Microbiology I.
Practical training

Diagnostics of important G+ cocci I. (staphylococci)

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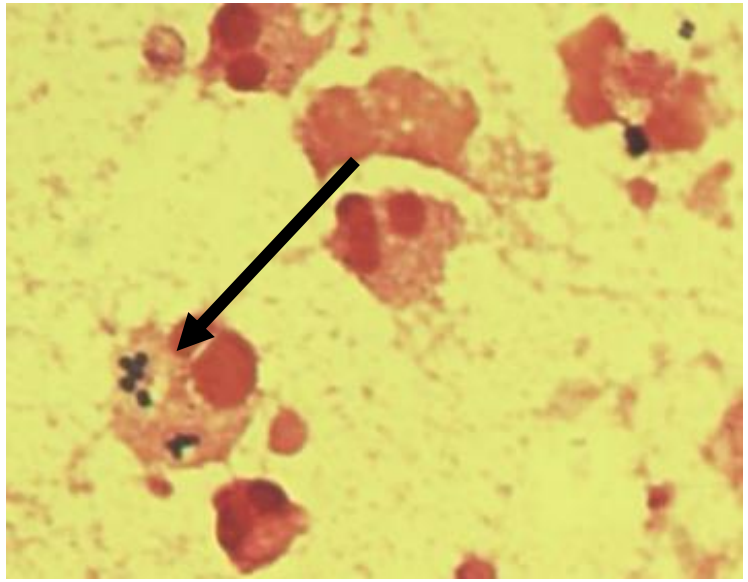
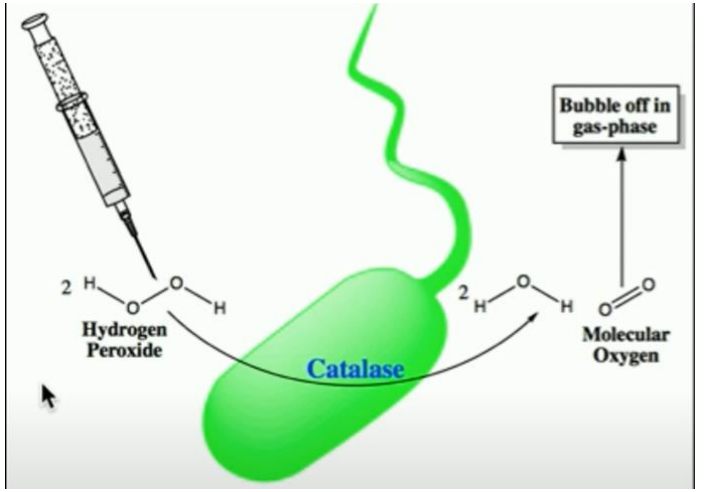
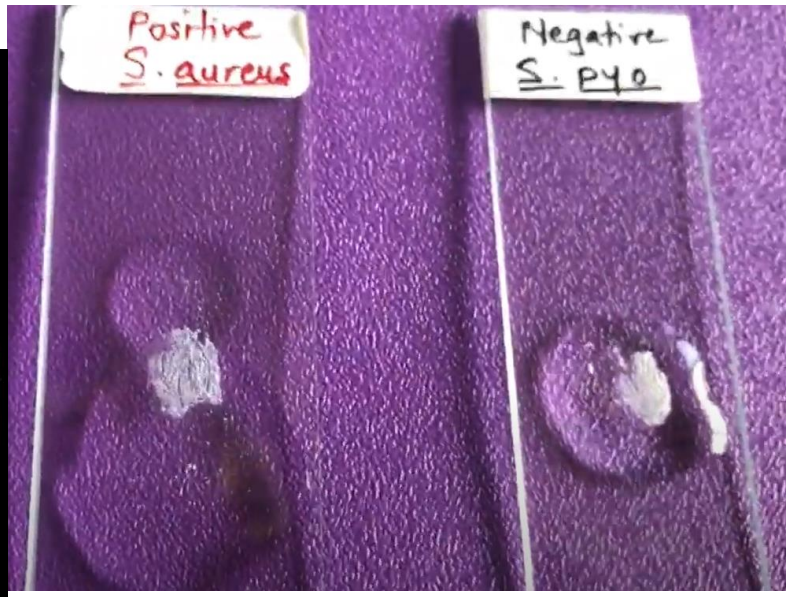
Who are they

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

- **Cultivation conditions**
  - Easy: at 37°C, 18-24 hours
  - No special atmosphere is required
- **Signifcance**
  - Part of the physiological microbiota
  - Colonisation of surfaces
  - Opportunistic pathogens







