Staphylococcus aureus – BACTERIAL PATHOGENESIS

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Path 417a

CASE

Elizabeth's pregnancy and the birth of Amanda had gone well however, Elizabeth and Amanda were now struggling with breastfeeding. Elizabeth was aware from her prenatal classes of the various reasons why breastfeeding might be difficult. On the advice of a friend she made arrangements for a lactation consultant to visit her at home. She continued trying to 'latch' and feed Amanda in the days leading up to the visit but stopped when she began to experience breast pain and noticed that her right breast was red all around the nipple. She was feeling stressed and tired, along with a feeling of general malaise that she attributed to the stress associated with trying to breastfeed her newborn baby.

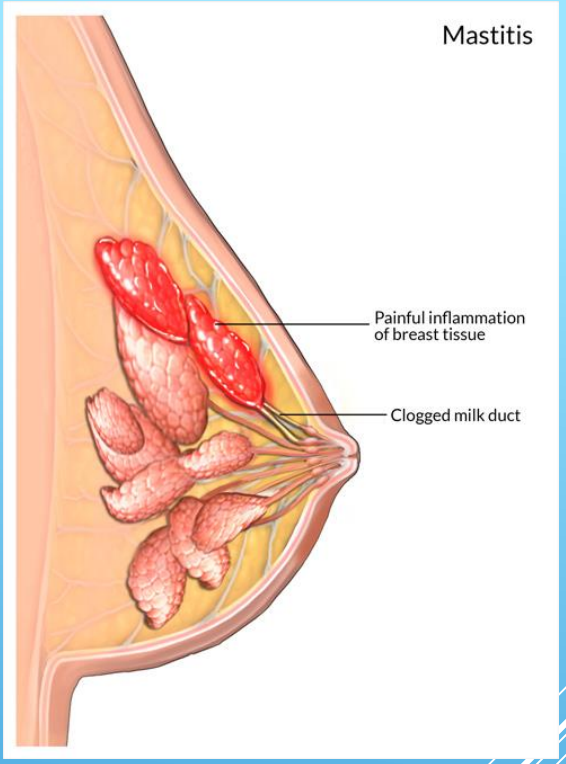
Based on Elizabeth's symptoms, the lactation consultant made a preliminary diagnosis of mastitis and suggested that Elizabeth see her doctor for a full diagnosis and possible antibiotic treatment. Do the symptoms that Elizabeth is experiencing concur with the preliminary diagnosis? What is the most likely bacterial cause and what are the antibiotics of choice to treat it?

1. ETIOLOGY

Common bacteria associated with the infection, likely cause

POSSIBLE BACTERIAL PATHOGENS

- Pathogens that cause mastitis prevent normal breastfeeding and change milk composition. Symptoms of infection such as redness and pain may appear around the nipple, and if not treated, can deteriorate to lactation failure, recurrent mastitis, or breast abscess
- Possible pathogens include bacteria (both gram positive and negative), mycoplasmas, fungi, and algae
- Common causative pathogens are: staphylococci, streptococci, *Esherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp.



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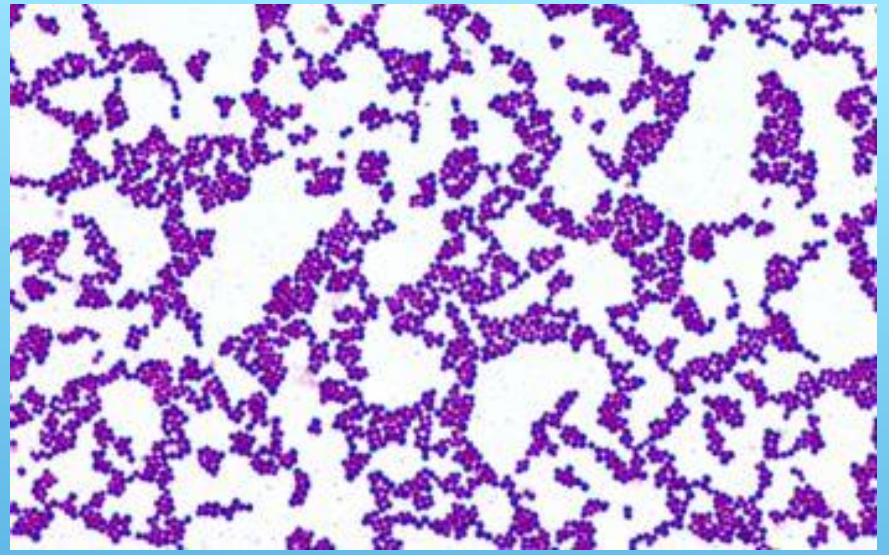
STAPHYLOCOCCI: *S. aureus*



