms
  - N. gonnorheae Gluc + Mal - Lac - Suc -
  - N. meningitidis **Gluc +** **Mal** + Lac - Suc -
  - N. lactamica Gluc + Mal + **Lac+** Suc-
  - M. catarrhalis Gluc - Mal - Lac - Suc -



- *N. gonorrhoeae* will NOT grow on 5% Sheep's blood agar
- *N. meningitidis* will grow on 5% Sheep's blood agar

CHO Fermentation Reactions

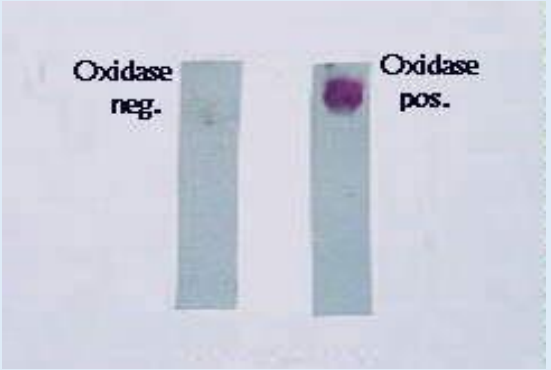


Growth on Chocolate Agar
Oxidase Enzyme Positive

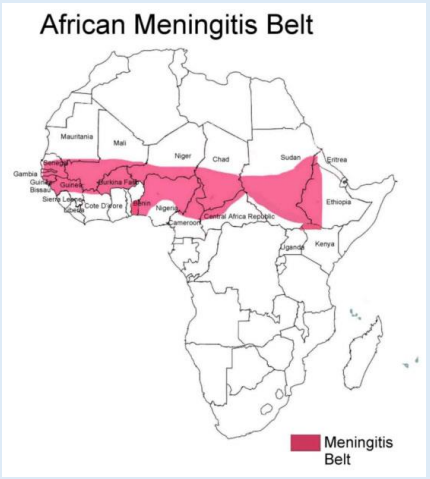
Glucose +
N. gonorrhoeae
No growth BAP

Glucose+/Maltose +
N. meningitidis
Grows on BAP

Oxidase enzyme spot test:
Detects production of enzyme cytochrome oxidase
Add reagent N,N trimethyl-p-phenylenediamine dihydrochloride to filter paper with organism smear
positive = blue to purple color



Neisseria meningitidis



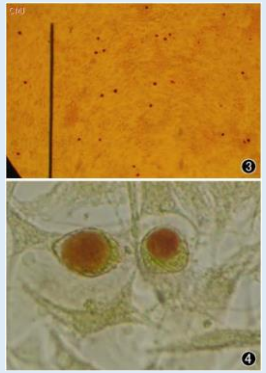
- Meningitis, usually occurring in children and young adults
  - Hallmark - petechiae (organisms crowd into capillaries) leads to tissue necrosis and disseminated intravascular coagulation(DIC) from production of endotoxin)
  - Infection can be rapidly fatal (<24hrs)
- Colonization in nasopharynx (10-20%)
- African meningitis belt – highest prevalence in world
- Capsular polysaccharide is primary virulence factor
- *N. meningitidis* serotypes A,B,C Y and W, most common
- Complement deficiencies in factors 7,8,and 9, Eculizumab, asplenia, and HIV predispose to infection
- Adrenal necrosis known as Waterhouse Friderichsen syndrome
- Immunization at ages 2m, 12 yr, 16 yr, and in HIV to prevent

Neisseria gonnorrhoeae

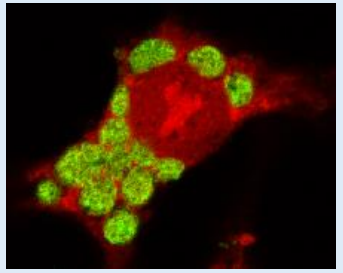
- Sexually transmitted infection: urethrae, endocervix, ocular, rectal, oropharynx, septic arthritis
- 10-20 % female ascend to PID but only 0.5% disseminate into bloodstream
- Gram stain of urethral discharge useful for male diagnosis
- Gram stain of cervix can be problematic due to normal flora look alike organisms, such as *Acinetobacter* species
- Culture: charcoal containing swabs at room temperature
  - Primary reason to culture is for susceptibility testing
- Media: Selective Thayer Martin or Martin Lewis agar, chocolate agars with increased nutrition and antibiotic trimethoprim
- Beta lactamase enzyme and Chromosomal resistance mechanisms
- Therapy: Ceftriaxone + Azithromycin or Doxycycline, combination therapy to prevent development of resistance

Molecular testing for *Neisseria gonnorhoeae*

- Molecular amplification methods are the standard of practice and combo testing for *Chlamydia trachomatis* is the norm due to high % of co-infections
  - Urine, cervix/vaginal, throat and rectal – sites most often tested
  - Molecular testing sensitive and specific @ 96%/99%
    - Female: most sensitive specimen is cervix
      - Urine <=10–15% less sensitive than cervix
    - Male: Urine has become the standard specimen
- The ancient way for diagnosing Chlamydia trachomatis infection:



C. trachomatis culture - Iodine staining of inclusions in McCoy cell culture



Fluorescent antibody stain of *C. trachomatis* infected cell – positive cell contains green staining Elementary bodies

Moraxella catarrhalis

- Colonizes the upper respiratory tract in children
- Infections: Pneumonia (COPD), sinusitis, Primary cause of otitis media in young children
- Gram stain of sputum can be helpful in diagnosis of pneumonia (PMNs with Gram negative diplo-cocci)
- Hockey puck colony – able to push colony across the agar surface without disruption
- Biochemical Tests:
  - Oxidase enzyme positive
  - DNA'ase enzyme positive
  - Produces a beta lactamase enzyme
  - Therapy: Augmentin or 3rd generation Cephalosporin