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FN M+H Fakultní nemocnice Motol a Homolka

Národní institut virologie a bakteriologie

Diagnosistics of significant G+ cocci I. (staphylococci)

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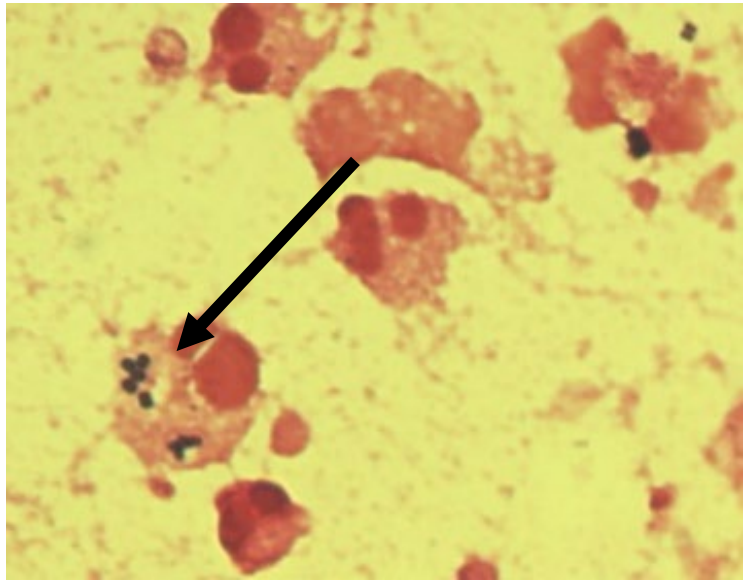
Microbiology I
Practicals

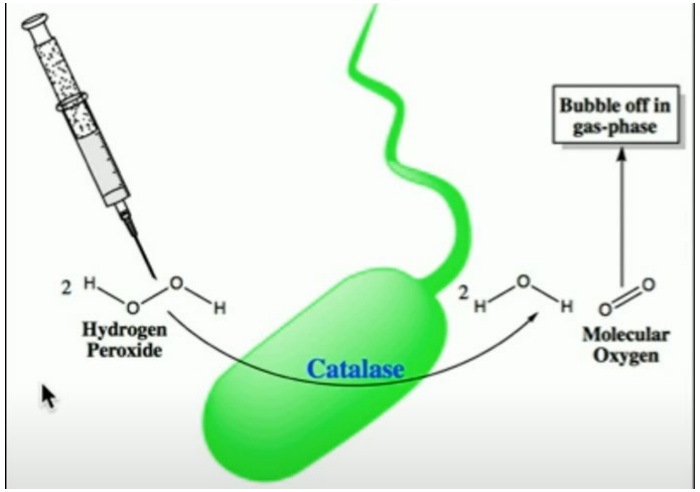
Who are they?

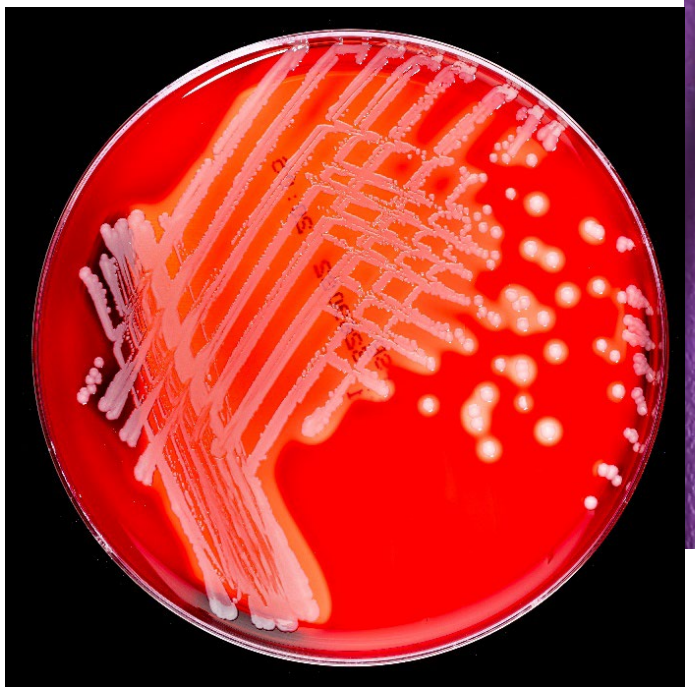
- **G+ cocci in clusters**
  - Facultatively anaerobic
  - **Catalase-positive**

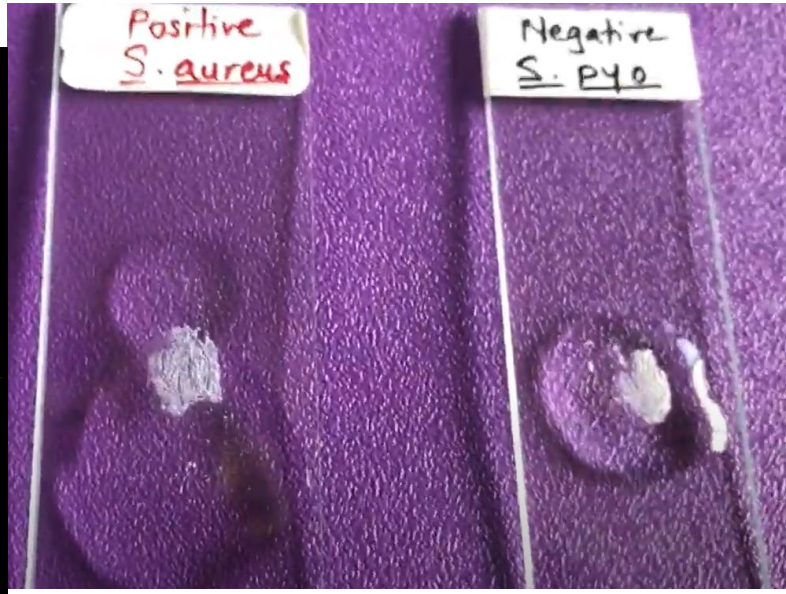
- Culture conditions
  - Basics: 37°C, 18–24 hours
  - No special atmosphere required