- The most common cause of mastitis
- Gram-positive, grows in pairs and clusters in a specific configuration
- Found on damaged skin and lesions such as boils and abscesses
- Transmission occurs through the nipple of the breast, and the bacteria infects the mammary glands through a cracked nipple
- Common signs and symptoms include pain and redness, while infection can lead to malaise and fever
- *S. aureus* have many virulence factors (eg. Biofilms), immune evasion strategies, and are resistant to some antibiotics to promote adherence and growth of the bacteria

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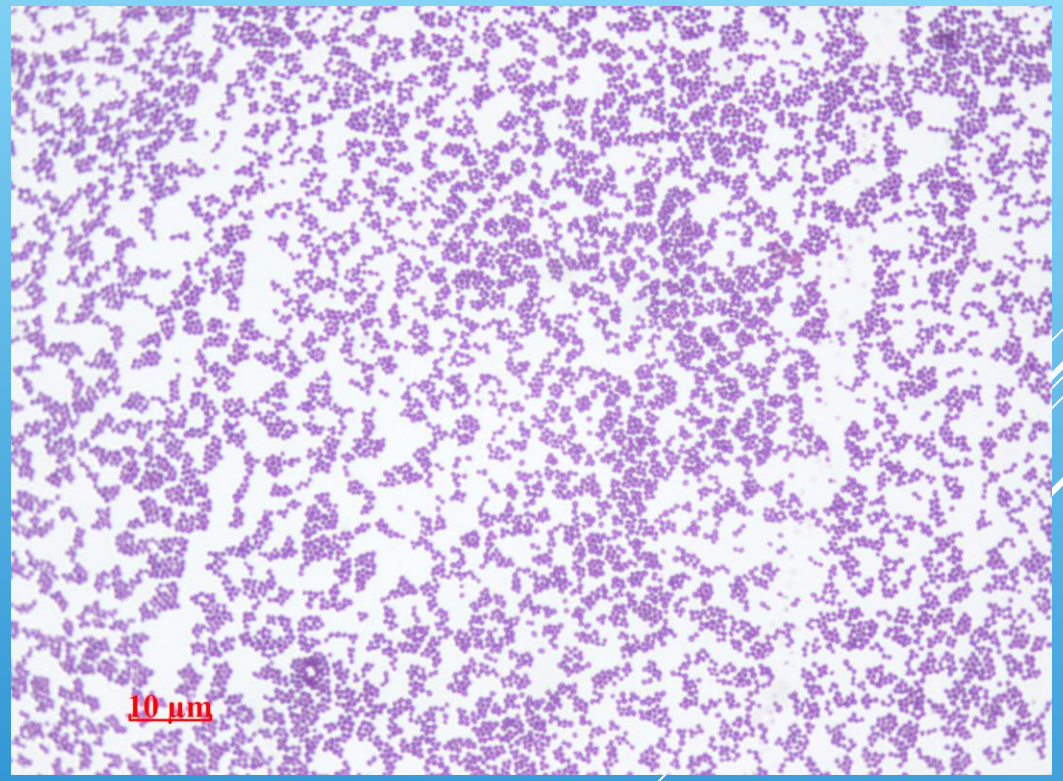
STAPHYLOCOCCI: *S. epidermidis*

- Gram-positive, grows in grape-like clusters
- Negative for coagulase enzyme, in contrast to *S. aureus*
- Opportunistic pathogen, resides in skin microbiome
- Also able to form biofilms, resistant to methicillin antibiotics



