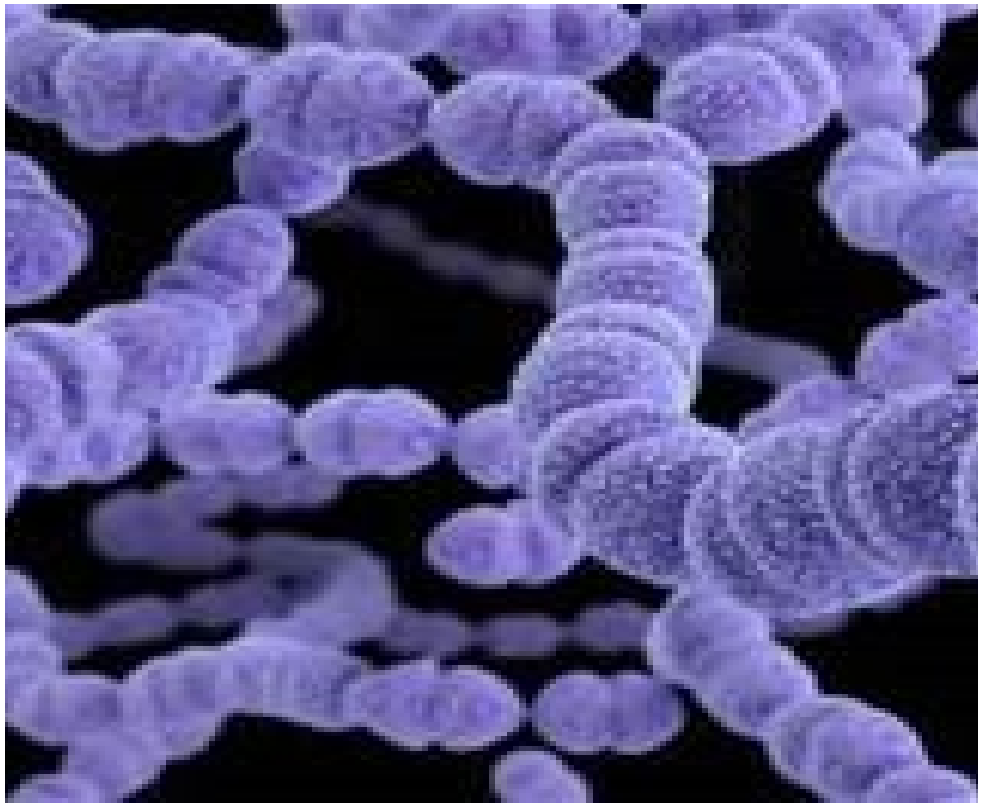
Streptococcus, Enterococcus & Other Catalase Negative Gram-Positive Cocci



Streptococcus and Enterococcus species

- Members of the Streptococcaceae family
- Catalase Negative
- *(remember: Staph is catalase positive)*
- GPC in pairs and or chains
- Facultative anaerobes or aerotolerant anaerobes
- Colonies are usually small + pinpoint
- All Streptococci are LAP positive
  - Hemolysis pattern varies
- Most are part of normal flora and considered opportunistic

Some strep are almost always pathogens:

i. Strep pyogenes
ii. Strep pneumonia
iii. Enterococcus

Streptococci can be classified by:

- Hemolytic pattern on blood agar
  - Alpha, beta, or gamma hemolysis
- Physiologic characteristics 4 groupings (old nomenclature)
  1. Pyogenic streptococci – mostly β-hemolytic & constitute most of the Lancefield group
  2. Lactococcus - mostly non hemolytic, Lancefield group N antigen, Often found in dairy products
  3. Enterococcus - mostly Gamma or alpha hemolytic, PYR positive, Skin and urinary tract pathogen
  4. Viridans streptococci - mostly C carbohydrate antigen negative, Not part of the Lancefield group
     - some may have A, C, F, G or N antigen
     - α-hemolytic (green), beta-hemolytic or non-hemolytic,

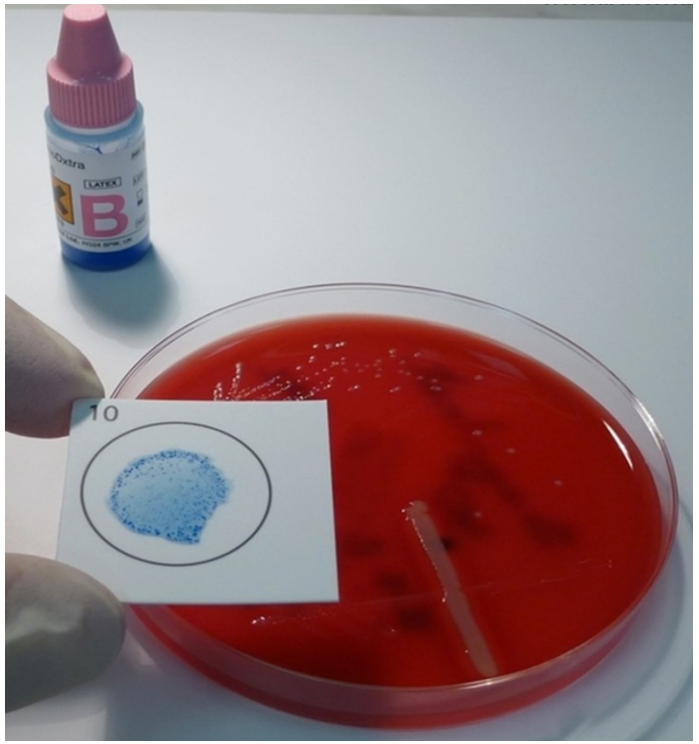
1. Serologic grouping or Lancefield classification
   1. proteins and carbohydrates that can be detected on the surface of streptococci

Lancefield Grouping – Streptococcus

- Group A - Streptococcus pyogenes
- Group B - Streptococcus agalactiae, Streptococcus halichoeri[5]
- Group C - Streptococcus equisimilis, Streptococcus equi, Streptococcus zooepidemicus, Streptococcus dysgalactiae
- Group D - Enterococcus faecalis, Enterococcus faecium, Enterococcus durans and Streptococcus bovis
- Group F, G & L - Streptococcus anginosus
- Group H - Streptococcus sanguis
- Group K - Streptococcus salivarius
- Group L - Streptococcus dysgalactiae
- Group M & O - Streptococcus mitis
- Group R & S - Streptococcus suis

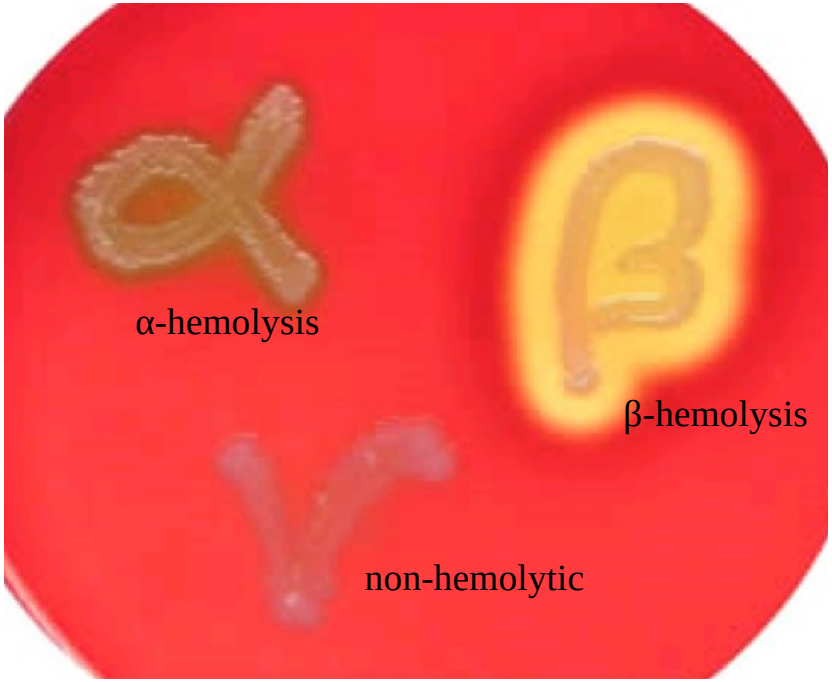
Cell Wall structure

- Most Streptococci (except viridans) haveC carbohydrate
  - polysaccharides in the cell wall for serologic classification
- This is called the Lancefield group—Strep A, B, C, D, F, G, N
  - Used for β-hemolytic Streptococci



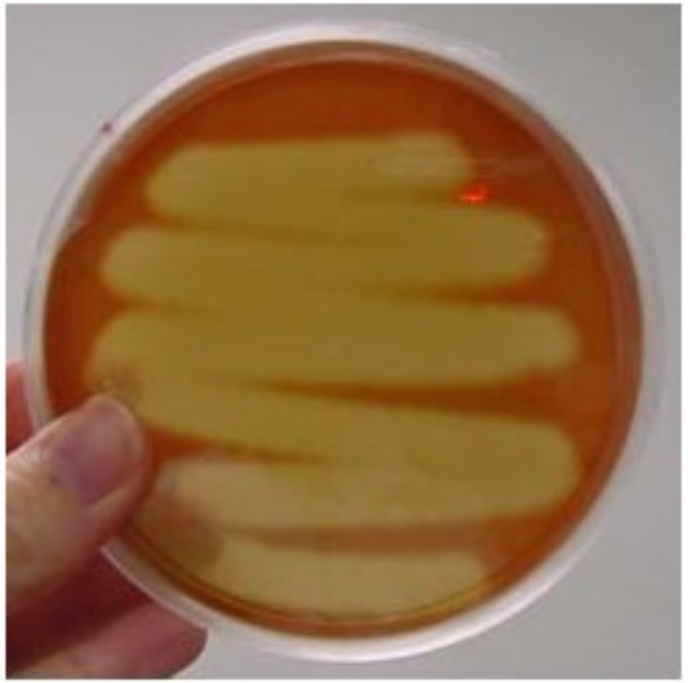
Hemolysis

- Exotoxins damage intact red blood cells.
- Clinically significant isolates are separated into two groups:
  - Pyogenic streptococci (most are β-hemolytic)
    - Group A, B, C, F, G
  - Non-pyogenic (Non-β-hemolytic)
    - Alpha and gamma