- Occurrence and significance
  - Part of the natural microbiota
  - Colonisation of surfaces
  - Opportunistic pathogens









Classification of staphylococci

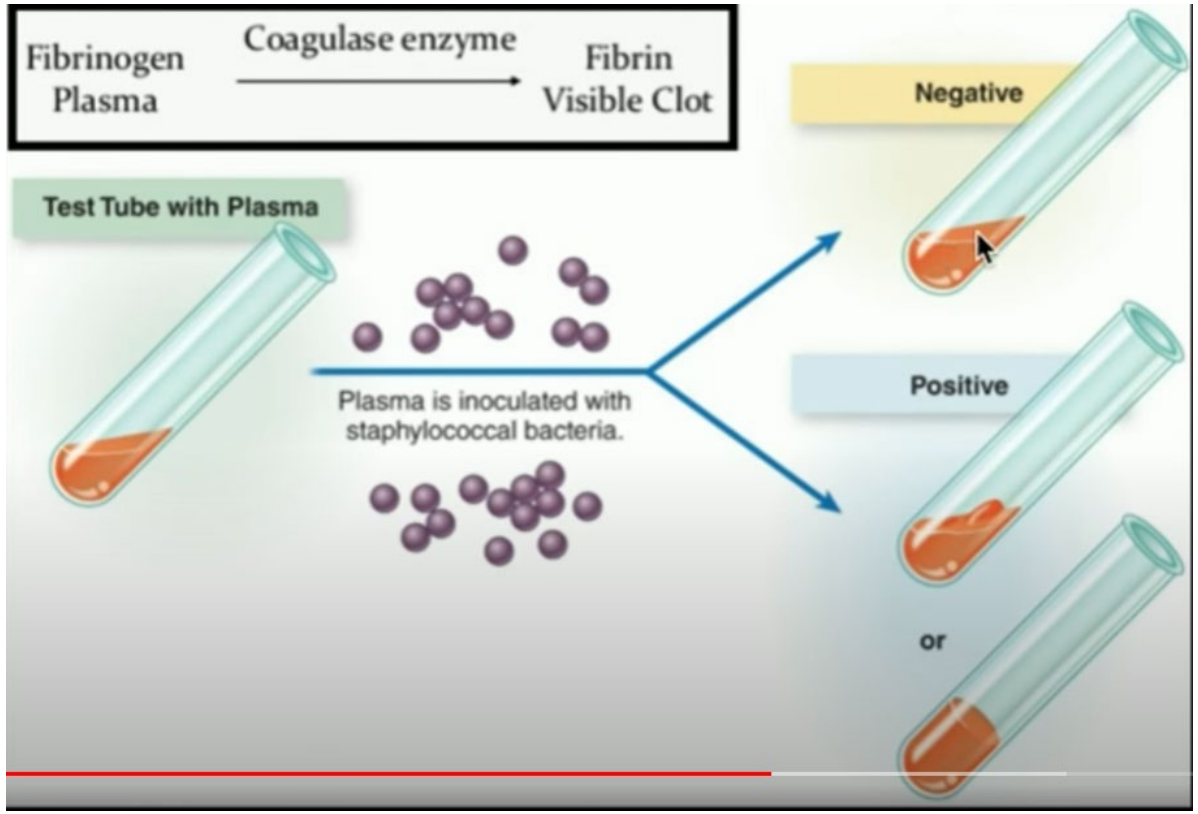
According to the plasma coagulase test

Coagulase-positive = *S. aureus*

- A common cause of a wide range of infections
- Toxin production

Coagulase-negative = CoNS

- Colonisation of foreign materials,
- Catheter-associated sepsis, urinary tract infections, biofilm,
- These strains do not produce toxins

