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Basics of Differentiation of Gram-positive Cocci

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Gram-positive Cocci

Classification: 4 Primary Families

- ***Micrococcaceae***
  - Includes *Micrococcus, Kocuria, Kytococcus, Rothia* spp.
  - Opportunists
- ***Staphylococcaceae***
  - Includes *Staphylococcus* spp.
  - Clinically relevant: *S. aureus, S. epidermidis, S. saprophyticus*
- ***Streptococcaceae***
  - Includes *Streptococcus* spp.
  - Clinically relevant: S. pyogenes, S. agalactiae, S. pneumoniae
- ***Enterococcaceae***
  - Clinically relevant: *Enterococcus faecalis* and *Enterococcus faecium*
- Miscellaneous genera

Differentiation of Gram-positive Cocci

- Colony morphology and pigmentation, if present
- Hemolytic reaction on Sheep Blood Agar*(sBAP)
- Gram's Stain Morphology
- Catalase
- Additional Identification assays

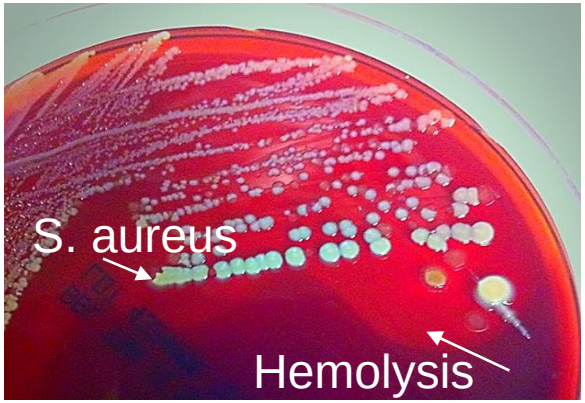
***Trypticase soy agar with 5% defribrinated sheep blood**

Differentiation of Gram-positive Cocci Culture Morphology

Micrococci

Pigmented *M. luteus*

Staphylococci



Mixed culture of *S. aureus* and *S. epidermidis*. Note golden pigment and beta-hemolysis of *S. aureus.*

Streptococci

Streptococci and enterococci often form small gray colonies. The hemolytic reaction varies depending on the species.

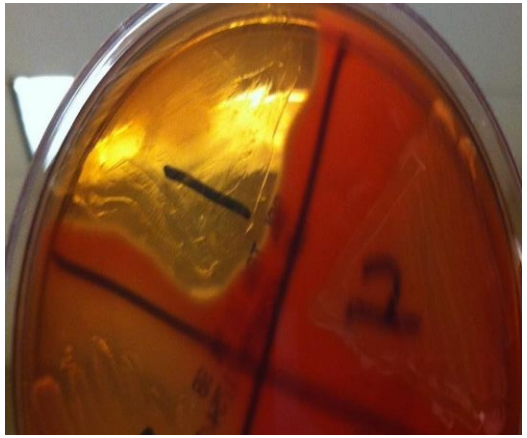
Hemolysis on sBAP

Hemolysis: destruction of erythrocytes in the agar medium by bacterial toxins produced by the growing organism

- Beta-hemolysis
  - » complete destruction(lysis)
- Alpha-hemolysis
  - » incomplete lysis (“greening”)
- Gamma-hemolysis
  - » no hemolytic reaction

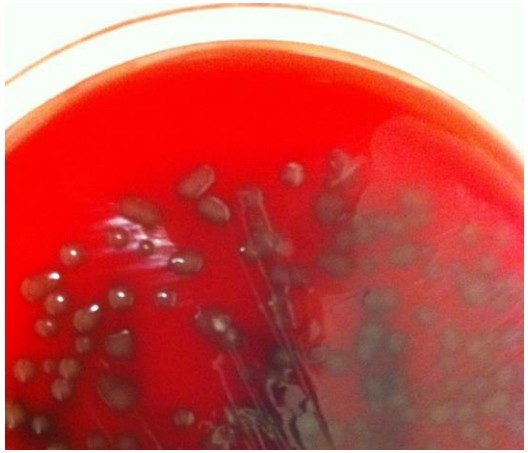
Differentiation of Gram-positive Cocci Hemolytic Reaction on sBAP