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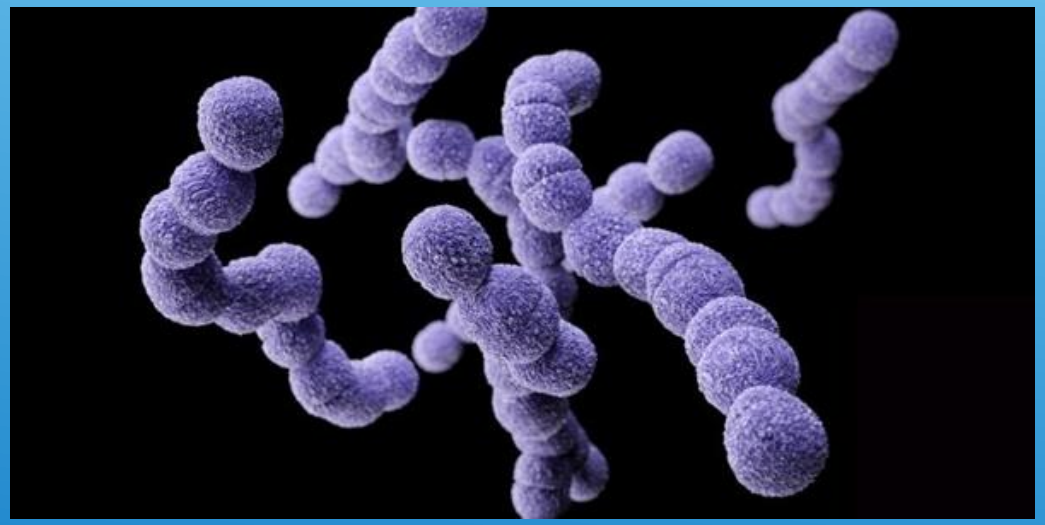
STREPTOCOCCI: GROUP B STREPTOCOCCI



- Gram-negative, non motile, non spore forming, in pairs or chains
- Different serotypes categorized by polysaccharide capsule, cell wall, pili antigens, or hemolytic
- Reside as normal flora in the vagina and intestines, are a cause of neonatal septicemia and meningitis
- Often resistant to tetracycline antibiotics. Penicillin, erythromycin, clindamycin, and macrolides are treatment options

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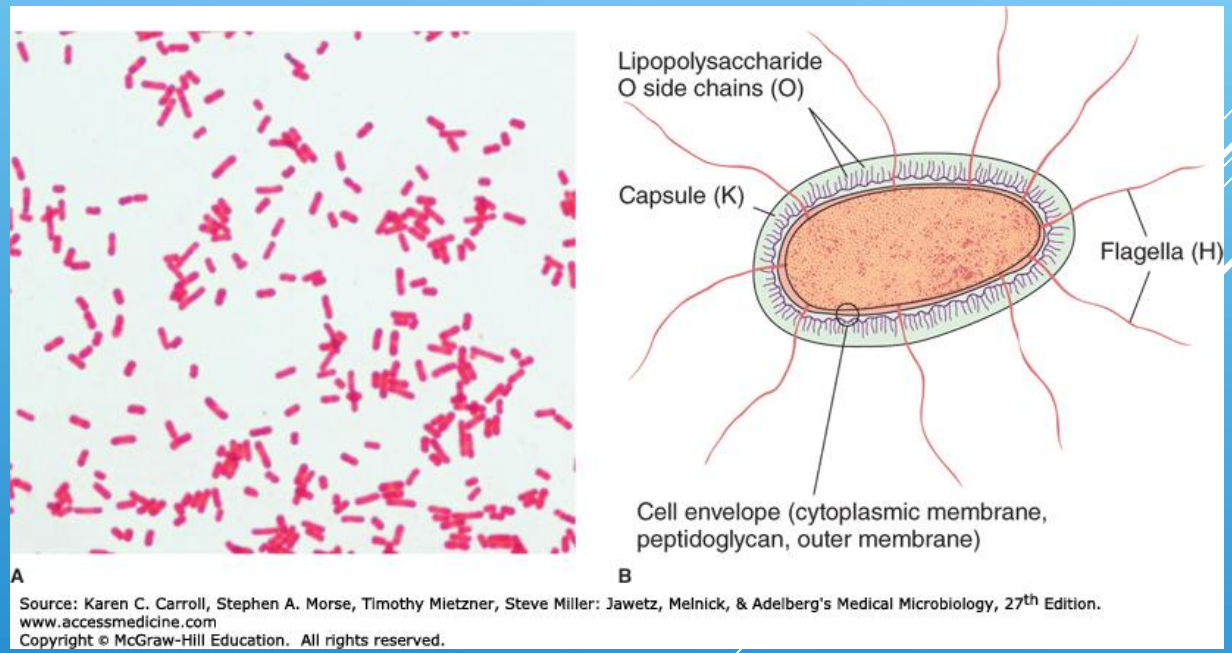
ENTEROBACTERIACEAE

- *Includes E. coli, Klebsiella spp, Serratia spp, Pseudomonas spp, Enterobacter spp, Shigella spp, and Citrobacter spp*
- Gram-negative, rod shaped, protective lipopolysaccharide cell wall
- Isolated on MacConkey agar
- Produce endotoxin
- Are not less of contagious pathogens but more of environmental pathogens for mastitis – reside in the environment instead of host to host transmission
- *E. coli* has shortest incubation period for mastitis, followed by *Klebsiella spp* and *Serratia spp*