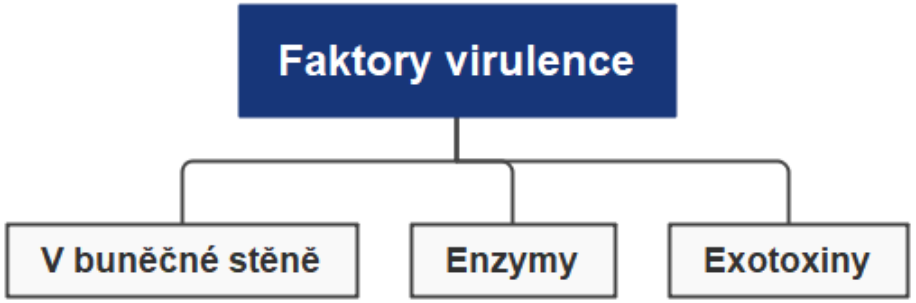


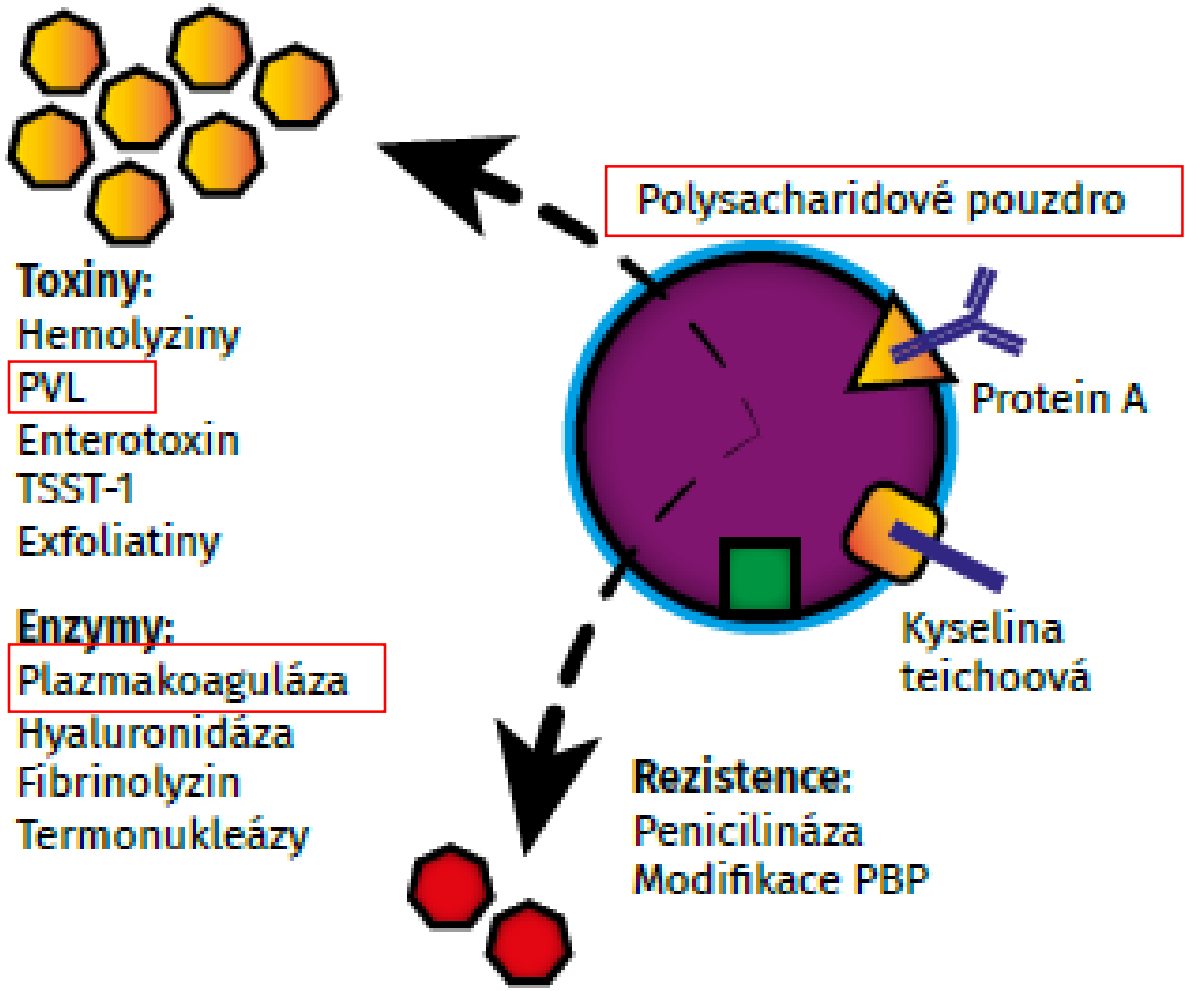
Title	Haemolysis on KA	Pathogenicity	
S. aureus	Yes	+++	Physiologically present in the nose (approx. 40–50%); Pathogenic potential: - IKMT, orthopaedic, pneumonia (! PVL+), BSI but not UTI - Enterotoxicosis, STSS, SSSS
S. capitis	Yes	+	**Physiologically on the skin**; Colonisation of catheters, prostheses and valves
S. epidermidis	No	+	
S. hominis	No	+	
S. haemolyticus	Yes	+	
S. lugdunensis	Yes	++	**Physiologically on the skin**; IKMT, orthopaedic, endocarditis, IKŘ
S. saprophyticus	No	++	Physiologically on the skin; IMC

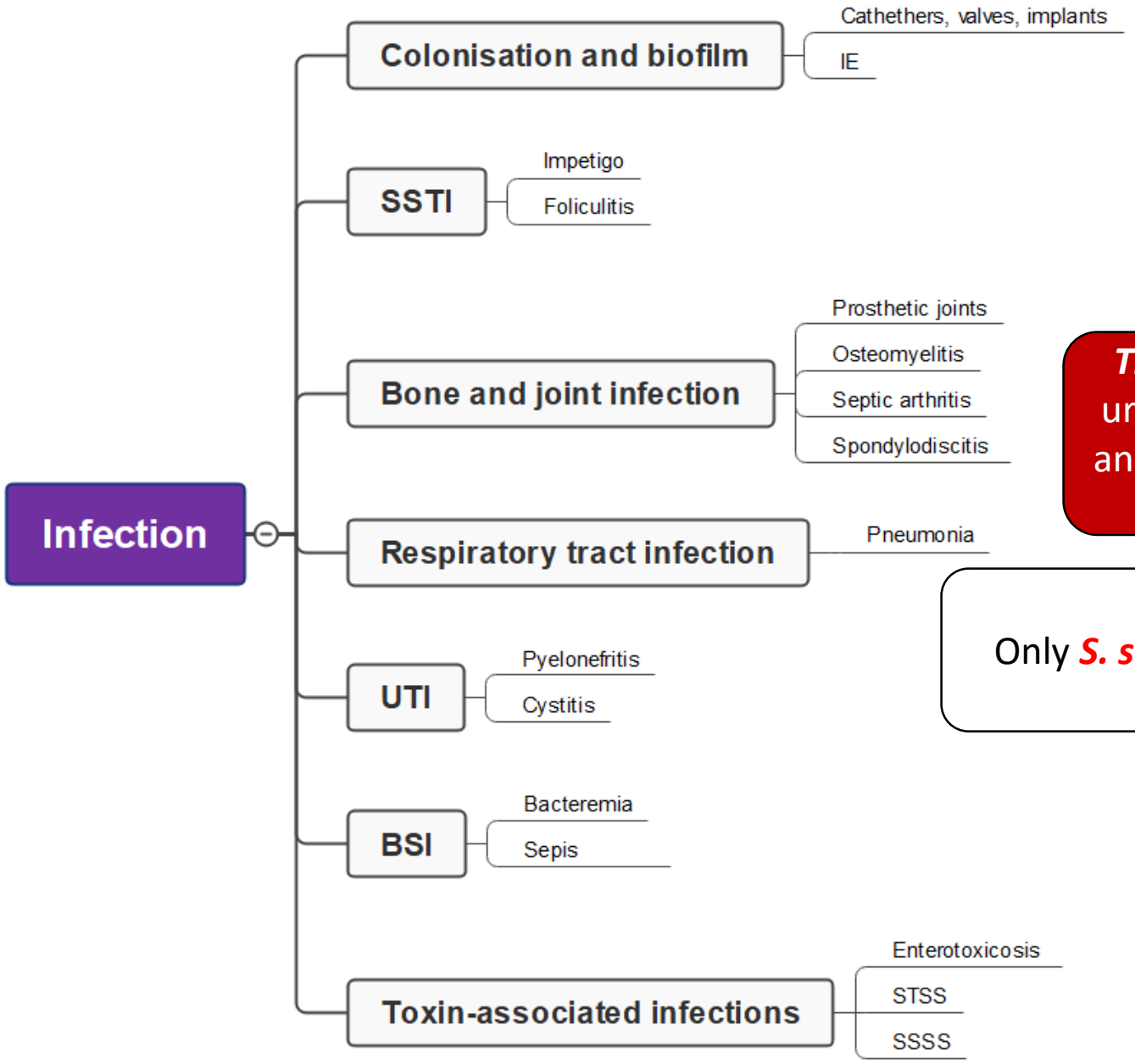
CoNS is not limited to *S. epidermidis and S. saprophyticus*