β-hemolysis

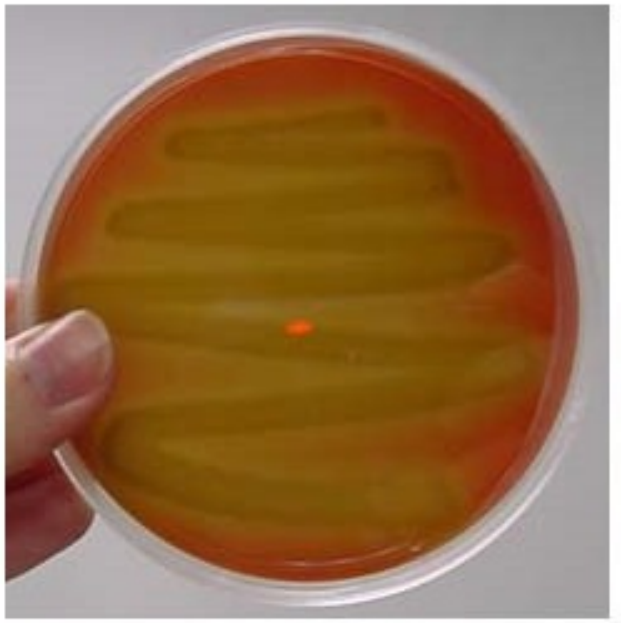
- β-hemolysis
  - complete lysis of RBCs in the agar
  - Results in clearing of area around colonies



Beta Hemolysis

α-hemolysis

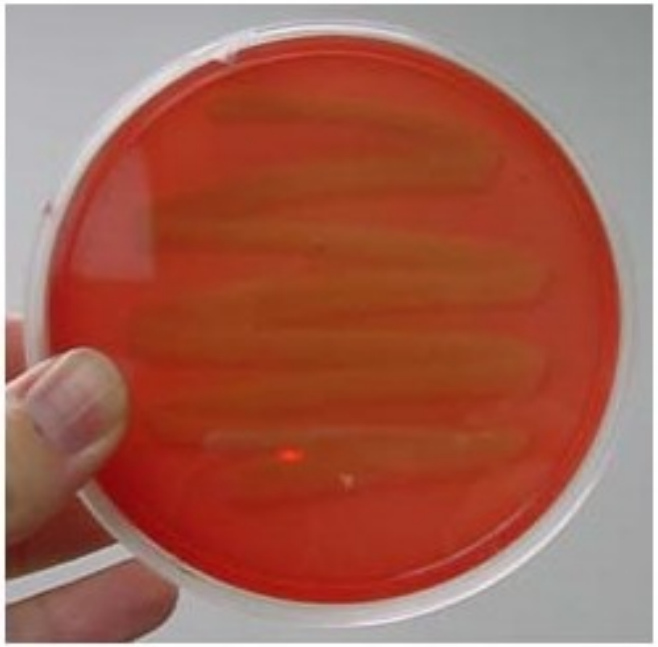
- α-hemolysis (alpha)-
  - Partial lysis of the RBCs
  - Results in a greenish discoloration of agar around colonies



Alpha Hemolysis

γ-hemolysis

- non-hemolytic –
  - an organism does not induce hemolysis
  - the agar under and around the colony is unchanged
  - Also called gamma hemolysis (γ-hemolysis).

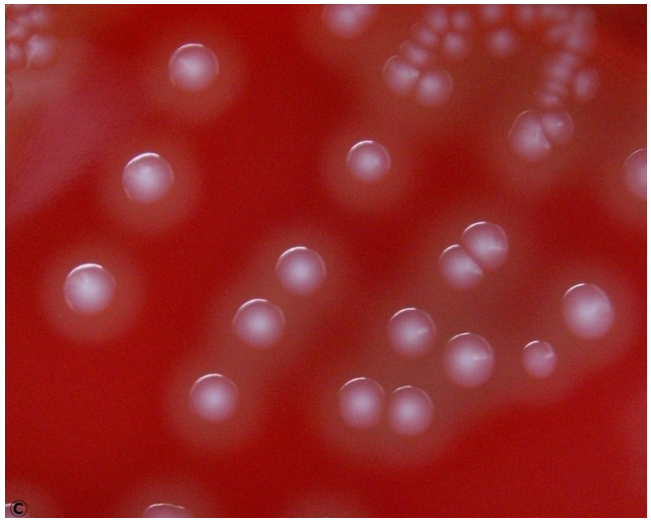


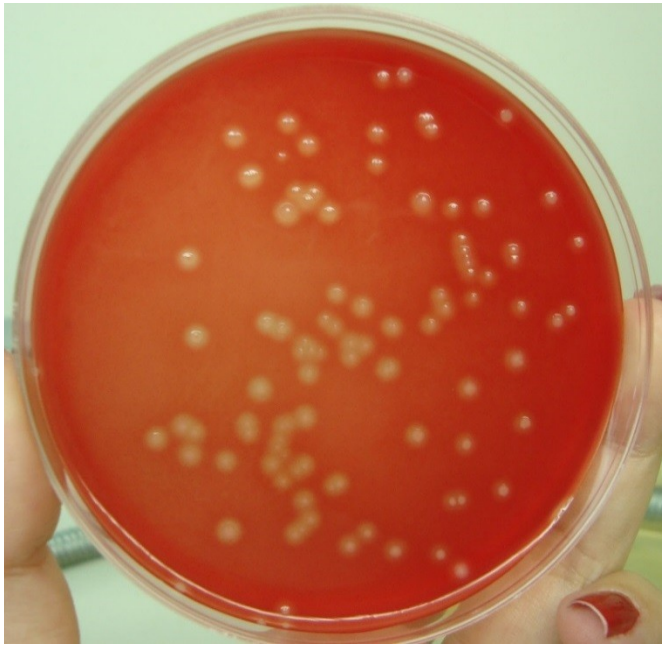
Gamma Hemolysis

Streptococcus spp

Streptococcus pyogenes (Strep A) - GAS

- Lancefield group A
- Colonizes throat and skin
- Virulence Factors
  - Streptolysin O
    - Responsible for the hemolysis on blood agar when incubated anaerobically
    - O refers to the hemolysin being oxygen **labile**—only activated under anaerobic conditions
  - Streptolysin S
    - Responsible for the hemolysis on blood agar when incubated aerobically
    - S refers to the hemolysin being oxygen **stable**





Streptococcus pyogenes (Strep A): Clinical Infections

- Bacterial Pharyngitis (Strep Throat)
  - Most common cases are caused by Strep A
    - Can be caused by Strep C and G but are less common
  - Most often seen in kids 5-15 years old
    - Spread by droplets and close contact / poor hygiene
  - Diagnosis relies on a throat culture or direct antigen test
  - 1/3 of children's sore throats are positive for Strep A

Strep Throat

Streptococcus pyogenes (Strep A): Clinical Infections

Pyodermal Infection

- Pyodermal Infection (skin infection)
  - Result in impetigo, cellulitis, wound infections, or arthritis.
  - Some Strep A produce exotoxins which results in scarlet fever.

Streptococcus pyogenes (Strep A): Clinical Infections

- Necrotizing Fasciitis (flesh-eating disease)
  - Uncommon but life threatening infection
  - Type 1-polymicrobial infection from aerobic and anaerobic bacteria
  - Type 2- Strep A only
  - Type 3- Strep A gangrene or Clostridial myonecrosis
  - Bacteria enter the body through a trauma such as a burn or lacerations

Necrotizing Fasciitis

Streptococcus pyogenes (Strep A): Clinical Infections

- Streptococcal Toxin Shock Syndrome
  - Condition in which the entire organ system collapses, leading to death.
  - A streptococcal pyrogenic exotoxin, notable SpeA is produced.
  - Young children with chickenpox and elderly adults are the greatest at risk

Streptococcal Toxin Shock Syndrome

Streptococcus pyogenes (Strep A): Clinical Infections

Poststreptococcal Sequelae (complications)

- Rheumatic Fever
  - Typically follows S. pyogenes pharyngitis
    - within 1 month after infection
  - Characterized by fever and inflammation of the heart, joints, blood, vessels, and subcutaneous tissues
    - Can lead to chronic progressive damage to the heart valves

Rheumatic Fever

Streptococcus pyogenes (Strep A): Clinical Infections

Poststreptococcal Sequelae (complications)

- Acute glomerulonephritis
  - Sometimes occurs after a cutaneous or pharyngeal infection
  - More common in children
  - Ag-Ab complexes deposit in glomeruli which can fix complement & inflammatory response occurs
  - Inflammatory response causes damage to the glomeruli resulting in impairment of kidney function

Acute glomerulonephritis

Streptococcus pyogenes (Strep A): Treatment

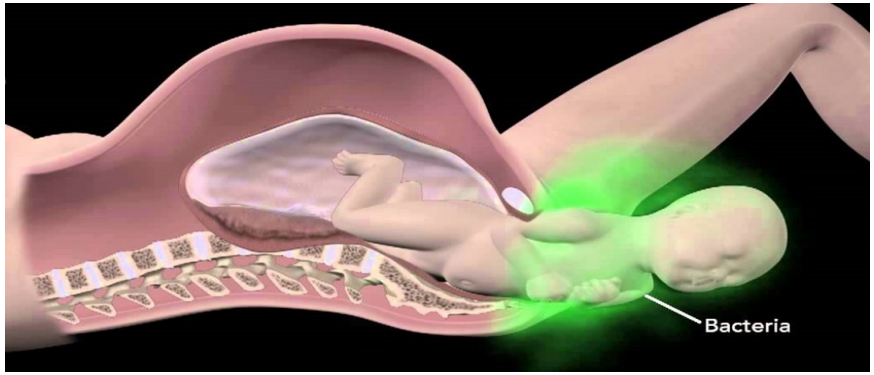
- Treatment
  - **Penicillin** is the drug of choice
  - If allergic to penicillin, **erythromycin** may be used