Source: Karen C. Carroll, Stephen A. Morse, Timothy Mietzner, Steve Miller: Jawetz, Melnick, & Adelberg's Medical Microbiology, 27th Edition.
www.accessmedicine.com

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LIKELY CAUSE OF MASTITIS INFECTION

- *Staphylococcus aureus* and *Group B streptococcus* seem to have the most pathogenic potential to cause mastitis, and are more commonly seen as the source of infection
- Coagulase-negative *S. epidermis* seems to be one of the most prevalent in bovine mastitis, and may be applicable to humans
- Knowledge is limited because most research on mastitis have been done more on cows than humans, and current information may be completely transferrable to human breast mastitis

2. LABORATORY SAMPLES

Samples for lab testing, implications in diagnosis

SAMPLES FOR LABORATORY TESTING

Breast milk samples

- May not provide the most accurate results: false-positives from normal bacterial colonization, false-negatives may fail to detect mastitis
- *S. aureus* and coagulase-negative *Staphylococcus spp* are most commonly found
- Antibody tests to determine infection or colonization against identified bacteria not routinely performed
- Milk samples must be taken if there's no response 2 days post-antibiotic therapy, patient is allergic to antibiotics, mastitis recurs, or the identified bacteria is Methicillin-resistant *S. aureus*

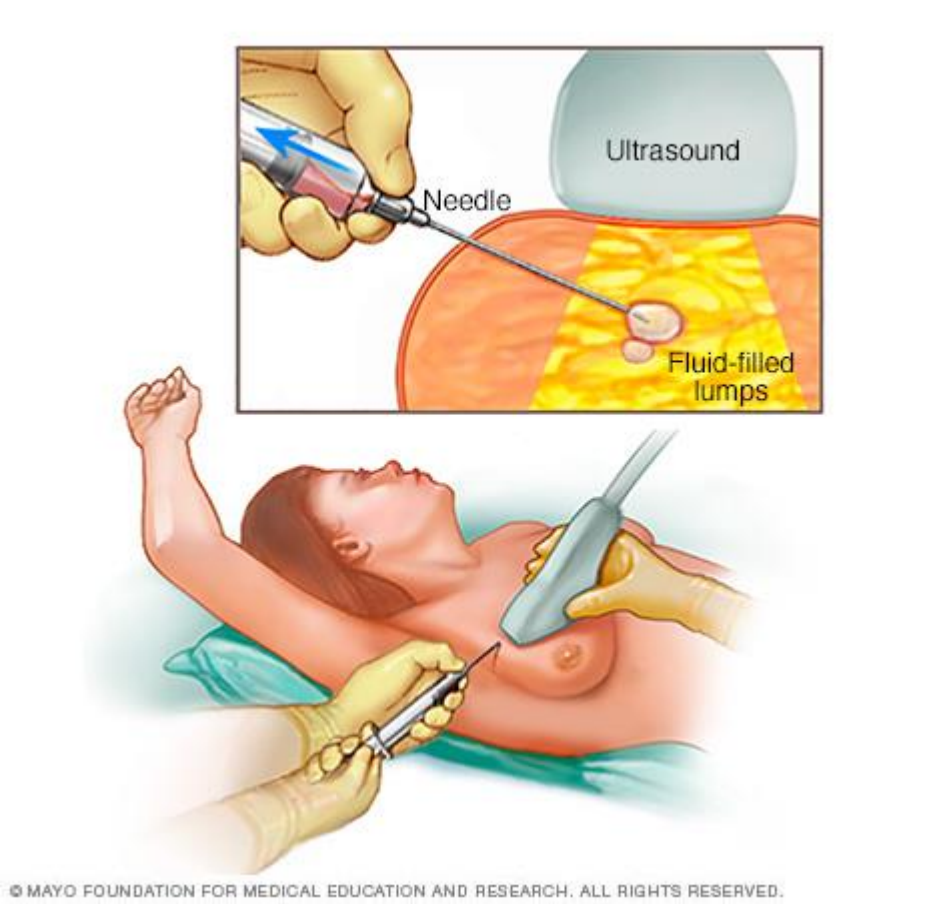
Culture for definitive diagnosis

- helpful during complications listed above, to identify specific bacterial agent

Staphylococcus aureus* and *Staphyloccocus epidermidis

http://www.healthhype.com/wp-content/uploads/staphylococcus_aureus_epidermidis_culture_colony.bmp