* SSTI = skin and soft tissue infection; BSI = bloodstream infection; UTI = urinary tract infection; STSS = staphylococcal toxic shock syndrome; SSSS = staphylococcal scalded skin syndrome; PVL = Panton-Valentin leukocidin)

Staphylococcus aureus







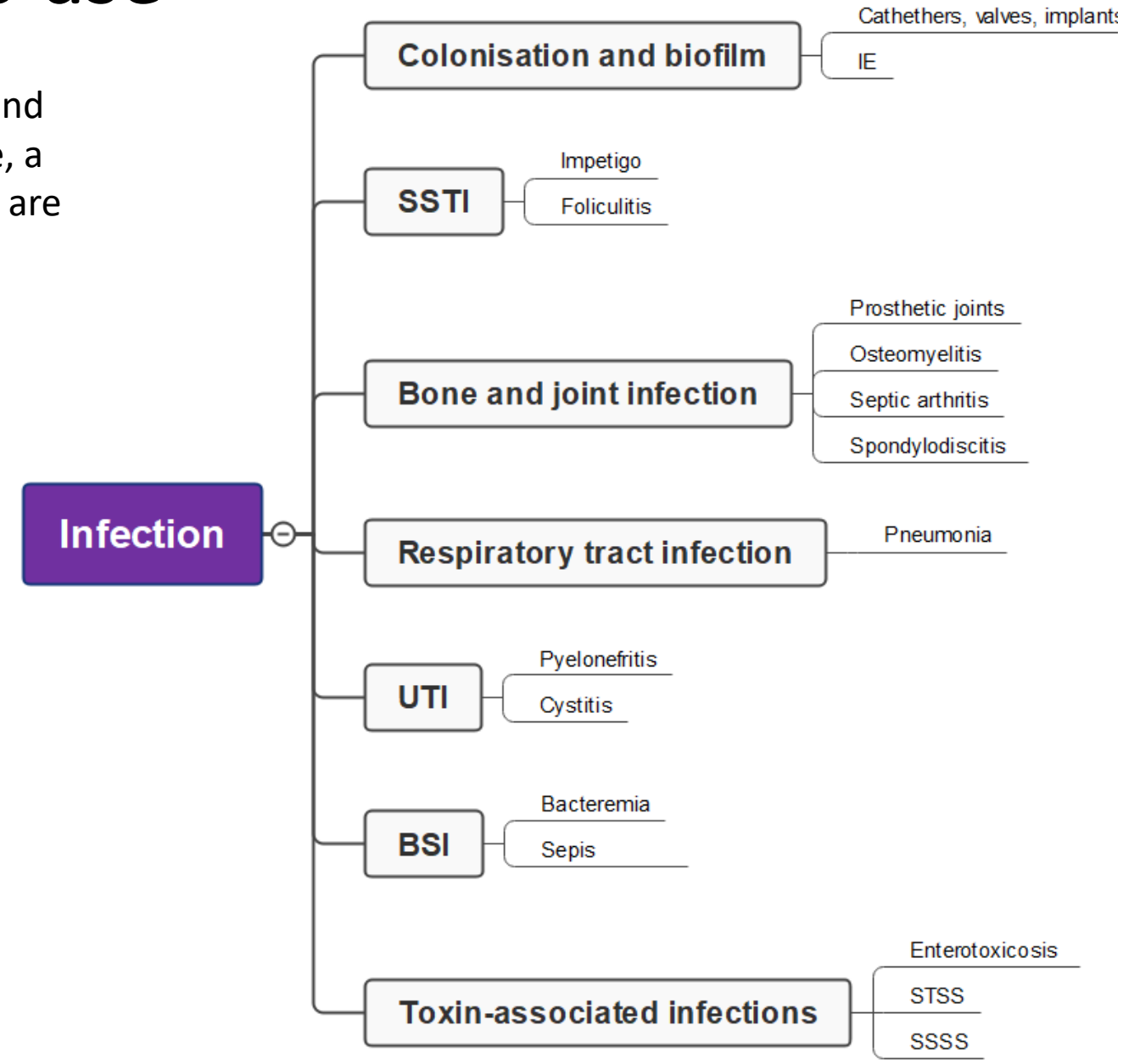
The presence of S. aureus in urine is a sign of bacteraemia, and the source must always be investigated.

Only ***S. saprophyticus***

Diagnosis of staphylococci

Samples to use

Due to the prevalence and spectrum of the disease, a large variety of materials are possible



Sample
Tissue / catheter / valve
Skin swab / abscess contents
Punctate / aspirate / tissue
Sputum / BAL / PCR for PVL toxin
Urine
BSI
Toxin test: agglutination / PCR for TSST-1, exfoliatin