Streptococcus pyogenes (Strep A): Laboratory Diagnosis

- Laboratory Diagnosis
  - Specimens should be plated to BA and Selective media (i.e. CNA or SSA)
  - Colonies on BAP are small and smooth with well-defined β-hemolysis
  - PYR+, LAP +, and CAMP –
  - Susceptible to bacitracin 0.04 units
  - (Taxos A)
  - A doctor's office can perform a rapid test directly from throat swab (EIA)- limited sensitivity but good specificity
    - Should follow up negative rapid strep screens with culture in children

Susceptible to Bacitracin

Streptococcus agalactiae (Strep B or GBS)



- Lancefield Group B
  - Neonatal GBS
    - GBS is the leading cause of death in infants in the US-colonization of GBS in the vaginal and rectal area is found in 10-30% of pregnant women.
    - Recommended that ALL pregnant women be screened for GBS.
    - Early-onset infection
      - Accounts for 80% of clinical cases
      - Infants that are <7 days old—occurs usually within 24 hours after birth
      - Cause by vertical transmission of the organism from the mother to fetus
        - obstetric complications, prolonged rupture of membranes, and premature birth
      - Manifests as pneumonia and sepsis
      - Mortality rate is high and treatment needs to be started ASAP.

Streptococcus agalactiae (Strep B or GBS)

- Late-onset Infection
  - Infants that are 7 days old to about 3 months old
  - Manifests as meningitis and sepsis
  - Uncommonly associated with obstetric complications—GBS is rarely found in the mother's vagina before birth.
  - Mortality rate is considerably less than that of early-onset infection.

Adult GBS infections

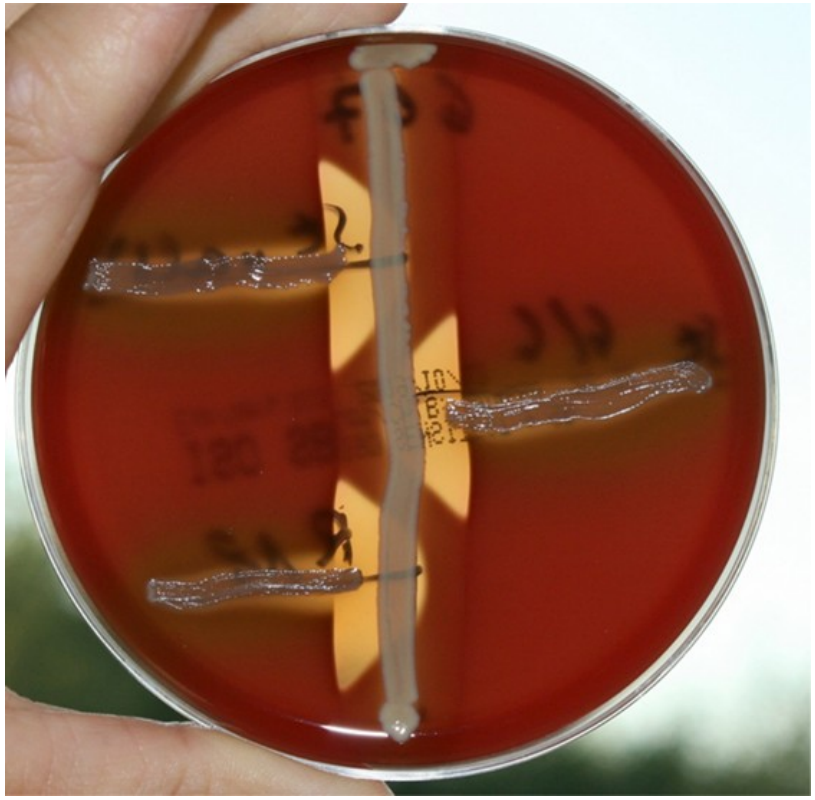
- Young healthy women becomes ill after childbirth or abortion—Endometritis and wound infections
- Young women undergoing obstetric procedures—tricuspid valve endocarditis
- Elderly patients with a serious underlying disease or immunodeficiency.

Streptococcus agalactiae (strep B): treatment and diagnosis

- Treatment
  - Penicillin is drug of choice
  - Can use cephalosporins:
    - clindamycin and vancomycin to treat pregnant women if they are allergic to penicillin.
- Laboratory Diagnosis
  - Colonies on BA are grayish white mucoid surrounded by a small zone of β-hemolysis—approximately 4% of GBS are non-hemolytic.
  - Strep B type positive, Bacitracin resistant, Hippurate positive, and CAMP test positive

Streptococcus agalactiae (Strep B)

- CAMP test +
- Used to presumptively identify Strep B—shows a classic arrow shape near staph streak.
- β-lysin + S. aureus is inoculated in a straight line in the center of BA and the unknown strep isolate is inoculated perpendicular to it.
- Can also drop a β-lysin impregnated disk
- **Enhanced** hemolysis occurs when the β-lysin and the hemolysis produced by Strep B are in contact

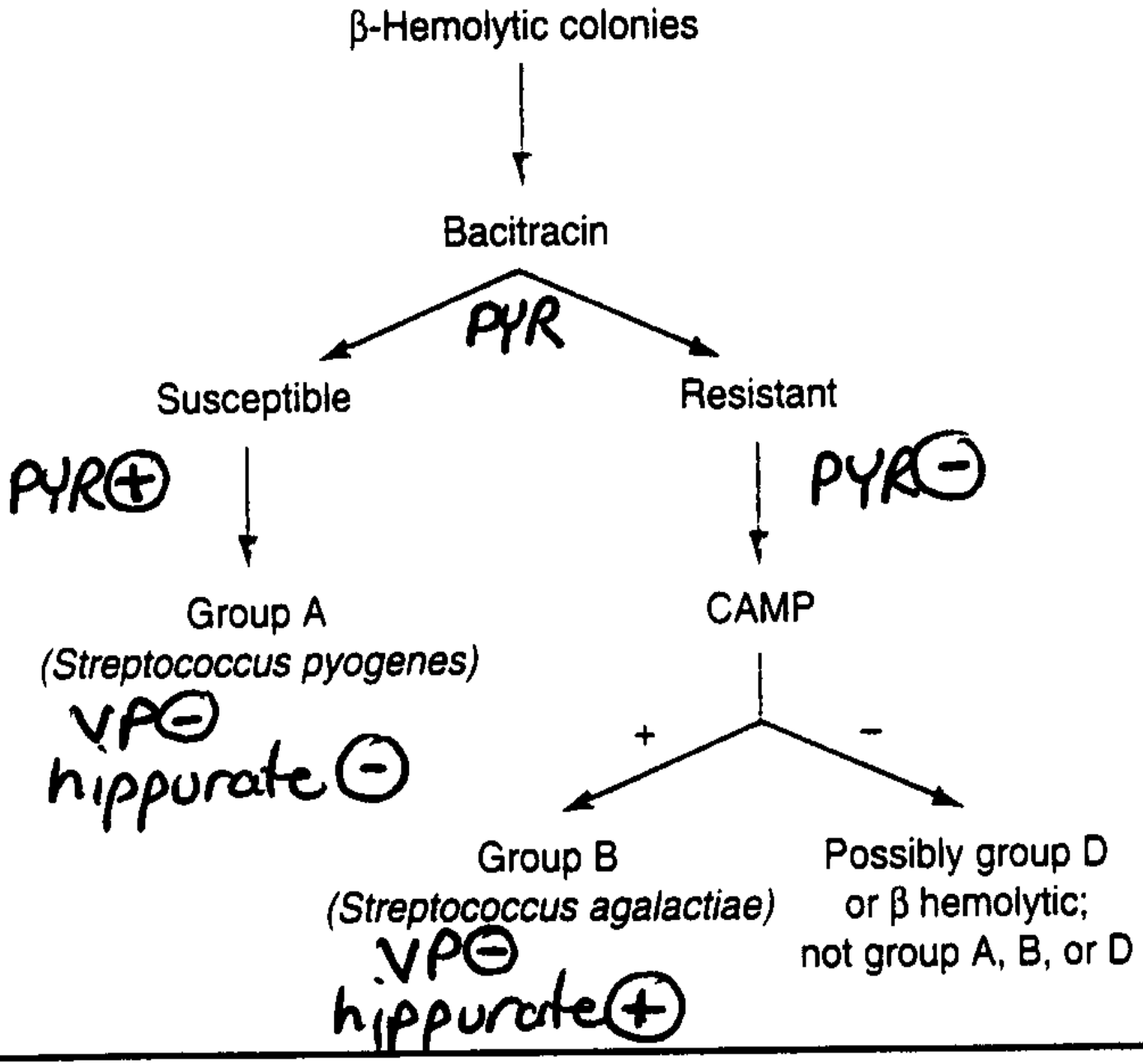


Streptococcus agalactiae (strep B):

- Pregnant women
  - GBS detection is accomplished by collecting a vaginal and rectal swab between 35 and 37 weeks of gestation.
  - Samples are inoculated into selective broth which is incubated at 35ºC for 18-24 hours. Culture with selective media or BAP, or a PCR test
  - CDC recommends:
  - All women who test positive for GBS must get prophylaxis
    - Any women who had a urine culture positive for GBS anytime during their pregnancy, a previous history of an infant with GBS disease, and an unknown GBS status at delivery time should be treated.
  - GBS should be reported in any urine with quant 10^4 of a women who is of childbearing age.
  - Carrot broth is a commercial broth for detection of GBS
    - Hemolytic strains produce orange or red pigment in the broth
    - Does not detect non-hemolytic strains of GBS (need to subculture the negatives)

