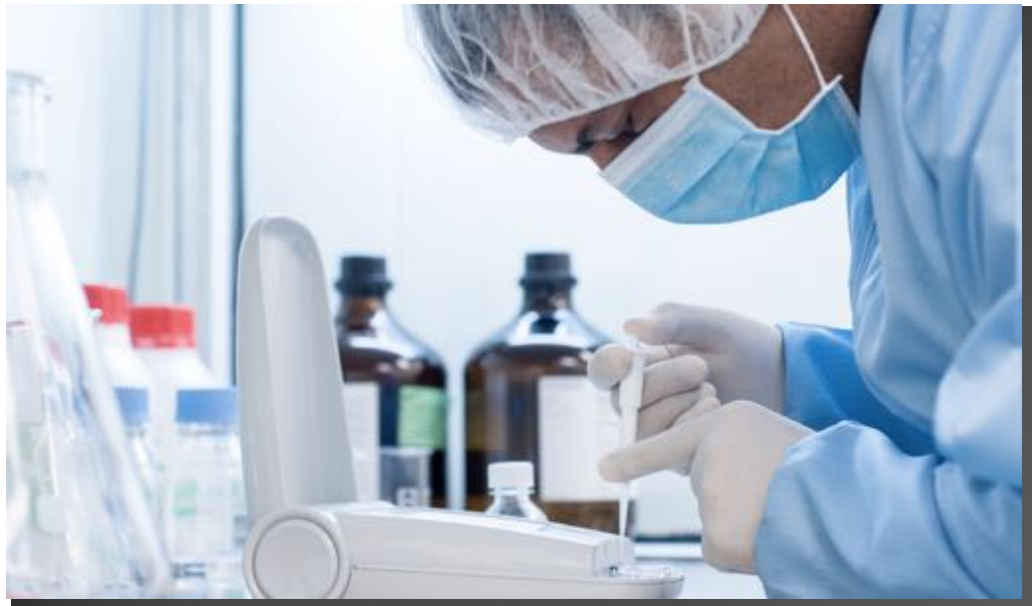
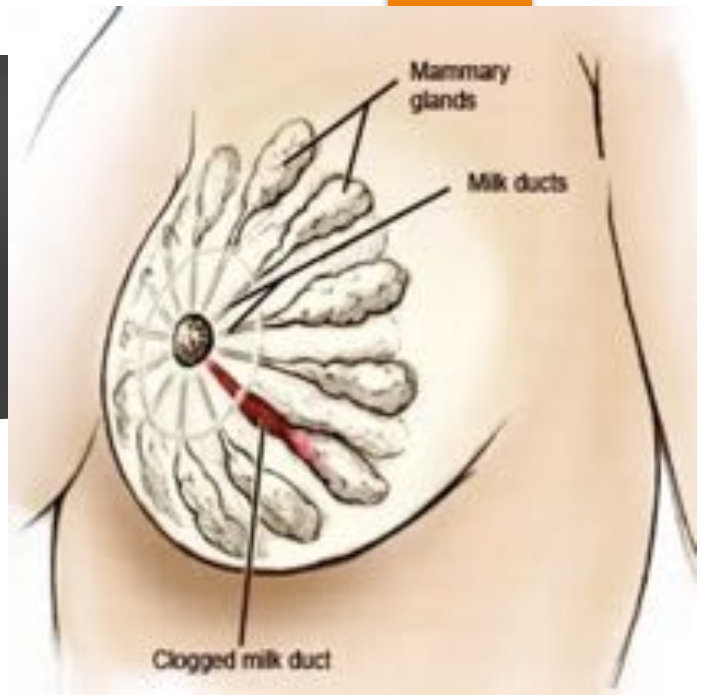