Beta-Hemolytic



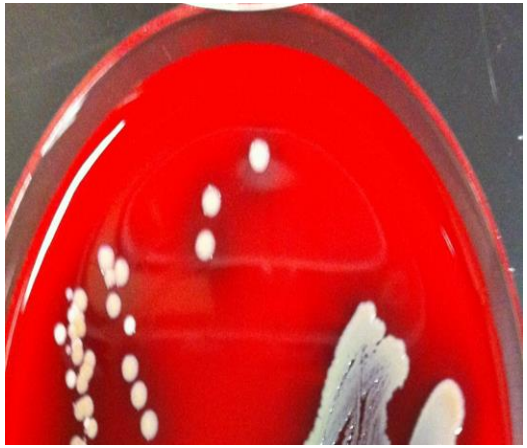
Examples:
S. aureus, b-hemolytic streptococci

Alpha-Hemolytic



Examples:
S. pneumoniae, viridans streptococci

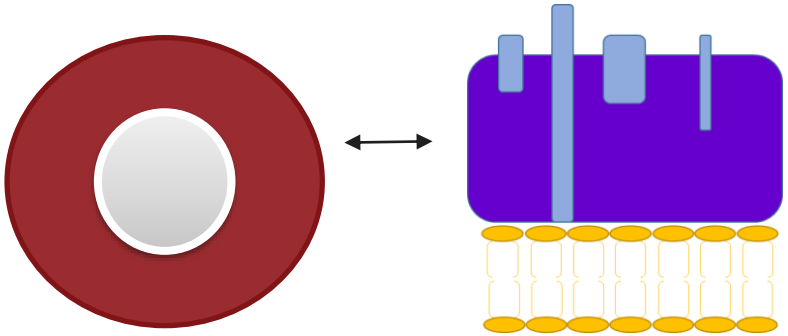
Gamma-Hemolytic



Examples:
Staphylococcus spp.
Some *Enterococcus spp*.