LIM Broth

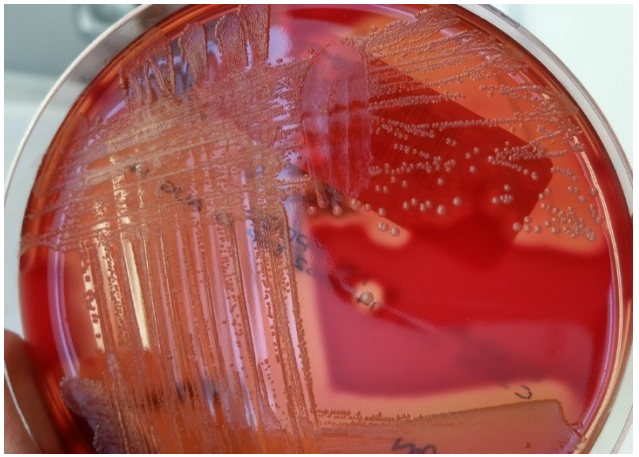
Carrot broth


β-Hemolytic colonies
Bacitracin
PYR
Susceptible
Resistant
PYR+
PYR−
Group A
(Streptococcus pyogenes)
VP−
hippurate−
CAMP
+
−
Group B
(Streptococcus agalactiae)
VP−
hippurate+
Possibly group D
or β hemolytic;
not group A, B, or D

Groups C and G

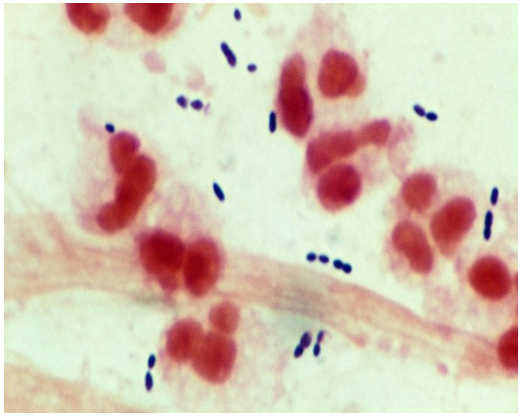
- These are subdivided into large-colony and small-colony
- Wide zone of beta hemolysis
- Can cause pharyngitis, bacteremia, endocarditis, meningitis, and respiratory and skin infections
- With Group G, infections often occur in patients with underlying malignancies
- Large-colony
  - Classified with pyogenic strep
  - Belongs to the subspecies *S. dysgalactiae*
- Small-colony
  - Belong to *S. anginosus* group (this is a viridians strep species)

Group C



Group G

Streptococcus pneumoniae



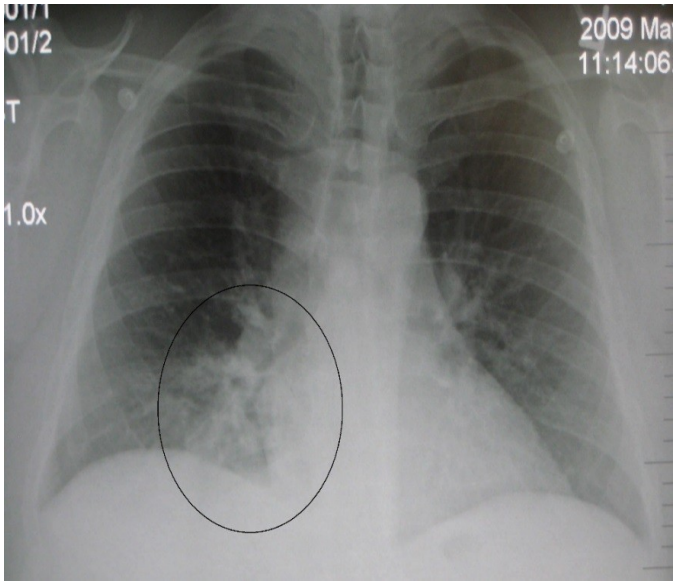
- Gram positive cocci seen in short chains or pairs
- Isolates on BA produce a large zone of **α-hemolysis** surrounding the colonies which can closely resemble Viridans strep.
- Young cultures have a round glistening, wet, mucoid, dome-shaped appearance “donut or cratered appearance”
  - autolytic changes result in the colony
- Grows best with 5-10% CO_2

S. Pneumo: Antimicrobial Resistance

- Antimicrobial Resistance
- Penicillin is drug of choice
- Penicillin-resistant S. pneumoniae occurs by alteration in the cell wall penicillin-binding proteins
- Strains that are resistance to penicillin are treated with erythromycin or chloramphenicol.
- Strains that exhibit resistance to erythromycin, tetracycline, chloramphenicol, and SXT have recently been reported.

Streptococcus pneumoniae: Clinical infections: *Pneumococcal pneumonia*

- Part of the normal respiratory tract flora when in small numbers
- Causes pneumonia, sinusitis, otitis media, bacteremia, and meningitis
- Number one cause of bacterial pneumonia
  - usually not a primary infection but rather a result of disturbance of the normal defense barriers allowing S. pneumo to grow
    - Travels from nasopharynx to lungs
- Infection begins with the aspiration of respiratory secretions which contain pneumococci.

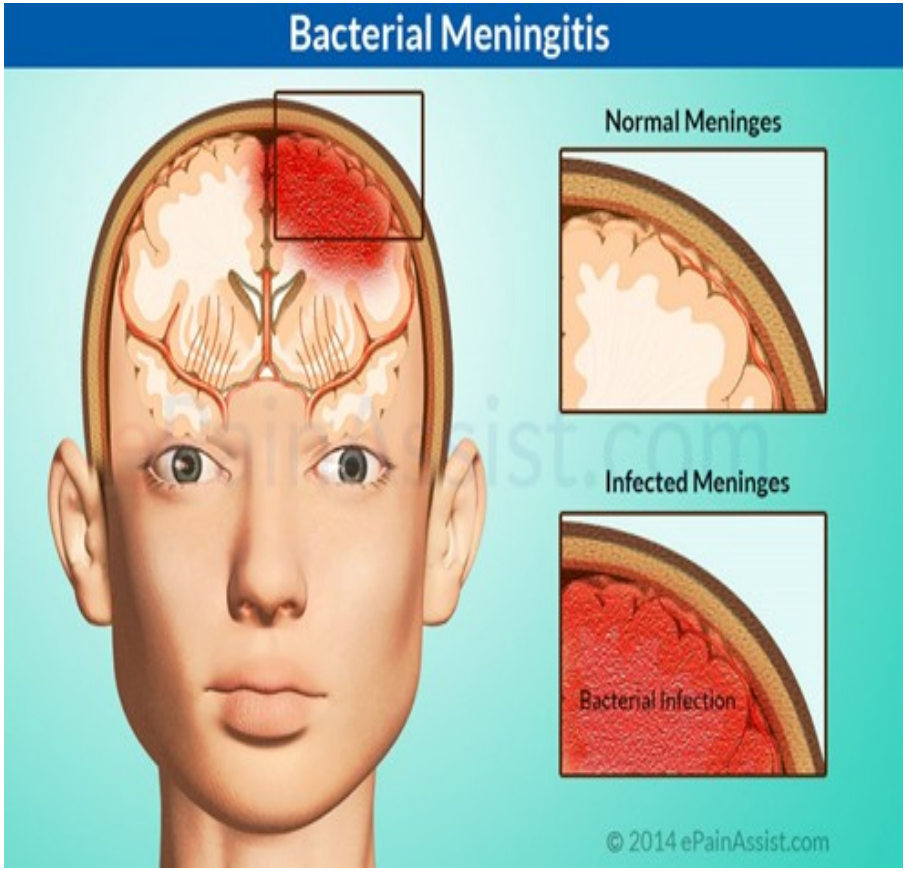


Pneumococcal pneumonia chest x-ray

Streptococcus pneumoniae: Clinical infections

- *Bacterial meningitis*

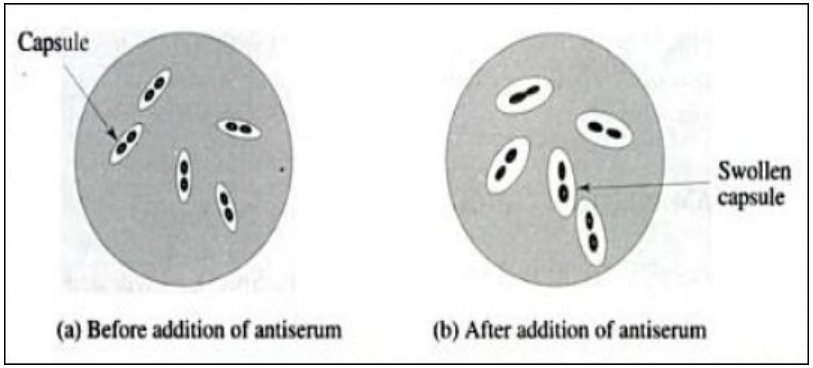
- Usually follows a S. pneumoniae infection such as otitis media or pneumonia.
- Mortality rate is near 40%
- *Secondary atypical hemolytic uremic syndrome*
- Reported in children as a secondary syndrome
- Diagnosis is critical with positive blood and spinal fluid cultures and a false positive Coombs' test.



Streptococcus pneumoniae:

- *<u>Antigenic Structure</u>*

- Cell wall contains an antigen known as C substance
  - Reacts with C-reactive protein in humans
- Can express 1 of 90 different capsular types (polymorphic)
  - Capsule is antigenic and can be identified with the appropriate antiserum in the Neufield test.
- This allows for ID and serotype specifically isolates.