Microbiology Laboratory

NESTOR CHEN

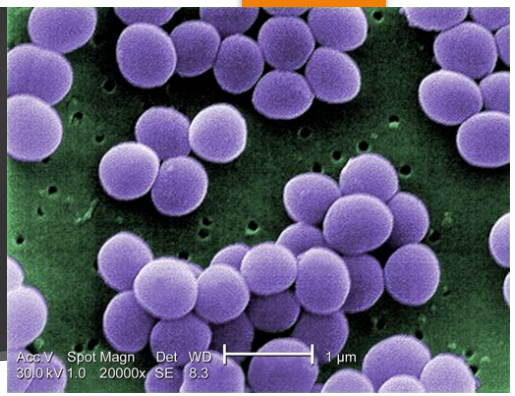
PATH 417A

Mastitis



- Inflammation of mammary glands by bacterial inflammation
- Changes milk composition and reduces secretion of milk
- Can be caused from poor breastfeeding practices or damage to the breasts, resulting in clogging of the milk duct
- Infection can be caused by a variety of pathogenic organisms

Pathogen: Staphylococcus aureus



- Main causative agent of human mastitis
- Transmission through udders, teat canals, and teat lesions
- Persist on damaged skin and teat lesions; causes superficial skin lesions
- Virulent factors: surface proteins and biofilms that promote adherence and growth of bacteria
- Resistant to many relevant antibiotics; respond poorly to antibiotic treatment

Pathogen: Staphylococcus epidermidis

- Most prevalent in animal forms of mastitis infections
- Coagulase-negative staphylococci
- Opportunistic pathogen in skin microbiome
- Adhesion mechanisms, creation of biofilms, greater rate of antibiotic resistance to methicillin than S.*aureus*

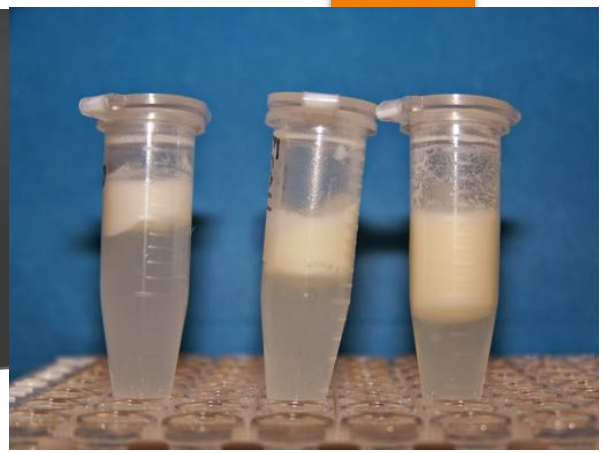
Pathogen: Group B Streptococci

- Supposedly play an active role during mastitis infection
- Group B categorized based on serologic grouping differences in cell wall carbohydrates and pili-associated proteins
- Non-motile, non-spore forming members of the normal flora
- No defined virulent factors
- Resistant to tetracycline antibiotics, but susceptible to penicillin and erythromycin

Pathogen: Enterobacteriaceae

- Bacteria family includes Klebsiella spp., Pseudomonas spp., Shigella spp., and E.coli
- Typically diagnosed in the lab with the use of MacConkey agar plates
- Gram-negative: cell wall with thick LPS, providing strength and protection
- Different family members have different pathogenicity in mastitis infections
- Klebsiella spp. occurs most frequently

Samples for Lab Testing

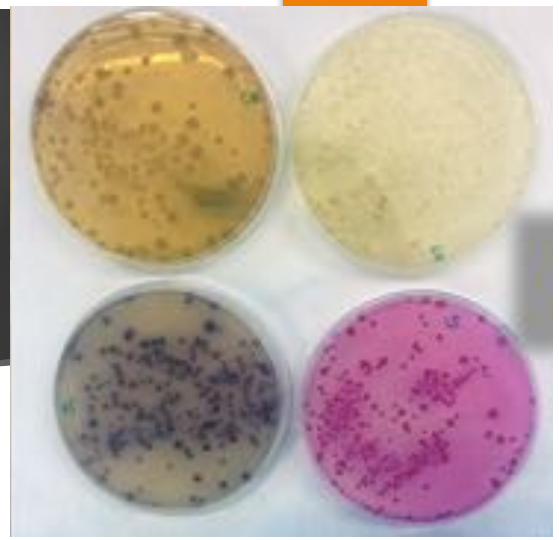


- Breast milk samples are an easy way to confirm bacterial infection
- Identification of pathogens in breast milk is not always possible
  - False positive or false negative results from contaminants of normal flora
- Acquiring culture of the breast milk can lead to identification of specific bacterial agent causing mastitis

