

**Gram-Positive Cocci (1):  
Staphylococci**

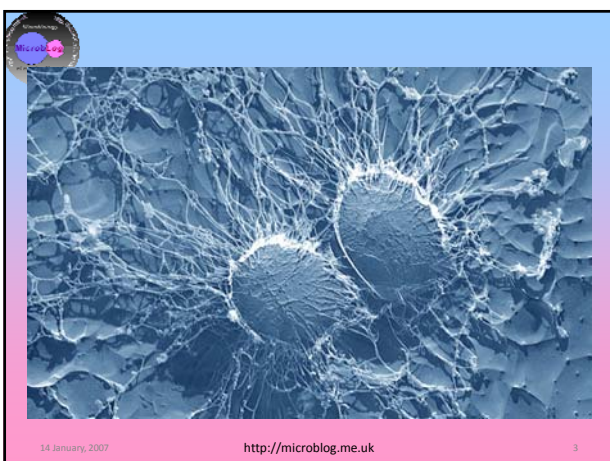
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SpR  
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**Topics**

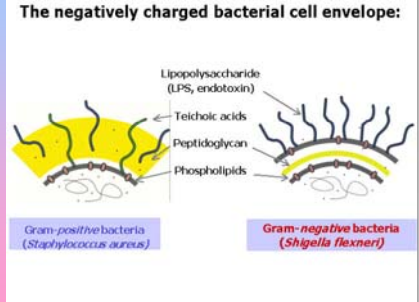
- Basic Structure
- Phenotypic profile
- Microscopy & Culture
- Laboratory Identification
- Pathogenesis
- Clinical Significance

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**Structure**


**The negatively charged bacterial cell envelope:**



Lipopolysaccharide (LPS, endotoxin)  
Teichoic acids  
Peptidoglycan  
Phospholipids

Gram-positive bacteria (*Staphylococcus aureus*)  
Gram-negative bacteria (*Shigella flexneri*)


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## Phenotypic Profile

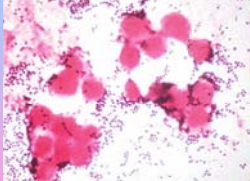
- 1-3mm long (comparatively large colonies)
- Smooth
- Low convex
- Opaque
- Buff or gold coloured
- +/-  $\beta$ -haemolysis

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## Microscopy

- Gram positive cocci
- Bunch of grapes appearance
- Binary fission in 3D
- Not a method of identification




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## Culture

- *Staphylococcus spp.* grow better on the aerobic plate.
- They will have more colourful and larger colonies than the anaerobic plate.
- *Staphylococcus spp.* will grow on any media. They are salt tolerant, as most organisms are killed by high [NaCl].

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## Media (1)

- Mannitol Salt agar (MSA)
  - 7-10% NaCl.
  - Indicator media containing **phenol red** turns SA colonies yellow (it turns pink in alkaline conditions). CoNS don't usually ferment mannitol, which aids ID.
- Baird-Parker
  - lithium chloride and **tellurite** (inhibit the growth of accompanying microbial flora)
  - pyruvate and glycine selectively stimulate the growth of staphylococci.
  - Opaque medium because of its egg-yolk content
  - characteristic zones and rings are formed as a result of lipolysis and proteolysis
  - reduction of tellurite to tellurium produces a black colouration

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## Media (2)

- Blood
- Chocolate
- CLED
  - Cystine Lactose Electrolyte Deficient
  - Bromthymol blue is used as a pH indicator. Media turned from green → yellow as lactose is fermented and pH ↓
- Chromogenic (MRSA)
  - Trade secrets (!) – 8mg/L oxacillin

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## Oxford Staph (Blood)



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## Oxford Staph (Baird-Parker)



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## Oxford Staph (Choc)




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### Oxford Staph (Chromo)




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### MRSA (Chromo)



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### Oxford Staph (CLED)



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### Oxford Staph (MSA)



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## Laboratory ID

- Catalase
- Coagulase
- DNase
- TNase
- Protein A
- PCR
  - Toxin typing
  - Phage typing
- MRSA

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## Catalase

- Catalase: Staphs (+), Streps (-)
- Method: Staph produce catalase:  $H_2O_2 \rightarrow H_2O + O_2$

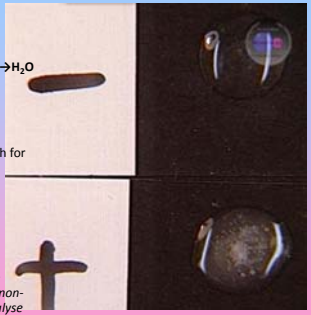
Slide Test:

- Drop of  $H_2O_2$  on a slide
- Smear colonies onto a coverslip – watch for immediate vigorous bubbles

Tube Method:

- 1ml  $H_2O_2$  in a tube
- Pick colony with a wooden stick
- Watch for immediate vigorous bubbles

*REMEMBER to pick the colonies from a non-blood agar plate, as blood contains catalase and can give a false positive. Choc agar is OK.*



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## Coagulase

- Enzyme in two forms
  - Bound (clumping factor)
  - Free (coagulase)
- Converts fibrinogen in plasma to a fibrin (clot)
- Plasma → Fibrin clot
  - ↑
  - Coagulase
- Need a thick suspension with a large inoculum
- Check saline alone for auto-agglutination



Slide test (clumping factor)  
Presumptive needs confirming with the tube test

Tube test (free coagulase)  
Check tubes at 1/2, 1, 2 & 4 hrs and overnight

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## Pitfalls

- Auto-agglutination: discard test
- -ve slide test: do a tube test
  - ? not a CoNS
  - Dilute plasma 1:10
  - Incubate @ 37°C
  - Check @ 1/2hr, 1hr, 2hrs, up to 4 hrs – keep checking as clot may lyse due to streptokinase – if this happens REPEAT
  - If no clot @ 4hrs – incubate overnight to be sure
  - Citrate consuming organisms give a false +ve

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### DNase

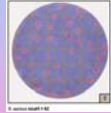
- Tests for DNase production.
- DNase agar (nutrient enriched, contains DNA, and methyl green as an indicator).
- Methyl green is a cation which binds to the negatively-charged DNA.
- When the DNA is broken down, it no longer binds to the methyl green, and a clear halo will appear around the areas where the DNase-producing organism has grown.