SAMPLES FOR LABORATORY TESTING



- Breast and nipple should be cleaned with alcohol, and express milk into sterile container
- Milk samples may only be sterile 50% of the time, due to bacteria part of skin flora
- If abscesses are present around the breast, abscess fluid can be collected for testing
  - Fine needle aspiration and drainage by catheter to collect samples for cytology

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SIGNIFICANCE OF LABORATORY TESTING

- Physical signs and patient symptoms may be sufficient for diagnosis and prescription of antibiotics
  - Redness, unilateral breast tenderness, abscesses
  - Malaise, fatigue, fever
- But lab testing is necessary if infection is severe, hospital acquired or doesn’t respond to antibiotic treatment
- Identifying specific bacterial strains may help guide the appropriate antibiotic treatment, and can be more effective than broad-spectrum antibiotics
- In addition, prevents antibiotic resistance, reduces patient suffering, and lowers cost

3. TESTING

Tests for pathogen identification

TESTING

Morphological Testing

- Cell shape, size, special structures e.g. endospores, granule and capsules

Gram-staining

- Determine if bacteria is gram positive/negative
- Performed on isolated species
- Gram positive: thick peptidoglycan layer
  Gram negative: peptidoglycan layer and outer lipopolysaccharide layer

Table 7.2 : Growths on nutrient agar (aerobic incubator at 37°C)

	Colony character	Morphology	Motility	Prov Diag	Further tests required
1.	Bluish or yellowish green pigment diffused into medium. Margin-irregular. Surface-Smooth or crenated or rugosed. Semitranslucent. Fruity smell. Raised colony. In liquid medium-surface pellicle.	Gram –ve slender short rods, discretely arranged	Motile	*Pseudomonas Spp.*	Oxidase, OF or Glucose fermentation, Extractibility of pigment with water (*aerogenes*) or both with water and chloroform (fluorescein). Confirmatory test for *Pseudomonas* — vide Table A.
2.	Dome shaped, butyrous, opaque, 2-3 mm diameter, golden yellow or white or non-pigmented colony. Pigment restricted to colony. In liquid medium uniform turbidity with deposit.	Gram +ve cocci in irregular cluster.	Non-motile	• *Staphylococcus Spp.*	Tests to differentiate the bacteria from *Micrococcus* and tests for species identification — vide Table B.
3.	Large opaque, raised, irregular surfaced and margined, non-pigmented colony. In liquid medium form pellicle.	Gram +ve stout long rods with unstained endospore space.	Motile or Non-motile	• *Bacillus Spp.*	Follow the scheme of table 'C' to confirm two pathogenic species *B. anthracis* and *B. cereus*.
4.	Flat or raised, or convex colony. Size moderate to large, smooth surfaced colony. Translucency — transparent to opaque. Usually non-pigmented (except *S. marcescens*).	Gram –ve rods, discretely arranged.	usually motile. Non-motile *Variants: Kleb, Shigella*	*Family* : O.F Oxidase Nitrate Follow • Enterobacteriaceae → F (–) + Table D • Vibrionaceae → F + + Table E • Afermentative Gram –ve rods → No reaction +/– +/– Table F • *Stenotrophomonas* • *Burkholderia* • Pigmented *Pseudomonas* • *P. stutzeri* → O + (except *P. maltophila*) + Table A

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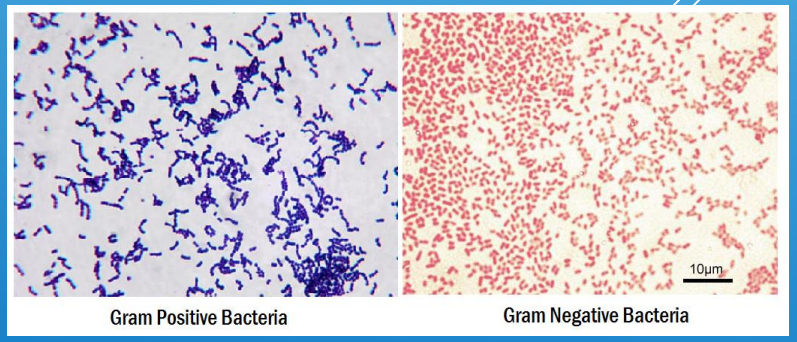