Sampling Methods

- Breast milk sample acquired in sterile container performed at breast milk banks or in hospitals
- Expression of milk without nipples touching to prevent contamination
- Despite careful sampling technique, only 50% of milk acquired may be considered sterile

Importance of Lab Testing



- Occasionally, a physical exam is sufficient in diagnosing a mastitis infection
- Laboratory identification of pathogen can help create a more effective and directed treatment plan
- Broad-spectrum antibiotics can lead to therapeutic failure and potential antibiotic resistance
- Important for diagnosing specific species and strain of bacteria

Laboratory Tests

Culturing

- A variety of growth agars are used to identify all the potential pathogens as different pathogens have different growth requirements

- Culturing and identification are done on milk samples to identify bacterial species present

Culturing: Columbia Blood Agar

- Identifies Streptococci, Staphylococci, and similar bacteria
- Indicates α-hemolysis, β-hemolysis, and γ-hemolysis
  - α-hemolysis: green discoloration
  - β-hemolysis: clear zone
  - γ-hemolysis: no change in medium / no destruction in hemoglobin
- Incubated at 37°C for 48 hours

Culturing: SDC Agar

- Sabouraud choloramphenicol agar
- Identifies fungi and yeast
- Contains choloramphenicol to inhibit many gram- and gram+ bacteria to allow growth of fungi and yeast
- Yeasts appear as white or creamy colonies
- Incubated at 25°C for 5 days

Culturing: Kanamycin Aesculin Azide Agar

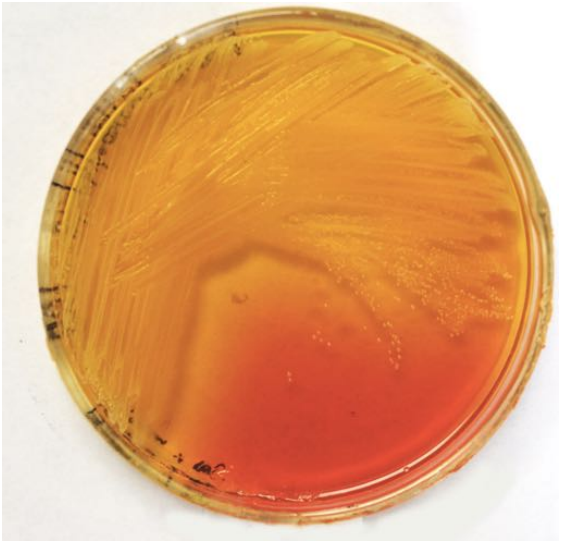
- Identifies Enterococci
- Sulphate and sodium azide are selective inhibitors that only allow the growth of enterococci
- Presence of round, white, or grey colonies surrounded by black zones indicates enterococci
- Incubated at 37°C for 48 hours

Culturing: Violet Red Bile Glucose Plate

- Identifies Enterobacteriaceae and other gram-negative bacteria
- Bile salts and crystal violet inhibit growth of gram+ bacteria
- Peptones, yeast extract, and glucose are present to stimulate bacterial growth
- Red and purple colonies means the bacteria is capable of fermenting glucose
- Incubated at 37°C for 48 hours