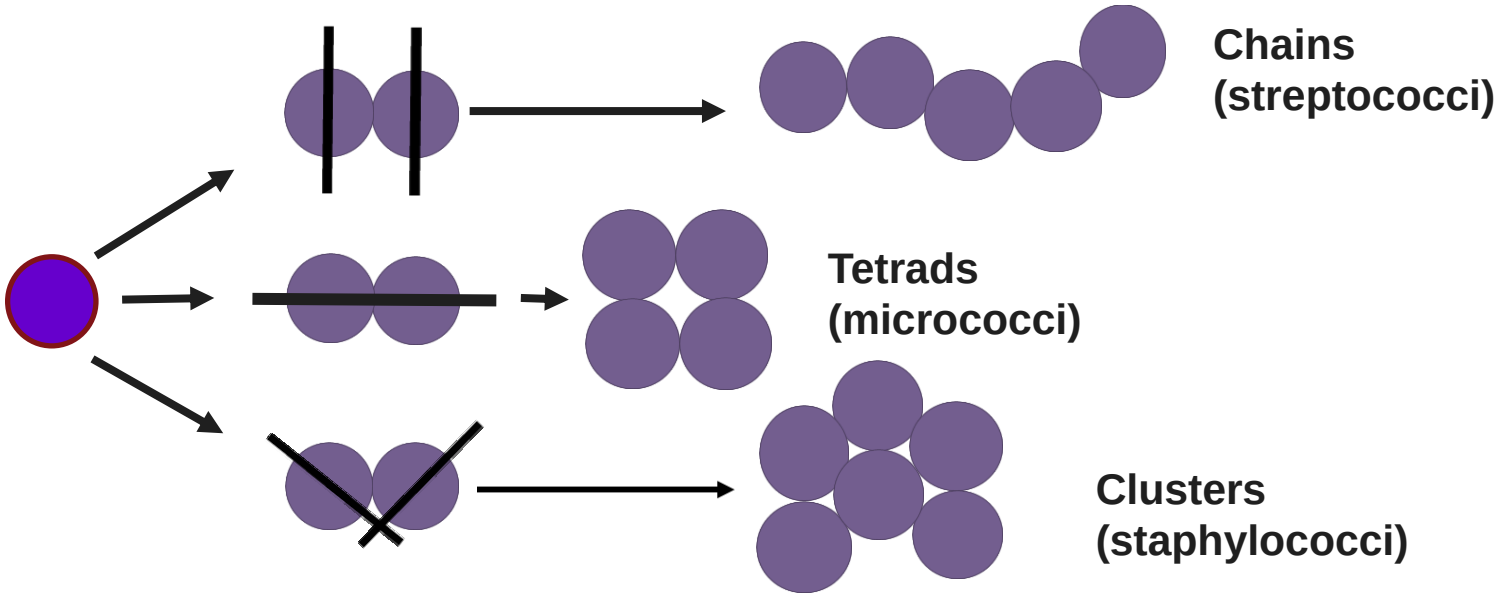
Microscopic Morphology

- **Gram Staining Characteristics**
  - Based on the bacterial cell-wall structure
  - Gram-positive organisms have thick cell walls
  - Cell wall retains the primary stain, crystal violet
  - A gram-positive organism appears purple



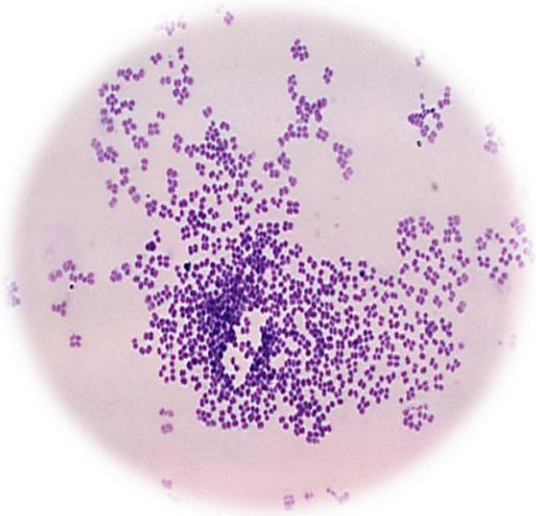
Microscopic Morphology

The microscopic morphology of Gram-positive cocci depends on the plane of cellular division of the different types of organisms



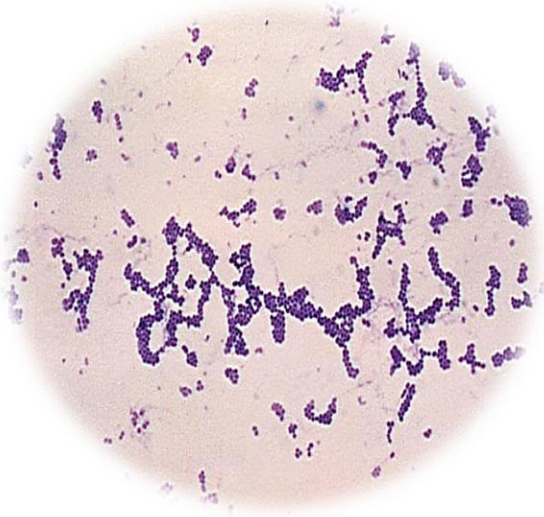
Differentiation of Gram-positive Cocci
Gram Stain Morphology