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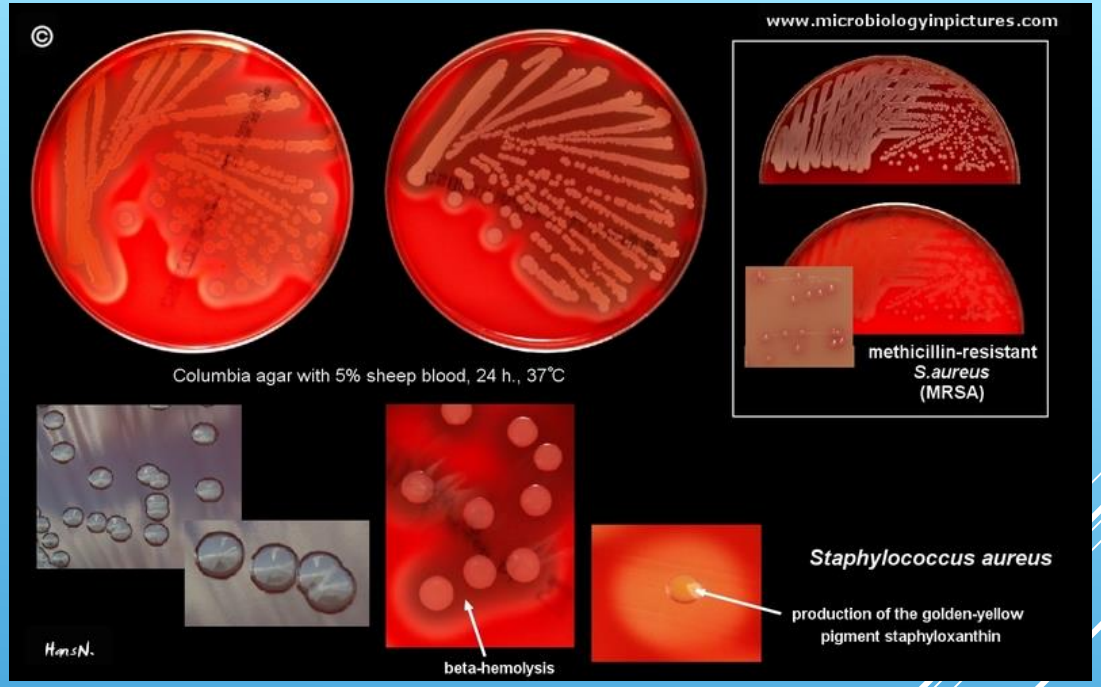
TESTING

Culture

- Milk samples are diluted with peptone water, then plated on multiple different agars to test the growth conditions of the bacteria
- Columbia blood agar: identifies streptococci, staphylococci, and similar bacteria
  - indicates α –hemolysis (green coloration), β –hemolysis (clear zone), or γ –hemolysis (no change, no hemoglobin destruction)
- Sabouraud chloramphenicol (SDC) agar: identifies yeast
  - Chrloramphenicol inhibits bacteria, allows fungal and yeast growth, appearing as cream-colored for yeast and multi-colored for mold



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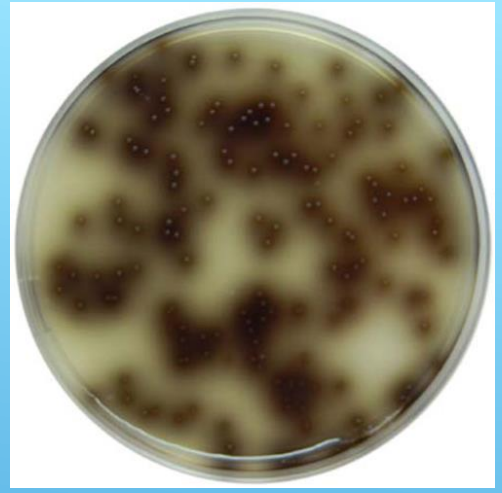
TESTING

- Kanamycin Aesculin Azide agar: identifies enterococci
  - Kanamycin sulphate and sodium azide select for enterococci, appearing as white or grey colonies surrounded by black zones
- Violet red bile glucose plate: identifies gram-negative bacteria that ferment glucose
  - Bile salts and crystal violet inhibit gram-positive bacteria, colonies look red and purple
  - Neutral red indicates pH
- Mannitol Salt Agar: identifies Staphylococci
  - Contains 7.5% NaCl and mannitol salt to select for Staphylococci that ferment mannitol (*S. epidermis*), colonies look yellow/gold