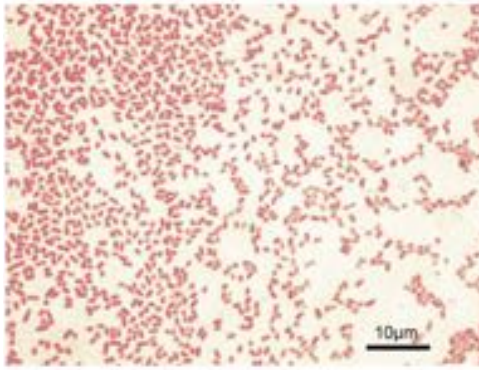
Culturing: Mannitol Salt Agar

- Identifies Staphylococci
- Selective growth medium used to select mannitol fermenting colonies which appear yellow or gold
- Grown on soy and 5% sheep blood agar
- Incubated at 37°C for 48 hours

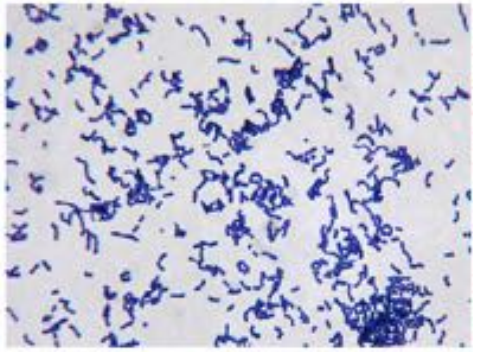


Gram Staining

- Used to determine if a bacteria is gram+ or gram-
- Gram+ bacteria stain purple due to presence of thick peptidoglycan cell wall
- Gram- bacteria stain red/pink due to thin peptidoglycan cell wall
- Can also reveal morphology and configuration of the bacteria (cocci, bacili, strepto, etc.)

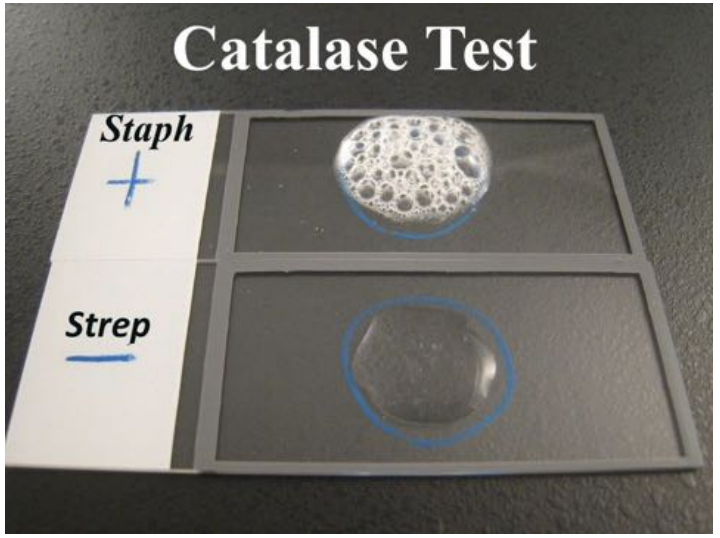


Gram Negative Bacteria


Gram Positive Bacteria

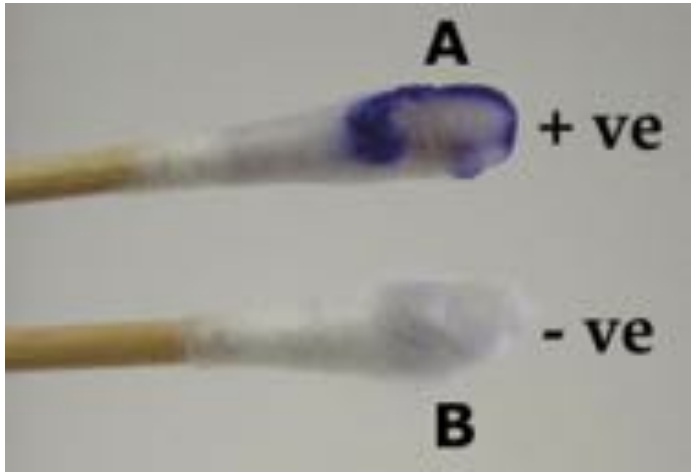
Biochemical: Catalase Test

- Respiratory test used to detect if catalase is present in the bacteria
- Indicates whether or not the bacteria can use oxygen as a terminal electron acceptor
- Positive test marked by occurrence of rapid bubbling, representing formation of oxygen gas