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### TNase

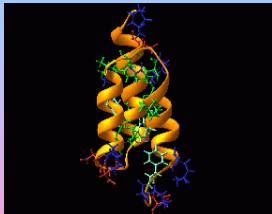
- Heat stable thermonuclease
- Broth heated prior to insertion in the agar
- Suspended colonies put into wells to allow diffusion, the indicator turns blue around each well (TNase+).



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### Protein A

- Found in *Staphylococcus aureus*
- Binds to the F<sub>c</sub> of IgG
- Better than a slide test
- Coloured clumps appear as protein A is found



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### PCR

- Toxin typing
  - PVL, TSST-1 etc.
- 16-S studies
  - Rapid molecular diagnostics
  - Taxonomy

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## Problems

- BORSA
  - Borderline oxacillin resistant *S. aureus*
  - Due to hyperproduction of  $\beta$ -lactamase
  - Cefotaxim sensitive
- Heteroresistance
  - two subpopulations (one susceptible and the other resistant) that may coexist within a culture.
  - incubate at 35° C for a full 24 hours before reading (this  $\uparrow\uparrow$  gene expression).

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## PATHOGENESIS

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### Virulence Factors


- **Surface proteins** that promote colonization of host tissues
- **invasins** that promote bacterial spread in tissues (**leukocidin, kinases, hyaluronidase**)
- **surface factors** that inhibit phagocytic engulfment (**capsule, Protein A**)
- **biochemical properties** that enhance their survival in phagocytes (carotenoids, catalase production)
- **immunological disguises** (**Protein A, coagulase, clotting factor**)
- **membrane-damaging toxins** that lyse eukaryotic cell membranes (**hemolysins, leukotoxin, leukocidin**)
- **exotoxins** that damage host tissues or otherwise provoke symptoms of disease (**SEA-G, TSST, ET**)
- **inherent and acquired resistance to antimicrobial agents.**

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### Superantigens

- Promote cytokine storms
- **Enterotoxins** (SE-A, B, C, D, E and G)
  - Gastroenteritis
  - enterotoxins B and C cause 50% of non-menstrual cases of TSS.
- **Toxic shock syndrome toxin (TSST-1)**
  - TSST-1 is responsible for 75% of TSS, including all menstrual cases.
- Superantigens stimulate T cells non-specifically without normal antigenic recognition.
- Up to one in five cells may be activated, whereas only 1 in 10,000 are stimulated during a usual antigen presentation

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## Staphylococcus aureus


- Gram-positive, cluster-forming coccus
- Non-motile, non-sporeforming facultative anaerobe
- fermentation of glucose produces mainly lactic acid
- ferments mannitol (distinguishes from *S. epidermidis*)
- catalase positive
- coagulase positive
- golden yellow colony on agar

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## CLINICAL SIGNIFICANCE


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## SSTIs

- **Colonization:** cell-bound (protein) adhesins
- **Invasion: Invasins:** staphylokinase
- Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
- **Resistance to phagocytosis:** coagulase, leukocidin
- **Resistance to immune responses:** coagulase
- **Toxigenesis:** cytotoxic toxins (haemolysins and leukocidin)


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## Pneumonia

- **Colonization:** cell-bound (protein) adhesins
- **Invasion: Invasins:** staphylokinase, hyaluronidase
- Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
- **Resistance to phagocytosis:** coagulase, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
- **Resistance to immune responses:** coagulase, antigenic variation
- **Toxigenesis:** Cytotoxic toxins (hemolysins and leukocidin)


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## Gastroenteritis

- **Toxigenesis:** Enterotoxins A-G


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## Septicaemia

- **Invasion: Invasins:** staphylokinase, hyaluronidase  
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
- **Resistance to phagocytosis:** coagulase, protein A, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
- **Resistance to immune responses:** coagulase, protein A, antigenic variation
- **Toxigenesis:** cytotoxic toxins (haemolysins and leukocidin)


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## Osteomyelitis

- **Colonization:** cell-bound (protein) adhesins
- **Invasion: Invasins:** staphylokinase, hyaluronidase  
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
- **Resistance to phagocytosis:** coagulase, protein A, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
- **Resistance to immune responses:** coagulase, protein A, antigenic variation
- **Toxigenesis:** cytotoxic toxins (hemolysins and leukocidin)


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## Toxic Shock Syndrome

- **Colonization:** cell-bound (protein) adhesins
- **Resistance to immune responses:** coagulase, antigenic variation
- **Toxigenesis:** TSST toxin, Enterotoxins A-G


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## Scalded Skin Syndrome

- **Colonization:** cell-bound (protein) adhesins
- **Invasion: Invasins:** staphylokinase, hyaluronidase  
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
- **Resistance to phagocytosis:** coagulase, leukocidin, hemolysins
- **Resistance to immune responses:** coagulase, antigenic variation
- **Toxigenesis:** Exfoliatin toxin

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## QUESTIONS?

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