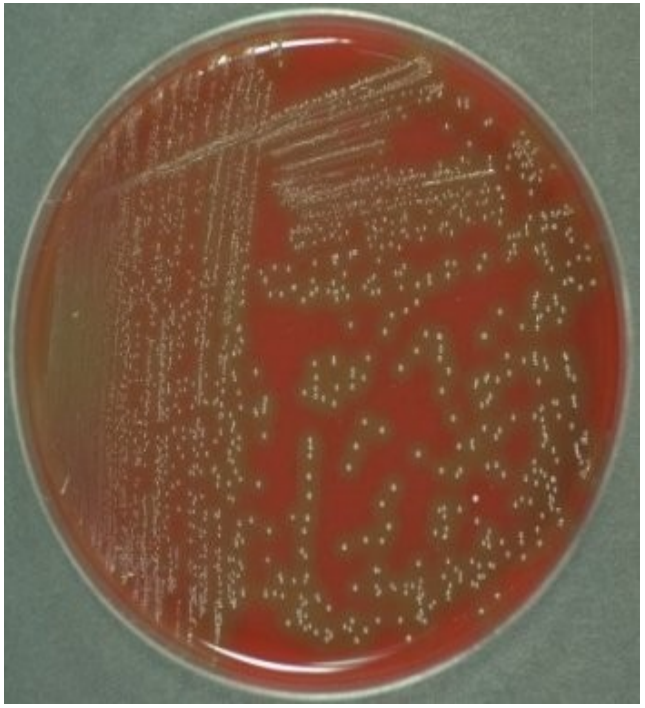
Neufield test (aka The Quellung reaction (swelling of the capsule)) is an antigen-antibody reaction causes a change in the refractive index of the capsule so that it appears "swollen" and more visible.

Streptococcus pneumoniae: Vaccines

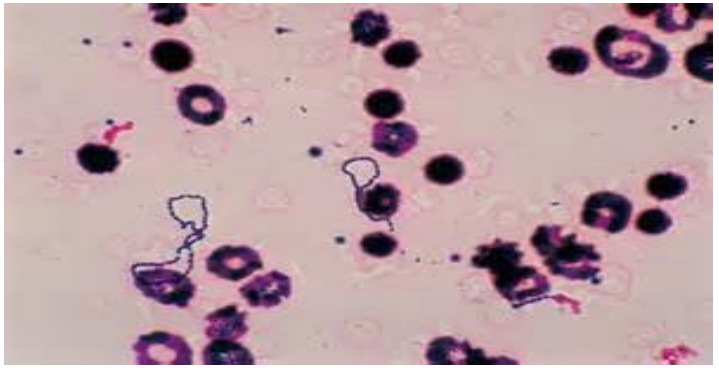
- **Pneumococcal conjugate vaccines (PCV)**
  - Composed of purified polysaccharides conjugated to a diphtheria protein
  - Given as part of the pediatric immunization schedule
  - PCV7 contains seven serotypes
  - PCV13 contains PCV7 serotypes plus six additional serotypes came out recently
- **Purified capsular polysaccharides (PS)**
  - Used in adults
  - PS23 which is composed of 23 serotypes
  - Recommended for people at risk to develop pneumococcal pneumonia.

Viridans Streptococci

- Constituents as normal flora of:
  - the upper respiratory tract, female genital tract, and the gastrointestinal tract
- Fastidious (some require CO_2 to grow)
- Viridans means “green” referring to α-hemolysis
  - May also be beta or gamma
- Viridans strep do not have carbohydrate antigen on cell surface
- More than 30 species are recognized with current classification into five groups:
  - S. *mitis* group
  - S. *mutans* group
  - S. *salivarius* group
  - S. *bovis* group
  - S. *anginosus* group

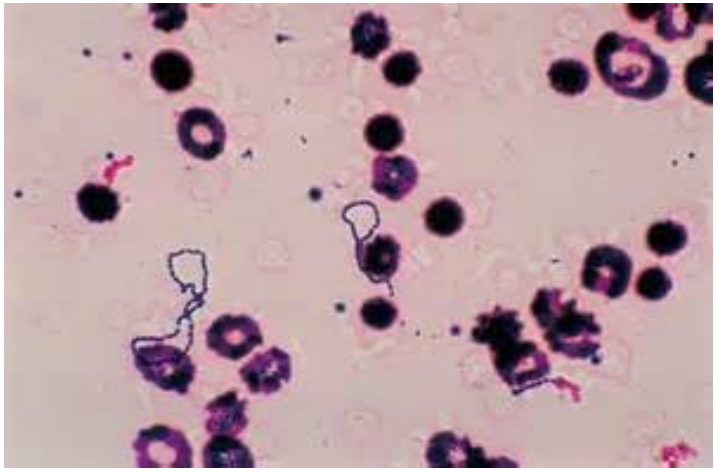


Viridians Streptococci



- Clinical infections
- Regarded as opportunistic pathogens.
- Most common cause of sub-acute bacterial endocarditis (SBE), a condition associated with transient bacteremia.
- Cause oral infections such as gingivitis and dental cavities, meningitis, abscesses, osteomyelitis and empyema
- Virulence factors
  - Extracellular dextran and cell surface-associated proteins have been implicated in adherence and colonization in endocarditis.

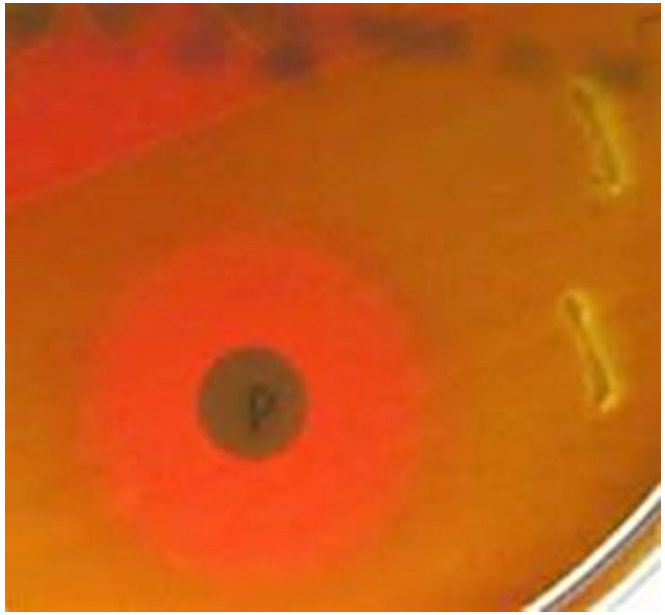
Viridians Streptococci



- Laboratory Diagnosis
  - Extremely difficult to identify to species level, hence the 5 groups
  - All are LAP +, PYR –, bile esculin – (except group D), 6.5% NaCl= No Growth
  - Gram stain is a typical strep
  - Colonies are small, surrounded by a zone of α-hemolytic. Remember some are β-hemolytic and non-hemolytic.

Differentiating *S. pneumoniae* from S. Viridans

- Optochin (P disk) susceptibility (ethylhydrocupreine hydrochloride)
- If the zone is at least 20 mm it is considered sensitive
- S. pneumoniae is sensitive and Viridans strep are resistant
- Isolates that produce a smaller zone size should be tested for bile solubility to confirm their identity
- Bile solubility
- S. pneumoniae is bile soluble
- Viridans strep is not bile soluble



Nutritionally Variant Streptococci

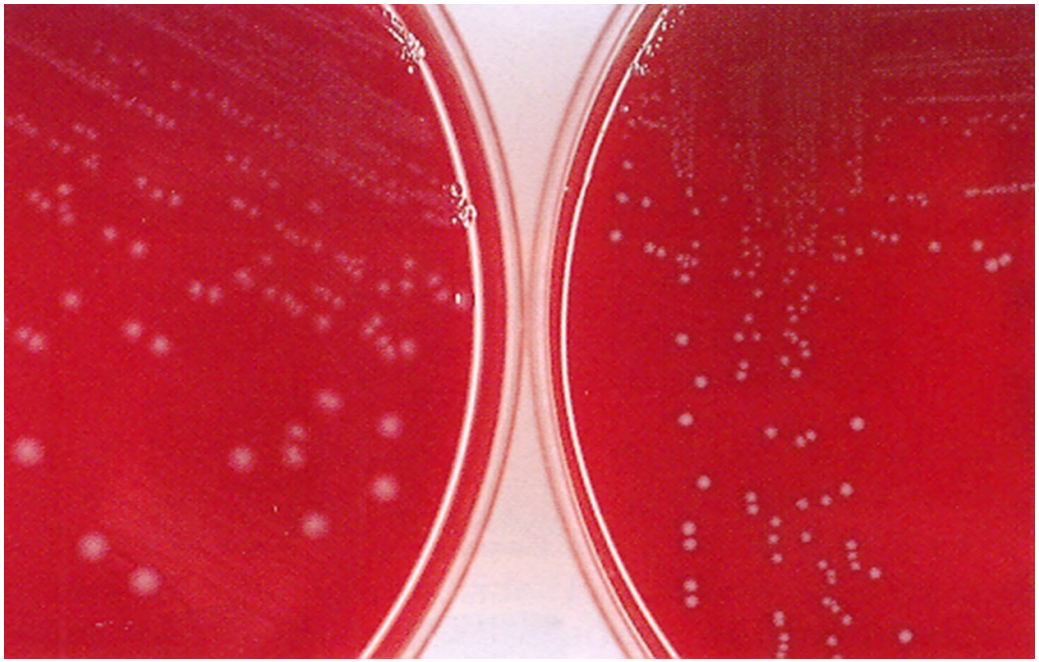
- Abiotrophia and Granulicatella spp.
- These bacteria grow as satellite colonies around other bacteria
  - require pyridoxal (Vitamin B6)
  - Can be supplied by:
    - Pyridoxal disk
    - Adding pyridoxal to media
    - Cross-streaking plate with S. aureus and examine for satellitism.
- Part of normal oral and GI flora (opportunistic)
  - Causes bacteremia, endocarditis, and otitis media
- Suspected organism when gram stain shows GPC in long chains but nothing grows on plates
- Most are susceptible to penicillin but they can be hard to treat-may even require surgical removal
- Bile esculin –
- No growth in 6.5% NaCl, and PYR +

Enterococcus spp.