Biochemical: Oxidase Test

- Respiratory test used to detect the presence of cytochrome oxidase in the bacteria
- Cytochrome oxidase is an important component of the electron transport chain and facilitates addition of electrons to oxygen
- Positive test marked by color change to dark purple



Biochemical: Coagulase Test

- Detects the presence of the enzyme coagulase which converts fibrin in plasma to insoluble fibrin
- Begins with emulsifying the isolate with rabbit plasma in a test tube
- Positive results indicated by the formation of a clot in the tube

Test Results

Staphylococcus aureus

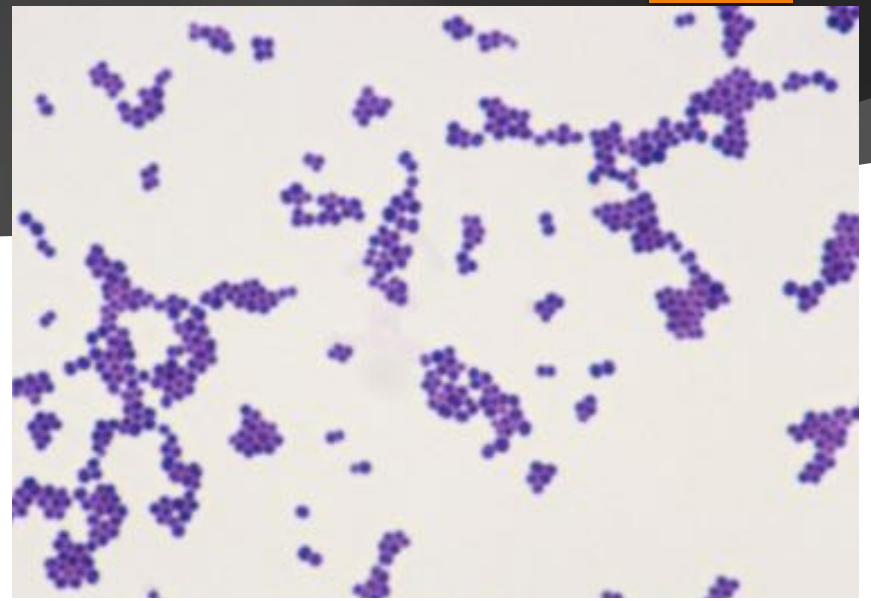
- Can ferment mannitol into acids, turning mannitol agar yellow

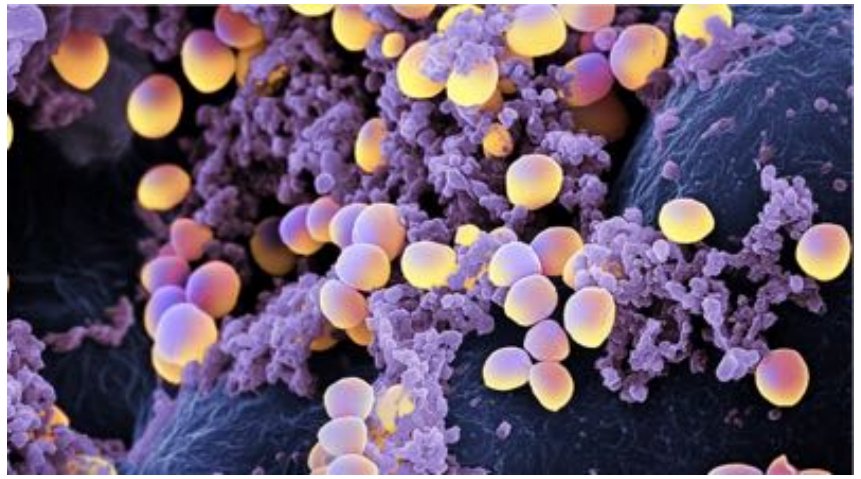
- Columbia blood agar: clear zones indicating β-hemolysis