Methods

Urine

Punctate / aspirate / tissue

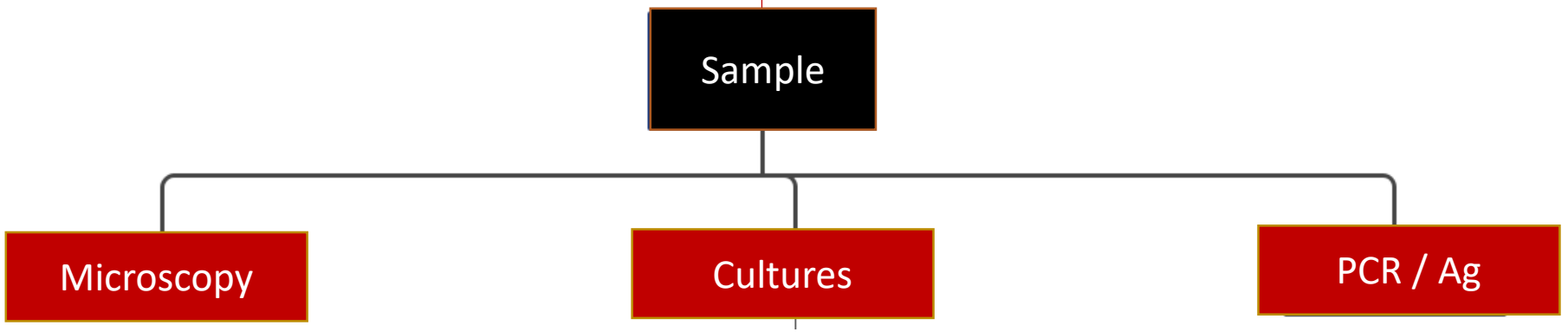
Skin swab / abscess contents

Toxin test:
agglutination / PCR
on
TSST-1, exfoliatin

Tissue / catheter / valve

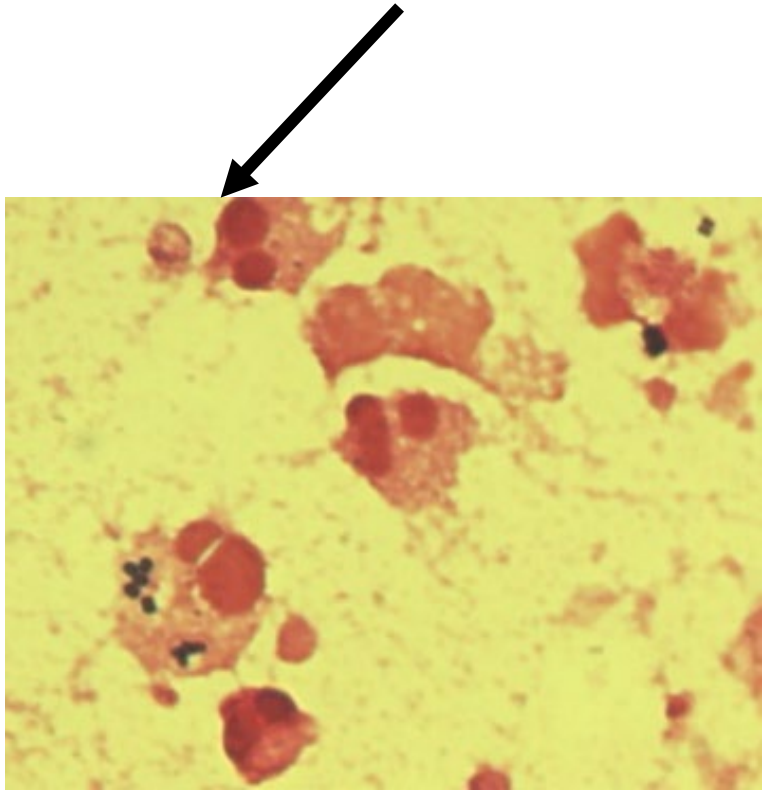
Sputum / BAL / PCR for PVL toxin

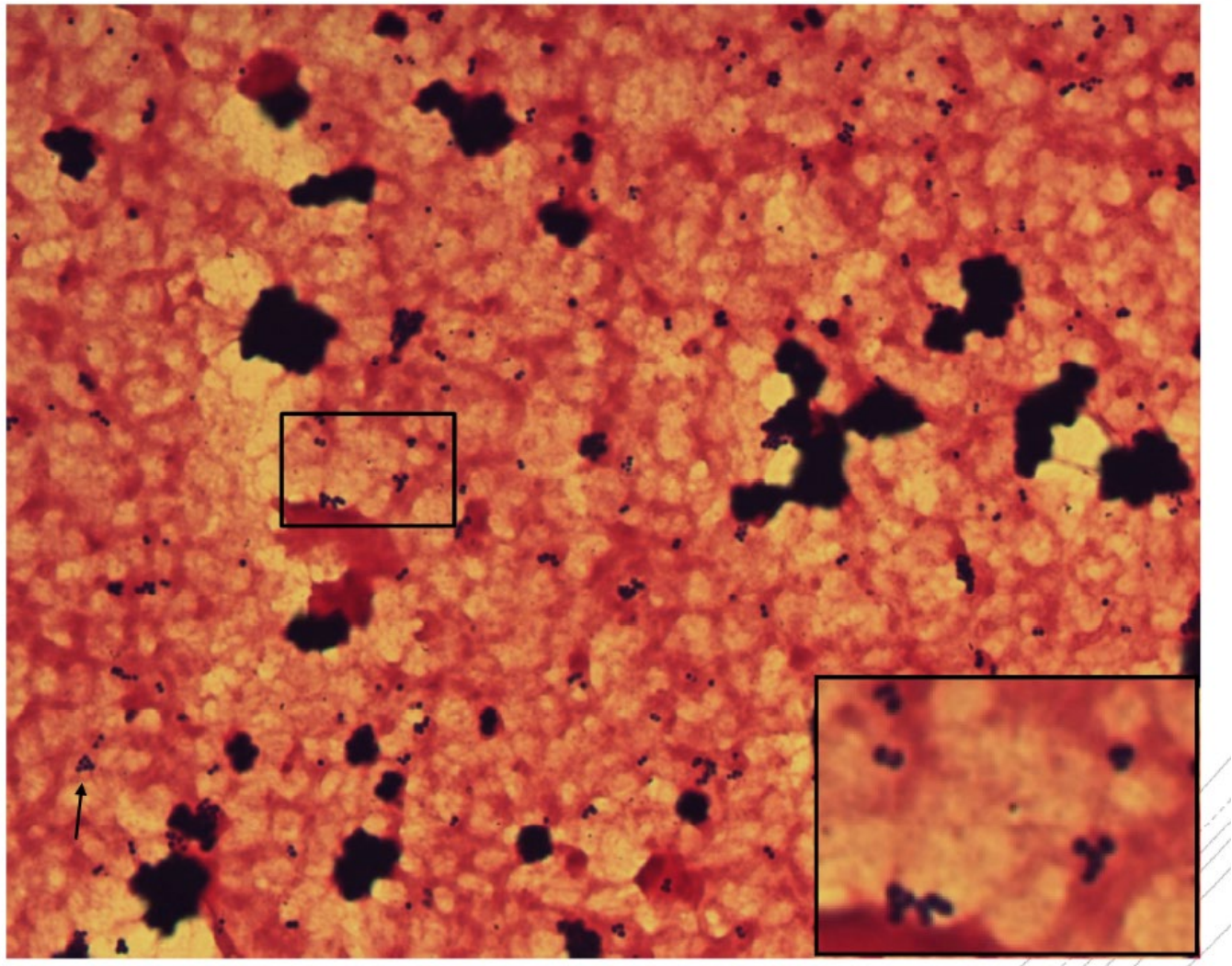
BSI



Microscopy

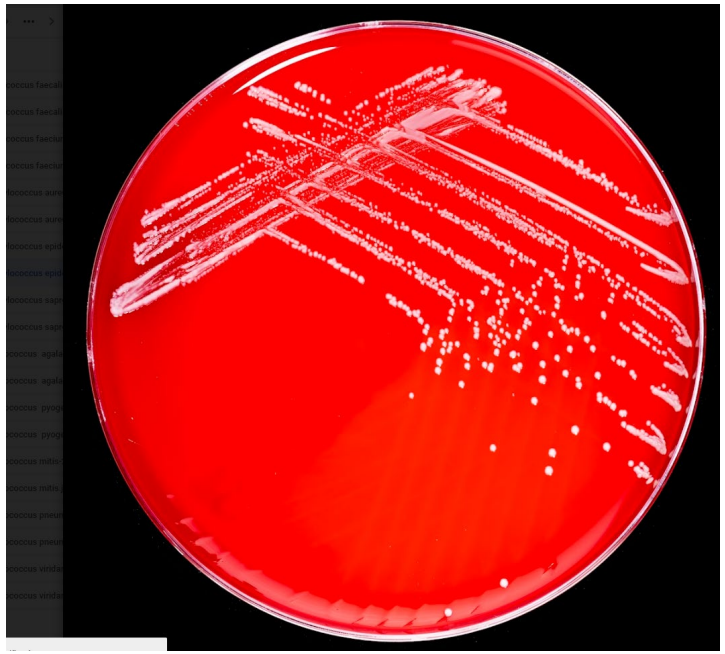
- **G+ cocci in clusters**

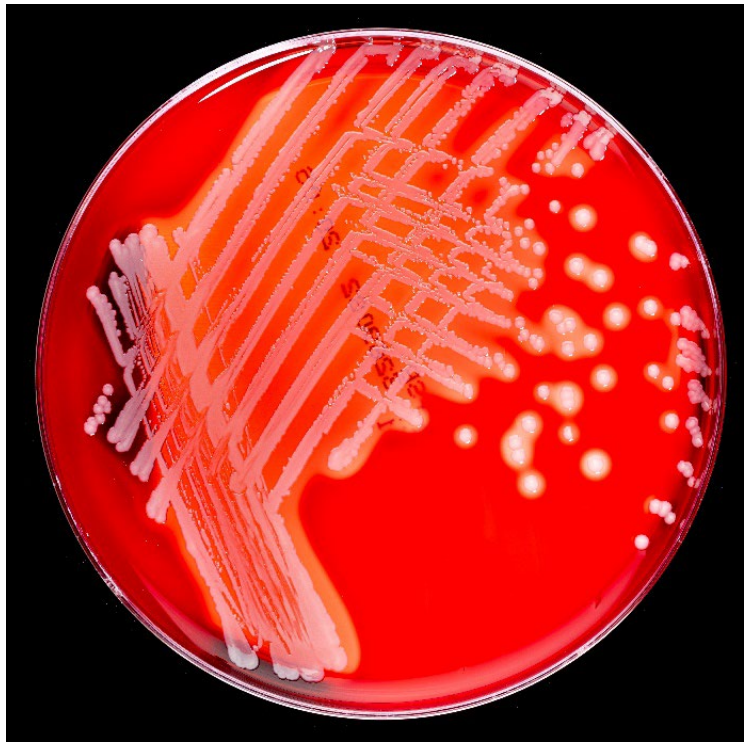




Cultivation

- Media:
  - blood agar
  - chocolate agar
- How long?
  - 18–24 hours
- What conditions?
  - 37°C, in normal atmosphere
- Evaluation:
  - Colony, size,
  - Pigment formation
  - Haemolysis
- Identification
  - **MALDI TOF MS**
  - Biochemical tests





Biochemical tests

- **Catalase**
  - Positive for all staphylococci
  - Using hydrogen peroxide – on a slide or in a test tube (video)