Cocci in Tetrads



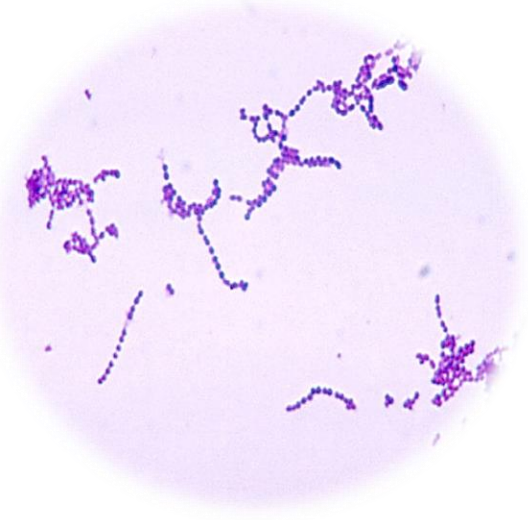
micrococci

Cocci in Clusters



staphylococci

Cocci in Chains/Pairs



streptococci/ enterococci

Images courtesy of Dr. Richard Facklam, Centers for Disease Control

Catalase Reaction

- **Catalase:** an enzyme produced by the bacterium which neutralizes the toxic effects of hydrogen peroxide, a metabolic by-product
  - If a sample of the bacterium is mixed with hydrogen peroxide, the enzymatic breakdown of the substrate to water and oxygen produces visible bubbles.
  - The production of bubbles indicates that the organism is a catalase producer (+)

Differentiation of Gram-positive Cocci

Catalase Test

Positive

staphylococci
micrococci

Positive catalase test

Negative

streptococci
enterococci
Miscellaneous GPC

Differentiation Within the Families of GPC

- ***Micrococcus spp.***
  - Differentiated from other GPC based on colony and gram-stain morphology
  - Rapid ID includes: susceptibility to bacitracin, furazolidone, and modified oxidase test
- ***Staphylococcus spp.***
  - Categorized within the genus primarily by using coagulase reaction
  - Other biochemical tests are used for more precise speciation
- ***Streptococcus and Enterococcus spp.***
  - Categorized on their hemolytic reaction on sBAP, the presence of unique carbohydrates in their cell walls (Lancefield typing), and other biochemical tests unique to the different species

Differentiation Within Families of GPC

- **Differentiation of Staphylococci: coagulase production**
  - Coagulase is an enzyme involved in the conversion of serum fibrinogen to fibrin (clot production)
  - Coagulase is used by *Staphylococcus aureus* as a virulence factor (coagulase (+))
  - Many other species of staphylococci do not produce coagulase (coagulase (-))

The Coagulase Test

The coagulase test is performed by mixing a sample of organism with rabbit plasma. Following incubation at 37ºC, if the plasma forms a clot, the organism is coagulase positive.

No clot formation: Coagulase (-)

Clot formation: Coagulase (+)

Differentiation of Gram-positive Cocci

Organism	Colony Morphology	Hemolytic Reaction on sBAP	Microscopic Morphology	Differential Diagnostic Tests
Micrococci	Often pigmented	gamma-hemolytic	Tetrads,	Catalase (+)
Staphylococcus aureus	Medium-large creamy, golden	beta-hemolytic	Clusters	Catalase (+) Coagulase (+)
Other Staphylococci	Small-medium Creamy, white	gamma-hemolytic	Clusters	Catalase (+) Coagulase (-)
Streptococci*	Small, gray	Species dependent	Pairs, chains	Catalase (-)
Enterococci*	Smooth, cream white-gray	Species dependent	Pairs, short chains	Catalase (-)

**The differentiation of streptococci and enterococci is based on more extensive biochemical testing*

Miscellaneous GPC

- A variety of Gram-positive cocci belonging to different genera can be found as opportunists or part of resident flora in clinical specimens
- These organisms can also be categorized based on the catalase reaction, colony morphology, and Gram-stain morphology
- The identification of these organisms relies on a list of biochemical assays performed in most clinical microbiology laboratories

References

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Disclosures/Potential Conflicts of Interest

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