- Does not grow on SDC agar, Kanamycin Aesculin Agar, or the Violet red bile glucose plate

Staphylococcus aureus

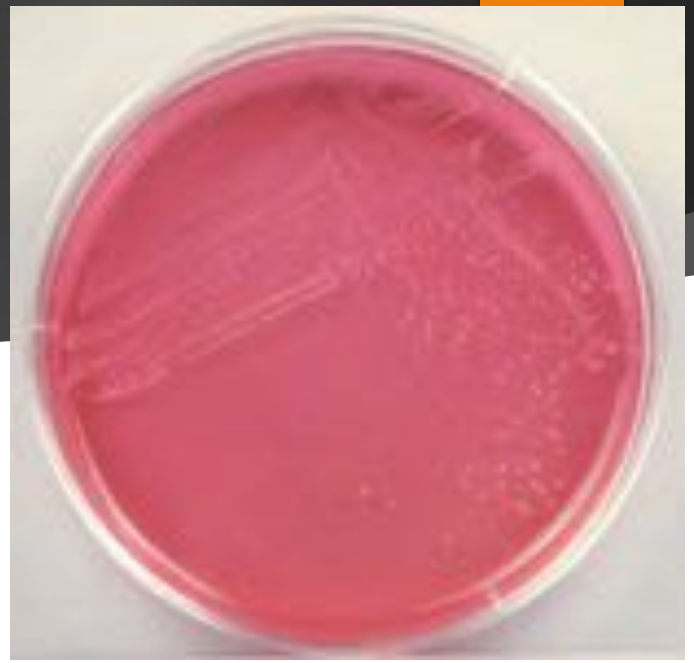
- Gram-positive bacteria
- Appears purple after gram stain
- Observed in round, grape-like clusters or pairs
- Confirmed with PCR if it matches the 16s rRNA sequence of S.*aureus*

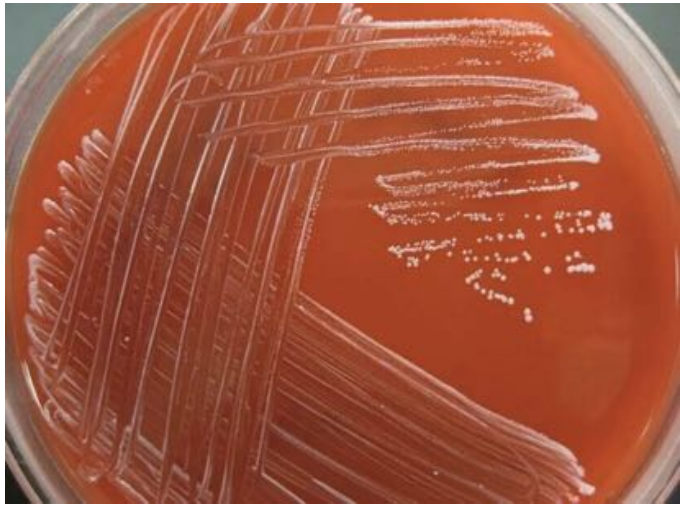




Staphylococcus epidermidis

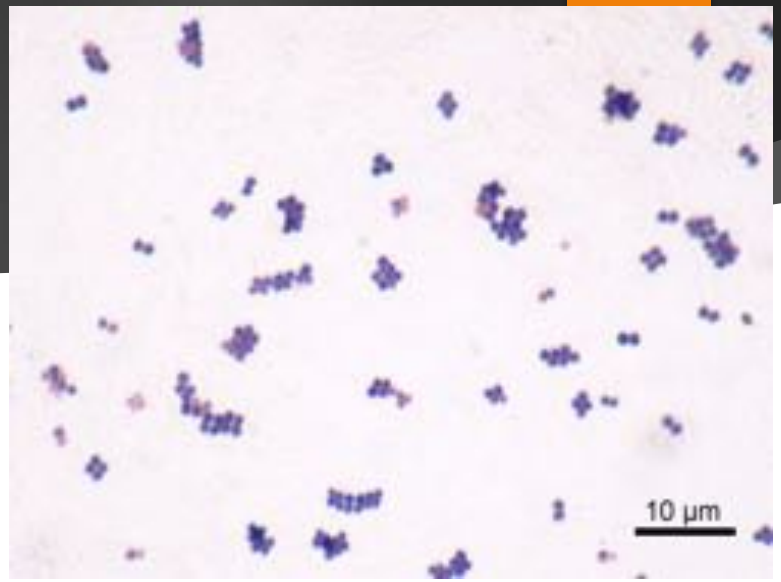
- Incapable of fermenting mannitol: no color change on mannitol salt agar
- Columbia blood agar: no destruction of hemoglobin, indicating γ-hemolysis
- Does not grow on SDC agar, Kanamycin Aesculin Agar, or the Violet red bile glucose plate