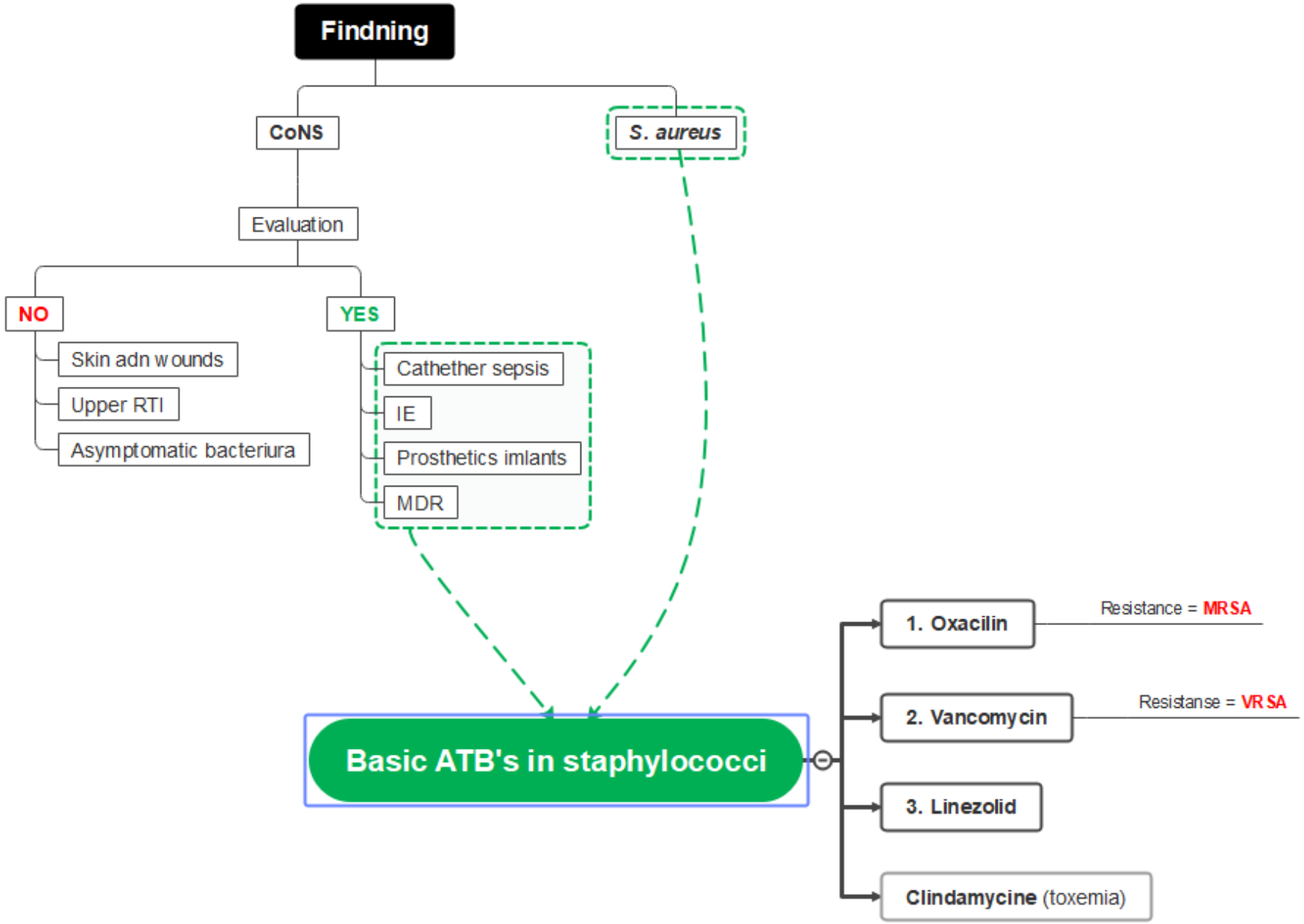
- **Plasma coagulase**
  - On the surface of the staphylococcus – agglutination – on a slide (video)
  - Sensitised plasma + staphylococcus → evaluate within 30 seconds → observe agglutinates

Antibiotic susceptibility testing

- For CoNS, assess the significance – always in cases of catheter-associated sepsis, endocarditis, prosthesis infections, etc., and for multidrug-resistant strains

- Crucial for *S. aureus* – prevalence of resistant strains

- MRSA – resistance to methicillin (in the Czech Republic, the oxacillin variant), also screening → susceptibility to oxacillin and cefoxitin
  - Resistance indicates reduced susceptibility to other agents
  - Follow-up care


Findning
CoNS
S. aureus
Evaluation
NO
YES
Skin adn wounds
Upper RTI
Asymptomatic bacteriura
Cathether sepsis
IE
Prosthetics imlants
MDR
Basic ATB's in staphylococci
1. Oxacilin
Resistance = MRSA
2. Vancomycin
Resistanse = VRSA
3. Linezolid
Clindamycine (toxemia)

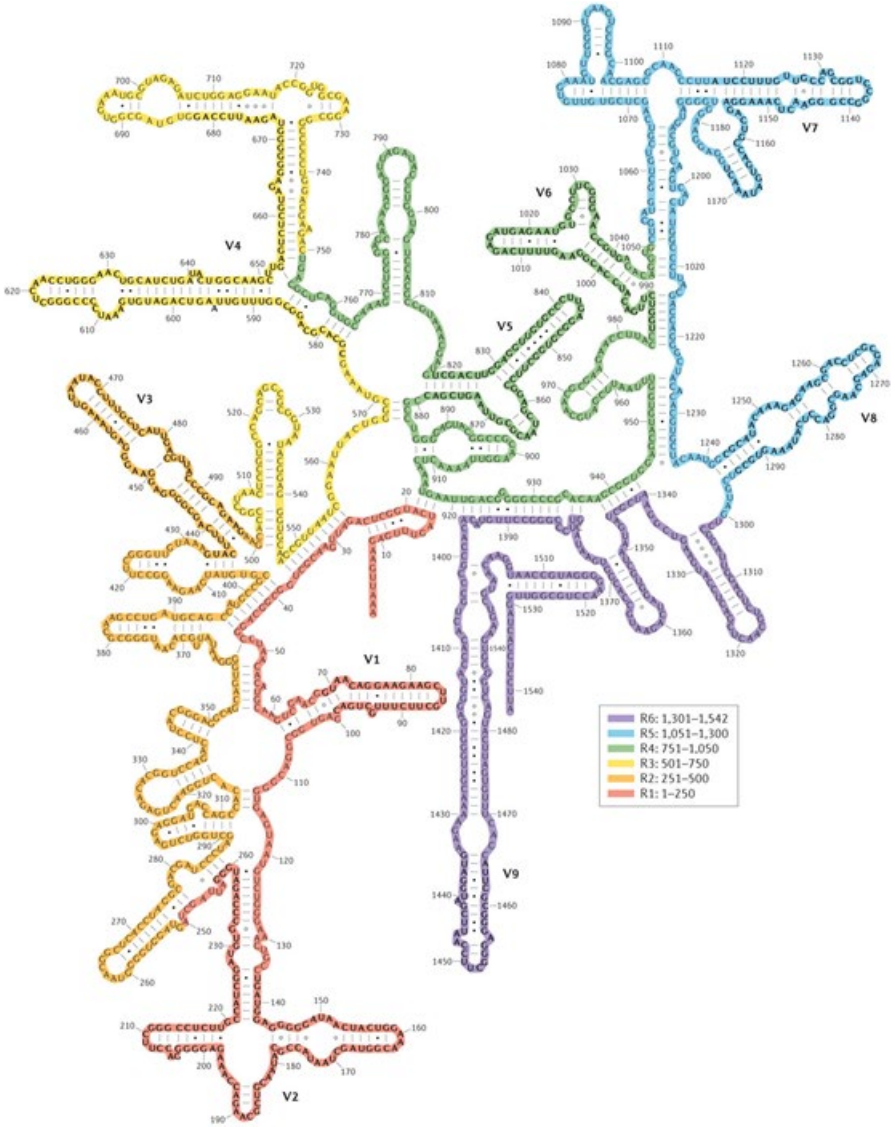
Toxins

- Some strains produce significant toxins:
  - Superantigens
    - Exfoliatins
    - TSST
    - Enterotoxins
  - **PVL**
- **Gene detection by PCR**

- **Add clindamycin (or linezolid) to the treatment**

What if the culture fails?