Enterococcus

- Expresses the Group D antigen
- Normal flora of the intestinal tract of humans and animals
- E. faecalis = most common
- E. faecium = 2nd most common
- Others:
  - *E. durans*
  - *E. avium*
  - *E. casseliflavus*
  - *E. gallinarum*
  - *E. raffinosus*

E. faecalis E. faecium



Salt tolerance

Enterococcus

Virulence Factors

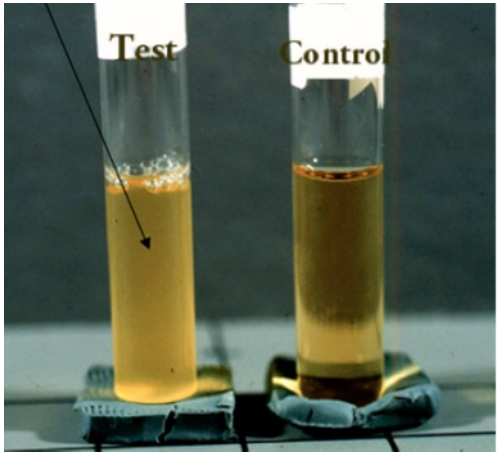
- Can grow in extreme conditions (bile)
- Very capable of acquiring & exchanging resistance genes

Clinical Infections

- Frequent cause of nosocomial infections in which UTI's (usually catheterization) are the most common followed by bacteremia.
- Frequently isolated from intra-abdominal and pelvic wound infections
- Rarely causes respiratory tract infections

ENTEROCOCCUS

- *<u>Laboratory Diagnosis</u>*
  - On BA, colonies are gray and mostly α-hemolytic or non-hemolytic but can be β-hemolytic
  - bile esculin +
    - Bile esculin detects growth of bacteria in presence of 40% bile
      - hydrolyzes esculin
      - All Group D strep & Enterococci = +
  - PYR +
  - 6.5% NaCl= Growth
    - Salt tolerance: Growth in 6.5% sodium chloride broth (Salt-tolerance test)
      - identifies Enterococcus and Aerococcus.
      - Group D Strep are unable to grow
    - Sometimes exhibits pseudo-catalase reaction- weak bubbling in the catalase test. (weak +)



Salt tolerance

Enterococcus

Antimicrobial Resistance

1. Intrinsic or acquired resistance to many antimicrobials
2. Vancomycin Resistance [VRE]
   i. There are 6 resistant phenotypes: VanA to VanE
   ii. VanA and VanB are the most frequently encountered
   iii. VanA is inducible and characterized by high levels of resistance
   iv. VanB is chromosomal mediated and characterized by variable levels of resistance.
3. Combo therapy used in serious infections like endocarditis
4. Cell-wall agent like ampicillin or vancomycin along with an aminoglycoside used for blood infections and endocarditis
   i. Need to do synergy testing to make sure there is no high level resistance

E. faecium

- Most resistant to penicillin and vancomycin, and usually has high level of resistance to aminoglycosides
- “VRE” (vancomycin resistant Enterococcus)
- “High level” resistance to vancomycin---- contains VanA and VanB genes
- Non-motile
- People can become carriers of VRE, mostly in their stool and rectum—screen for VRE is done on inpatients.
- Can spread its resistance genes to other organisms therefore patients who test positive for VRE are placed into isolation.
- Considered a nosocomial pathogen
- VRE can be very difficult to eradicate

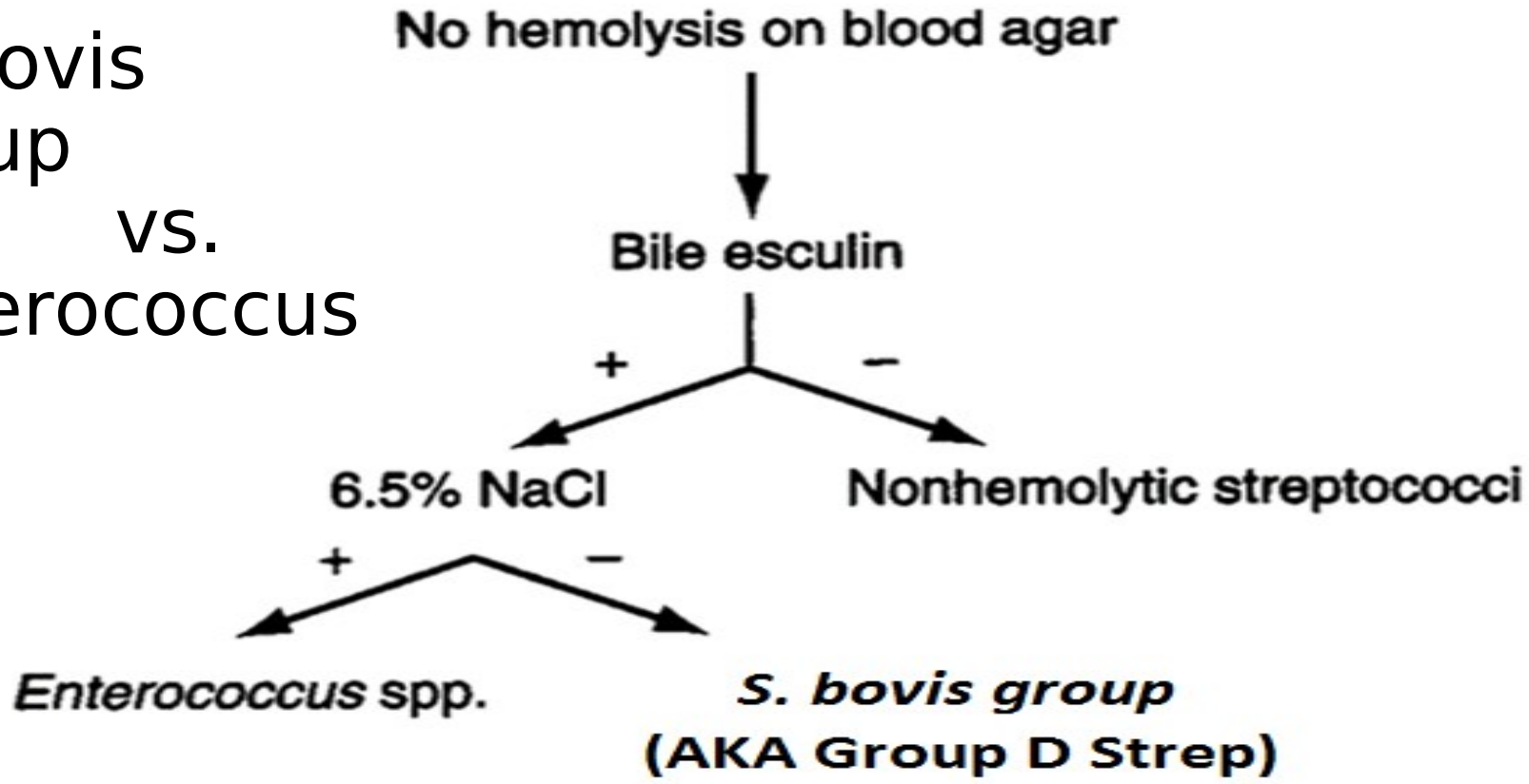
S. bovis group (AKA Group D Strep)

- Group D antigen expressed by both Enterococcus and the S. bovis group
- Bacterial endocarditis, UTI, abscesses and wound infections
- S. bovis group associated with GI malignancies
- Usually non-hemolytic (but may be α or β)
- Antimicrobials
  - Generally relatively susceptible

