

# Identification of Bacteria

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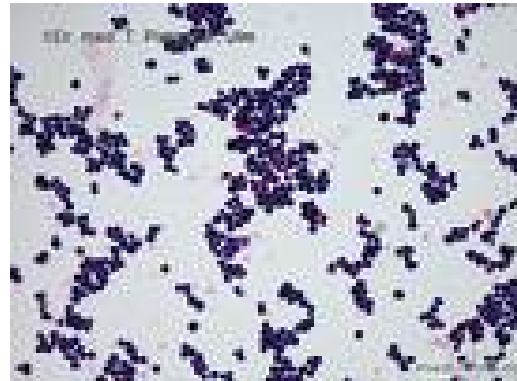
University hospitals of Leicester

Junior Doctors Tutorials

(6/9/06)

# Genus Staphylococcus

Microscopy



Gram positive cocci  
in clusters

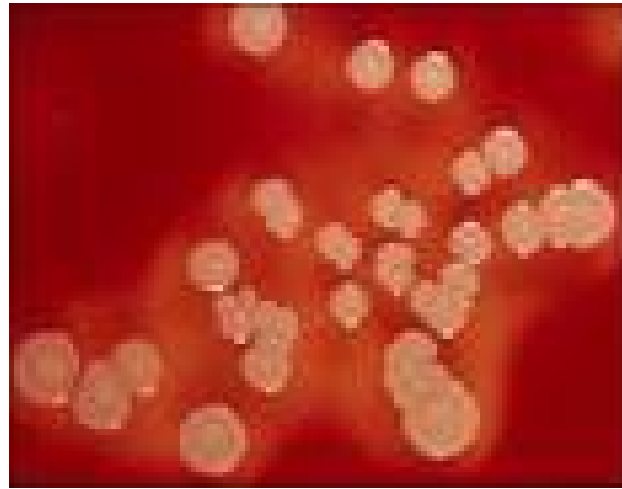
Gram Positive ?

Clusters

Problems ?

# Genus Staphylococcus

Culture



1-3 mm

Smooth

Low convex

Opaque

Buff or gold

+/- B hemolysis

Incubation

Other culture media...

# Other culture media

- Selective / Enrichment media  
7-10% salt containing broth and agar  
Salt cooked meat broth  
Salt agar
- Differential agar  
Mannitol(1%) salt(7.5%) agar with phenol red as indicator
- Baird - Parker medium
- Methicillin(10mg/l) mannitol salt agar
- Cefoxitin salt agar



# What media do we use for MRSA screening?

- Chromogenic agar with 8mg/l Oxacillin
- Pink colonies
- Confirm with a 10mg Cefoxitin disc (BSAC <22mm = MRSA) - why confirm with cefoxitin??

# How to proceed with the identification

- Catalase test - what, why and how
- Coagulase test - what why and how

# Catalase test

- Principle - tests the ability of the organism to liberate  $O_2$  from  $H_2O_2$  by the action of catalase
- Method
- 3%  $H_2O_2$  & few colonies from a non blood agar medium
- Controls (positive and negative)
- Immediate vigorous bubbling

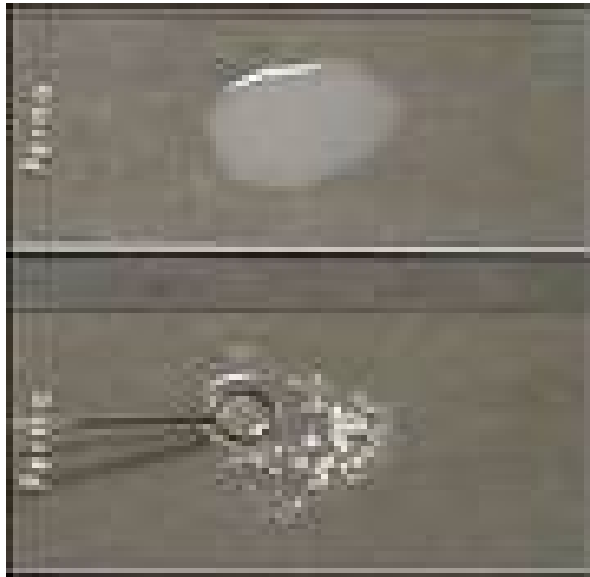


# Coagulase test

- Enzyme
- Two forms Bound (clumping factor)  
Free (coagulase)
- Converts fibrinogen in plasma to a fibrin (clot)
- Plasma  $\xrightarrow{\text{coagulase}}$  Fibrin clot

# Methods

Plasma (human, rabbit, horse) & lot of colonies and controls



Slide test (clumping factor)

Presumptive needs confirming with the tube test



Tube test (free coagulase)

Check tubes at  $\frac{1}{2}$ , 1, 2 & 4 hrs and overnight

# Interpretation

- auto-coagulation of slide test - discard
- Negative slide tests - tube test
- Negative tube test at 4 hrs - overnight
- Lysis of clot in the tube test - repeat
- Citrate consuming organisms and false positive coagulase test - Gram negative bacilli and some *E faecalis*

# Coagulase positive Staphylococci

- *Staphylococcus aureus*
- *Staphylococcus intermedius* (dogs mouth)  
(positive for both clumping factor and free coagulase)
- *Staphylococcus lugdunensis*  
(positive for clumping factor - CNS)
- Other organisms ?

# Coagulase negative Staphylococci

- *Staphylococcus epidermidis* - slime production
- *Staphylococcus lugdunensis* - ornithine decarboxylase
- *Staphylococcus haemolyticus* -  $\beta$  haemolysis
- *Staphylococcus saprophyticus* - resistant to 5 $\mu$ g novobiocin (< 15mm zone diameter)

# Other tests

- Deoxyribonuclease (DNase) test
- Heat stable thermonuclease (TNase) test
- Protein A
- API StAPH
- Toxin tests
- Gene tests
- Sensitivity tests