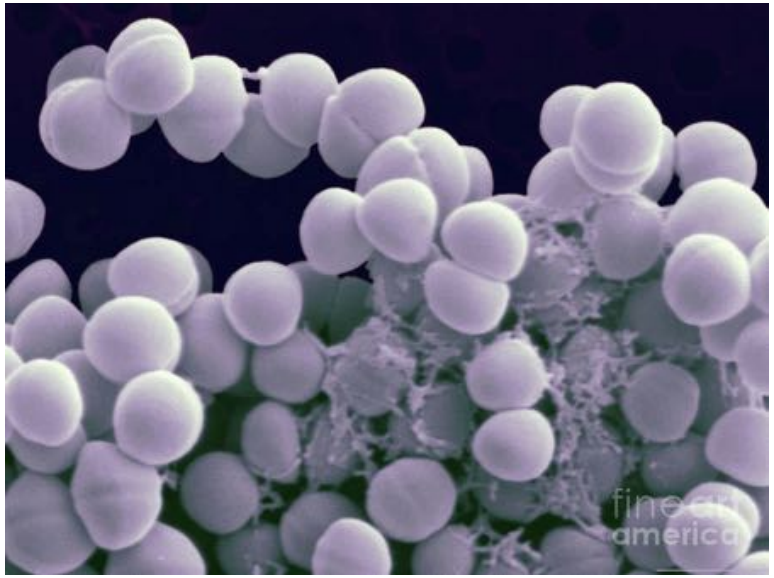




Staphylococcus epidermidis

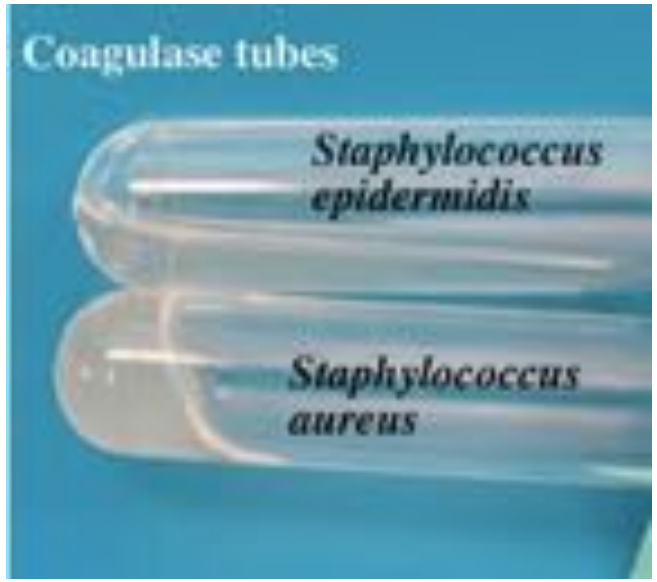
- Gram-positive bacteria
- Appears purple after gram stain
- Round, grey or greyish-white color
- Observed in pairs or tetrads





S. *aureus* vs. S. *epidermidis*

	Catalase Test	Oxidase Test	Coagulase Test
S.*aureus*	+	+	+
S.*epidermidis*	+	-	-



S. *aureus* vs. S. *epidermidis*