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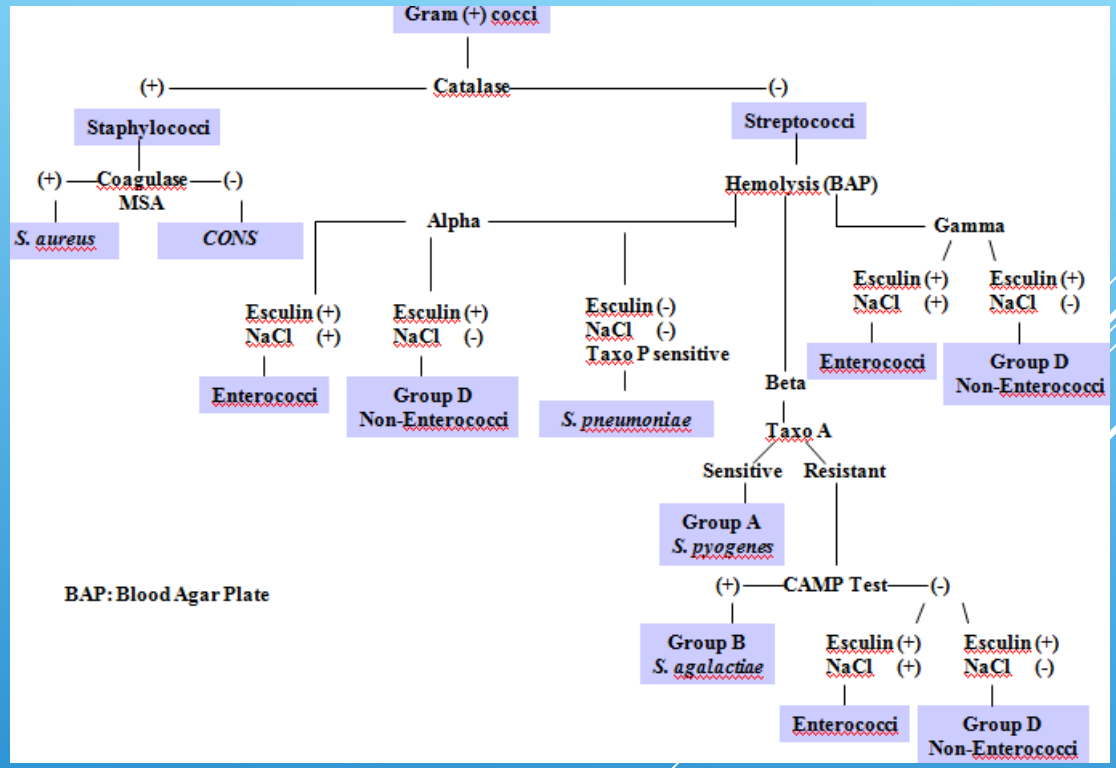
TESTING

Biochemical Testing

- Catalase test: catalase hydrolyzes hydrogen peroxide into water and oxygen
  - Positive test: bubbling
- Oxidase test: cytochrome oxidase adds electrons to oxygen at the electron transport chain
  - Positive test: dark purple coloration
- Coagulase test: coagulase converts plasma fibrin to insoluble fibrin
  - Positive test: forms clot
- Hemolysin test: hemolysins lyse red blood cells, degrade hemoglobin
  - Beta-hemolysin breaks down red blood cells and hemoglobin completely -> clear zone around colony; Alpha-hemolysin partially breaks down red blood cells -> green color; no hemolysin -> no clearing around colony



TESTING

Genotypic/Molecular Testing

- Faster than biochemical tests
- Identifies housekeeping genes through extraction, PCR, and sequencing
  - 16S rRNA: separate broader bacterial genuses
  - Cpn60 (chaperonin/heat shock protein 60), dnaJ (heat-shock protein 40), rpoB (beta subunit of RNA polymerase), sodA (superoxide dismutase A), and tuf (elongation factor Tu)

Antibiotic Susceptibility Testing

- VITEK 2 system: place swabs of bacterial culture in test tube, and following contact with different antibiotics, the machine measures fluorescence, turbidity, and colorimetric signal
- Diffusion antibiotic disc assay: bacterial broth culture spread on agar plate, treated with antibiotic disks and zones of inhibition measured to determine if bacteria is resistant, intermediate or susceptible to the antibiotic