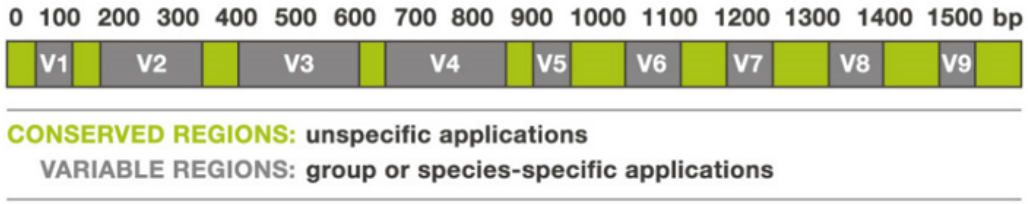
- A clear suspicion of infection, but negative on microscopy and culture too.
- What can be done?



Pan-bacterial PCR

- **16S rRNA gene**
  - Size: 1542 bp
  - Structure: conserved regions (where the primers bind) and nine hypervariable regions V1–V9 (most commonly V3–V4)
- **Methodology**
  - 1. 16S rDNA PCR
  - 2. Amplicon sequencing
  - 3. Comparison with reference databases (GreenGenes, RDP, Silva)

16S rDNA is a linear structure - > transcribes into a linear rRNA, and folds.



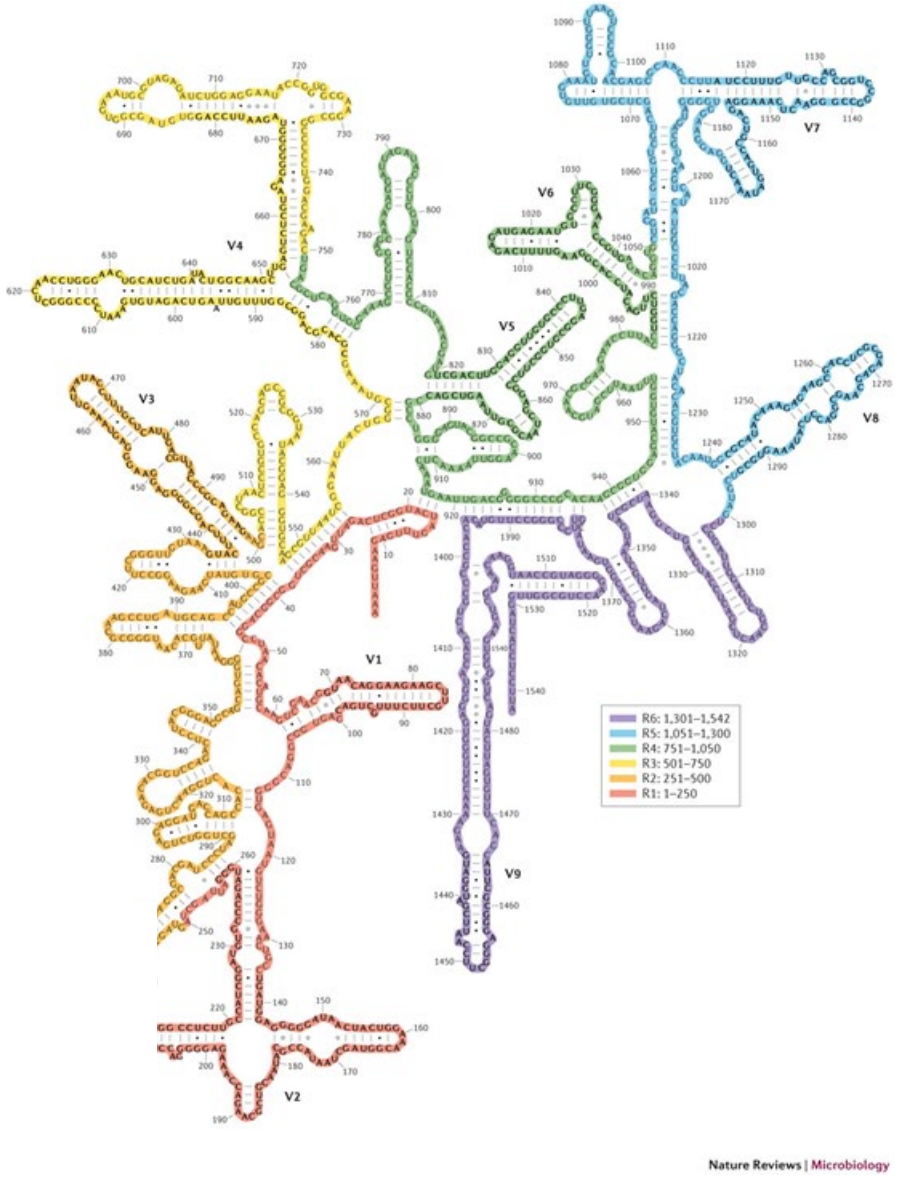


Figure 1: An example of a 16S rRNA gene. The regions in green are conserved in all microorganisms. These are the sites that are targeted by primers for PCR amplification so that all the 16S rRNA genes in a sample are amplified. The grey regions are the species-specific regions that-- when sequenced-- allow for scientists to see which species are present in a community. Image courtesy of: http://www.alimetrics.net/en/index.php/dna-sequence-analysis

Pan-bacterial PCR

Use:
Primarily sterile materials