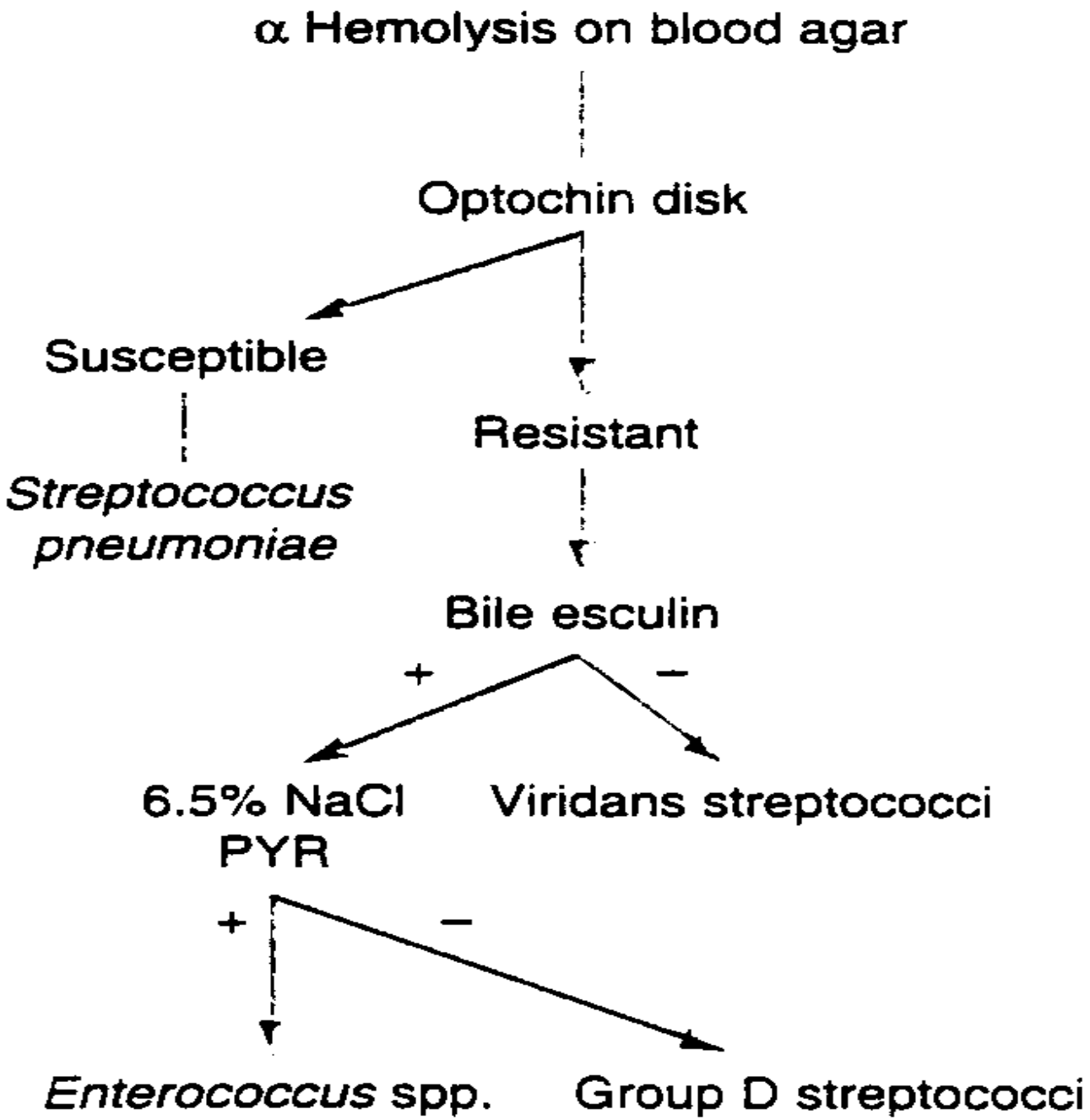
S. bovis group

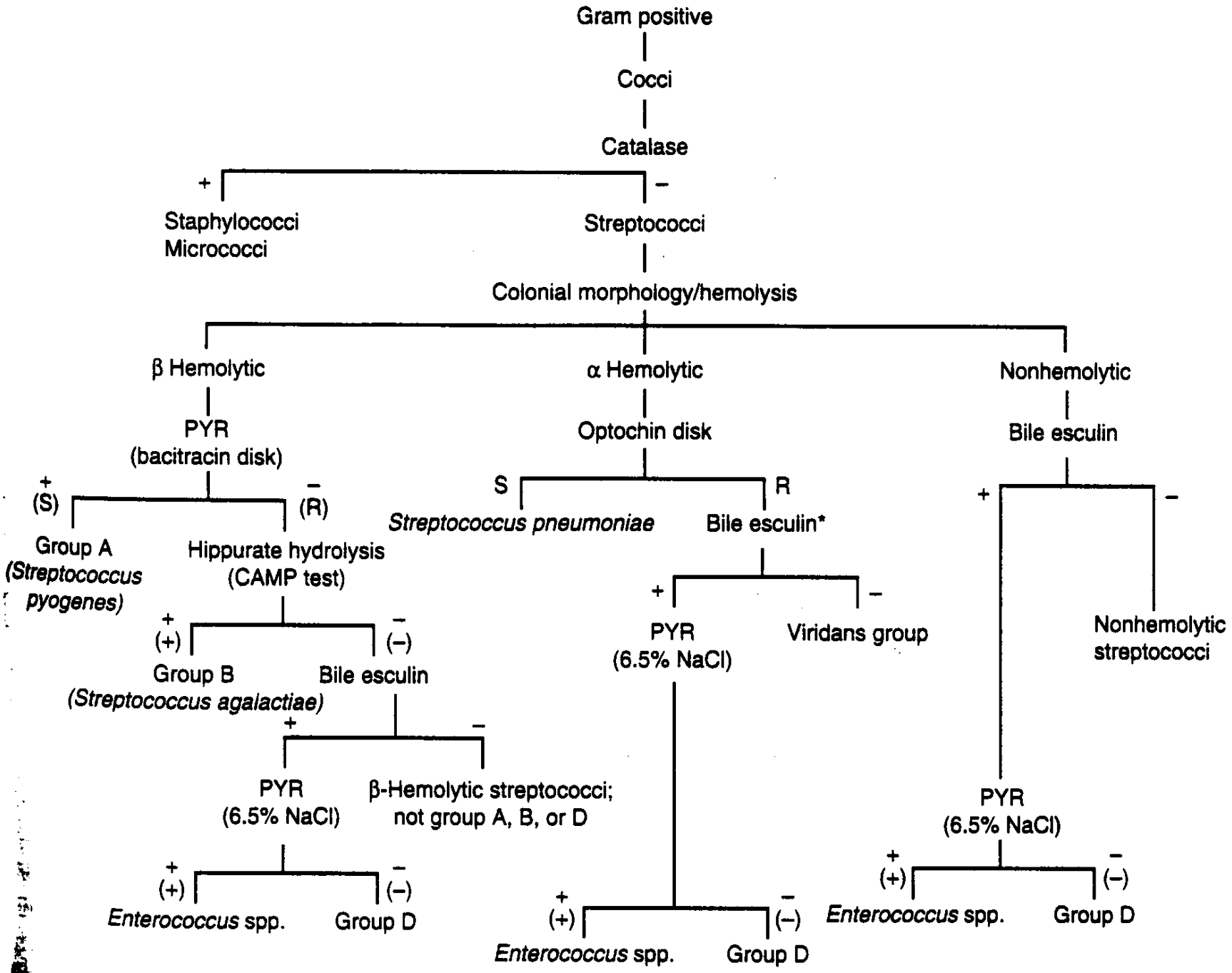
vs.

Enterococcus



	Bile esculin	PYR	6.5% NaCl	Penicillin
S. bovis group	+	-	No Growth	Susceptible
Enterococcus	+	+	Growth	Resistant

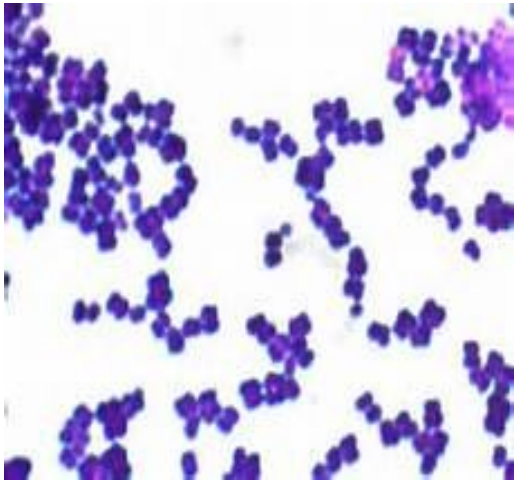

α Hemolysis on blood agar
Optochin disk
Susceptible
Resistant
Streptococcus pneumoniae
Bile esculin
+
−
6.5% NaCl PYR
Viridans streptococci
+
−
Enterococcus spp.
Group D streptococci

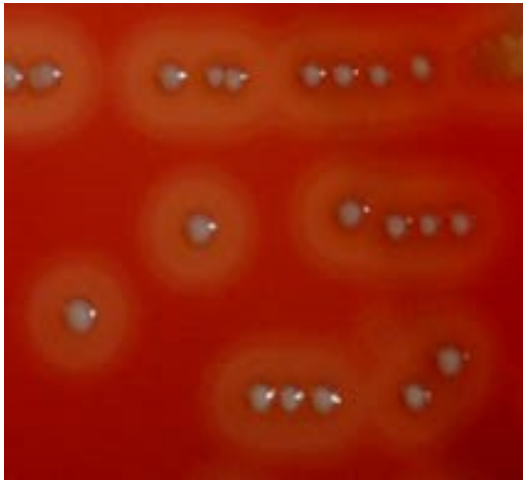

Gram positive
Cocci
Catalase
+
–
Staphylococci
Micrococci
Streptococci
Colonial morphology/hemolysis
β Hemolytic
α Hemolytic
Nonhemolytic
PYR
(bacitracin disk)
+
(S)
–
(R)
Group A
(Streptococcus pyogenes)
Hippurate hydrolysis
(CAMP test)
+
(+)
–
(–)
Group B
(Streptococcus agalactiae)
Bile esculin
+
–
PYR
(6.5% NaCl)
β-Hemolytic streptococci; not group A, B, or D
+
(+)
–
(–)
Enterococcus spp.
Group D
Optochin disk
S
R
Streptococcus pneumoniae
Bile esculin*
+
–
PYR
(6.5% NaCl)
Viridans group
+
(+)
–
(–)
Enterococcus spp.
Group D
Bile esculin
+
–
Nonhemolytic streptococci
PYR
(6.5% NaCl)
+
(+)
–
(–)
Enterococcus spp.
Group D

Aerococcus spp and other odd ball GPC

Aerococcus spp.