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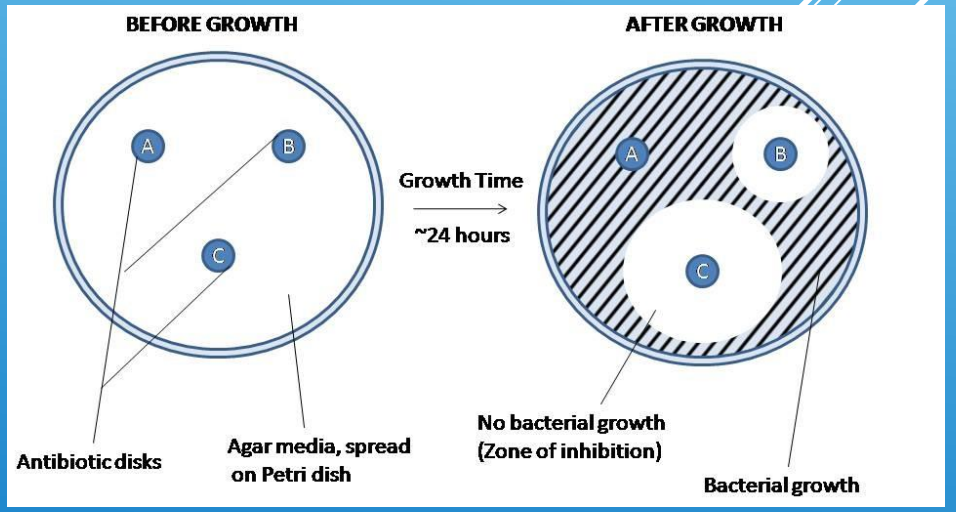

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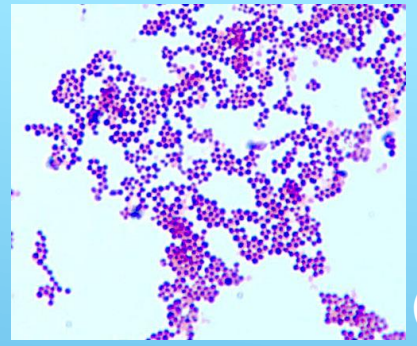
4. TESTING RESULTS

Expected results for *S. aureus*

EXPECTED RESULTS

(1) 

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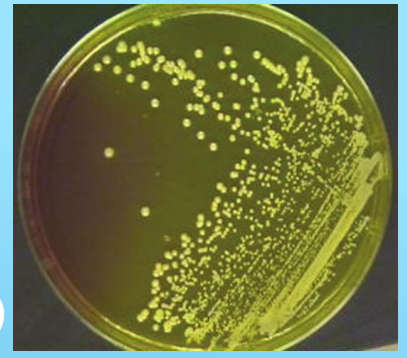
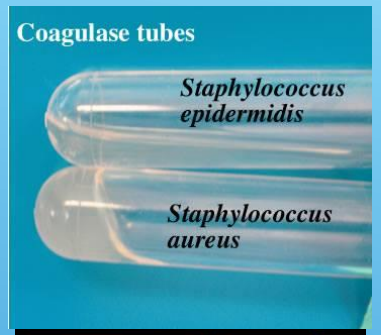
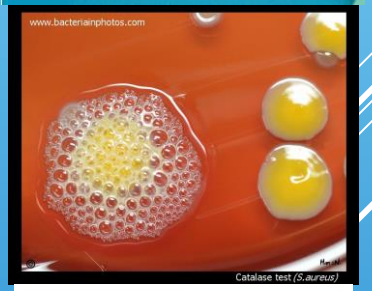
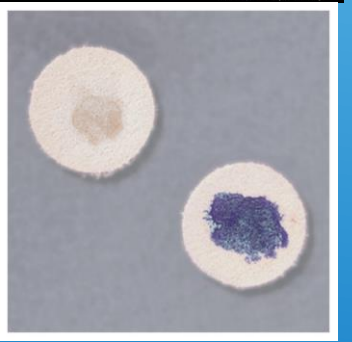
(3) 

- *S. aureus* morphology: pairs, clusters, or short chains of circular cocci, that are convex and 0.5 -1um in diameter

1. Gram-staining would result in gram-positive purple bacteria
2. See clear clear zones to indicate ß-hemolysis on Columbia blood agar
3. Able to grow on MSA, ferments mannitol into acids and lowers the pH, the phenol red indicator will turn yellow
4. Does not grow on SDC, the Kanamycin Aesculin Azide, or the Violet red bile glucose plate
5. *S. aureus* is coagulase-(+) with clot formation, catalase-(+) with rapid bubbling, oxidase-(+) with color change to dark purple
6. Sequencing will match the 16S rRNA of *S. aureus*
7. *S. aureus* is sensitive to ampicillin, streptomycin, erythromycin, and cefoxitin, although the antibiotic disc assay depends on the specific strain of *S. aureus*







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