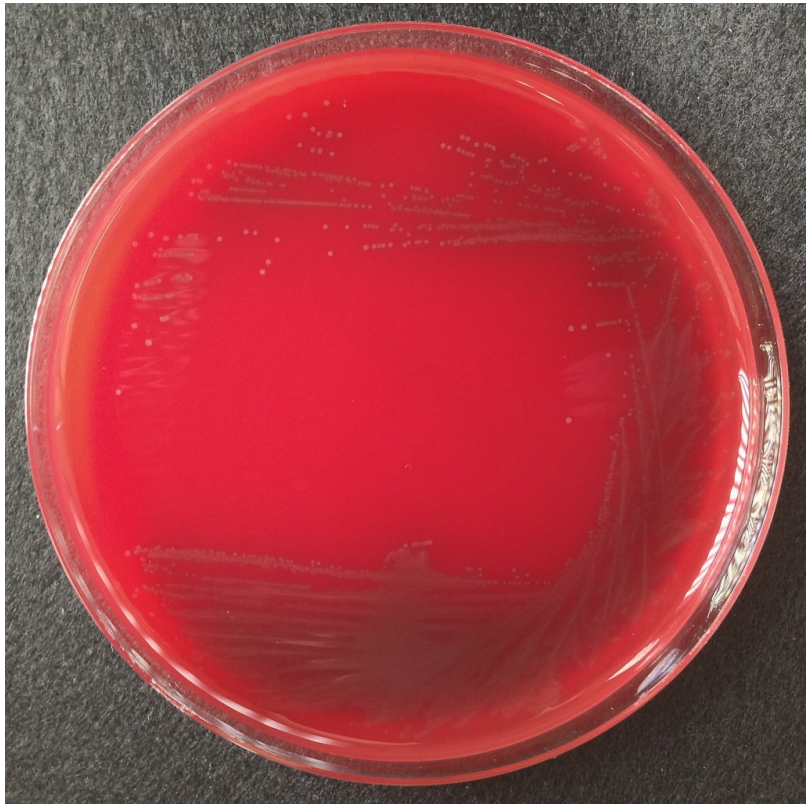
- Found on skin and upper respiratory tract; common airborne organism - Opportunistic pathogen
  - Can cause endocarditis, bacteremia, UTI
- GPC in tetrads/clusters on a gram stain
- Colony morphology look very similar to viridans strep but tend to be more Beta hemolytic
- Catalase negative or weakly positive
- LAP
  - Aerococcus spp = +
  - Leucinistoc spp = -
  - Other Strep = +
- *A. urinae* found in UTI, endocarditis, lymphadenitis, and peritonitis - LAP + & PYR negative and beta hemolytic
- *A. viridans* found in bacteremia and endocarditis- LAP neg & PYR pos





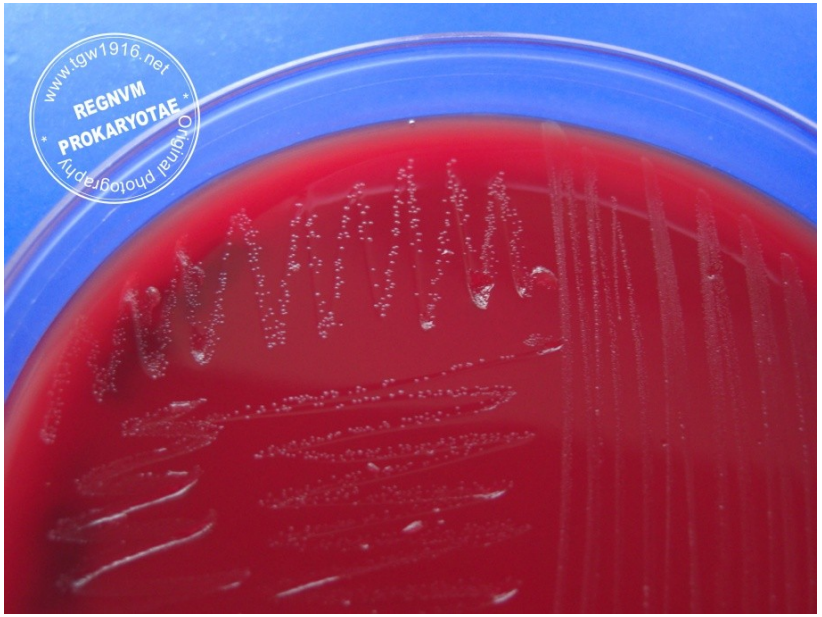
Gemella spp

- Similar to viridans streptococci
- α-hemolytic or no hemolytic
- Easily decolorized so may appear gram negative in pairs, clusters, tetrads or short chains on a gram stain
- Endocarditis, wounds and abscesses



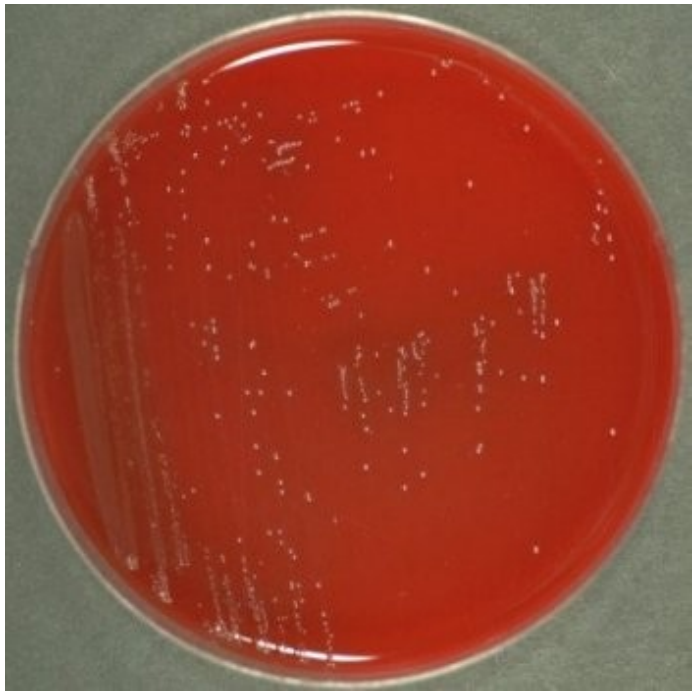
Lactococcus

- GPC occurring singularly, pairs, or in chains on gram stain
- LAP positive
- Isolated from patients with UTI & endocarditis
- Physiologically similar to Enterococci



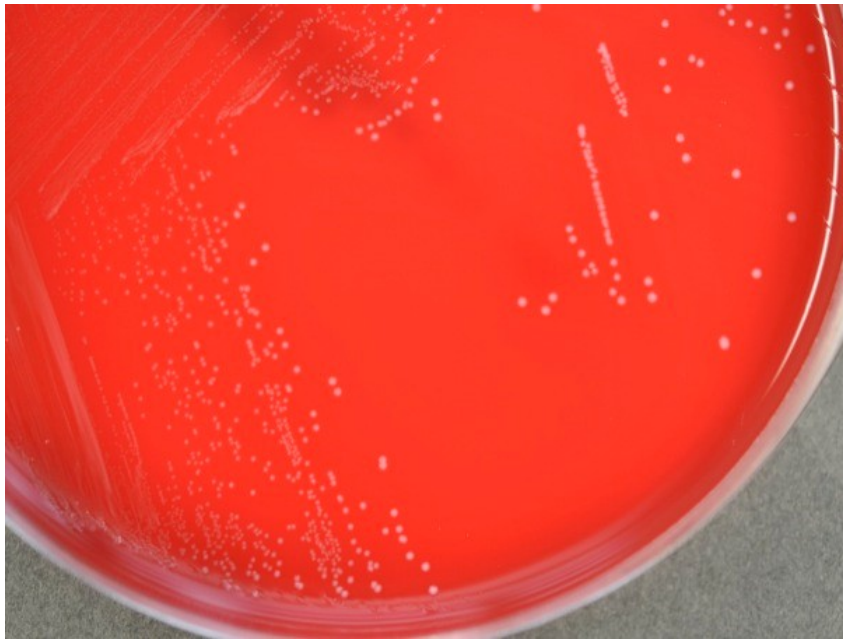
Leuconostoc

- Opportunistic infections in immunocompromised patients
- Causes meningitis, endocarditis, septicemia, and UTIs
- Found in the environment
- Resistant to vancomycin
- LAP-, Catalase -, PYR-
- Gram positive irregular shaped coccoid morphology on a gram stain
- May produce α-hemolysis and can resemble viridans strep



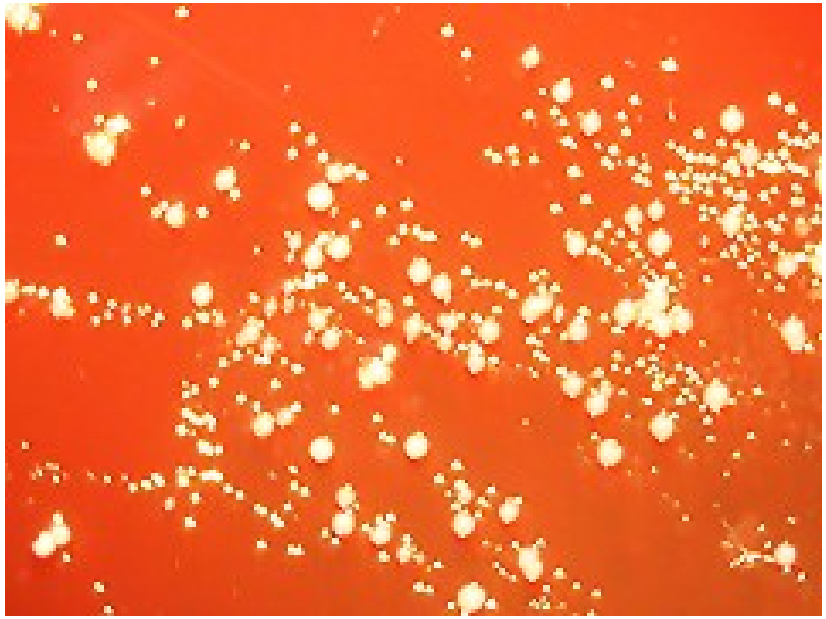
Pediococcus

- Only very rarely causes infections in immunocompromised
- Associated with GI abnormalities or people who have had abdominal surgery
- Facultative anaerobe that grows at 45°C
- Resistant to vancomycin
- GPC in pairs, tetrads and clusters
- Isolated from saliva, stool, urine and wounds
- LAP +, PYR-



Alloiococcus

- Associated with otitis media in children
- Non-hemolytic but can show α-hemolysis with prolonged incubation
- PYR-, LAP+



Globicatella

- Causes sepsis, meningitis, bacteremia and UTI's
- α-hemolytic, PYR+, LAP-
- Sensitive to vancomycin