Classification of staphylococci

According to the plasma-coagulase test

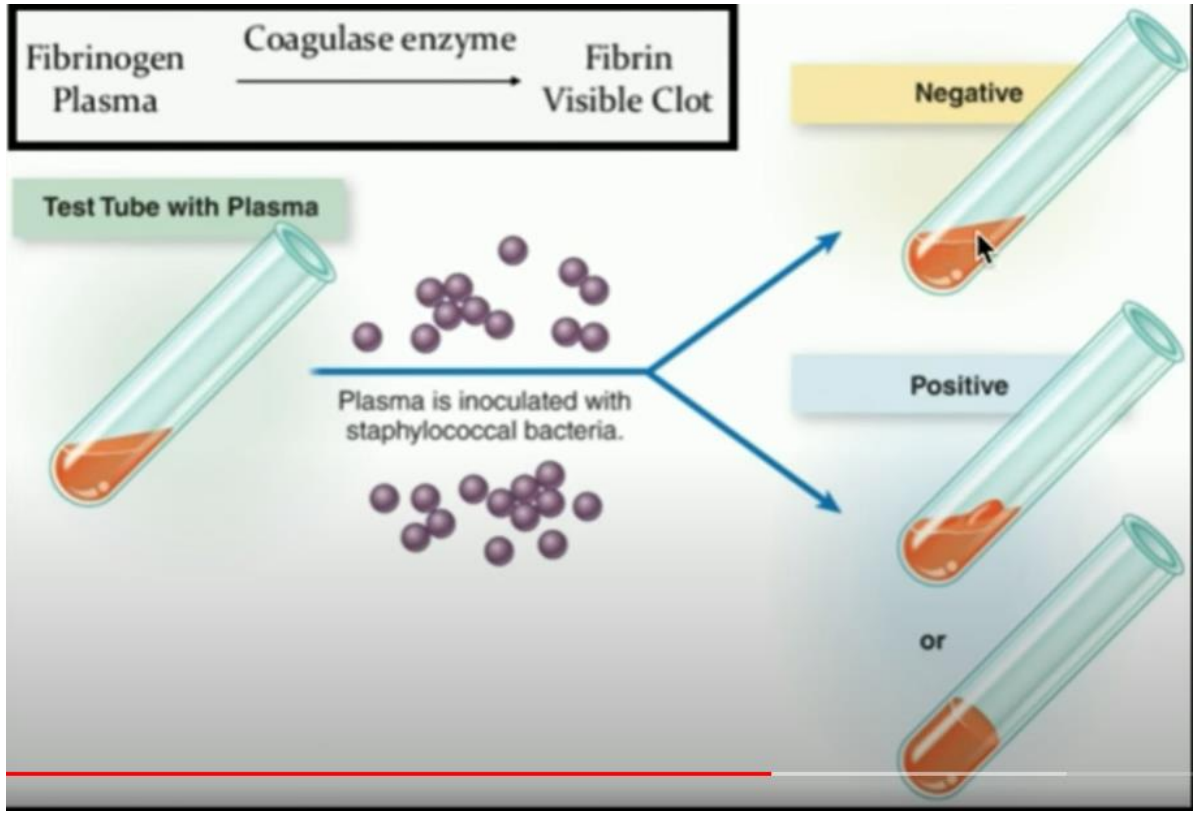
Coagulase-positive = ***S.aureus***

Common agent of a wide range of infections

- Toxin production

Coagulase-negative = CoNS

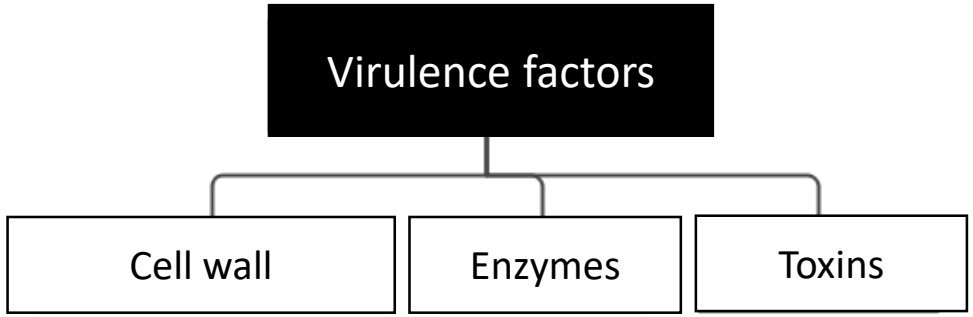
- Colonization of foreign materials,
- Catheter sepsis, urinary infections, biofilm,
- These strains do not produce toxins

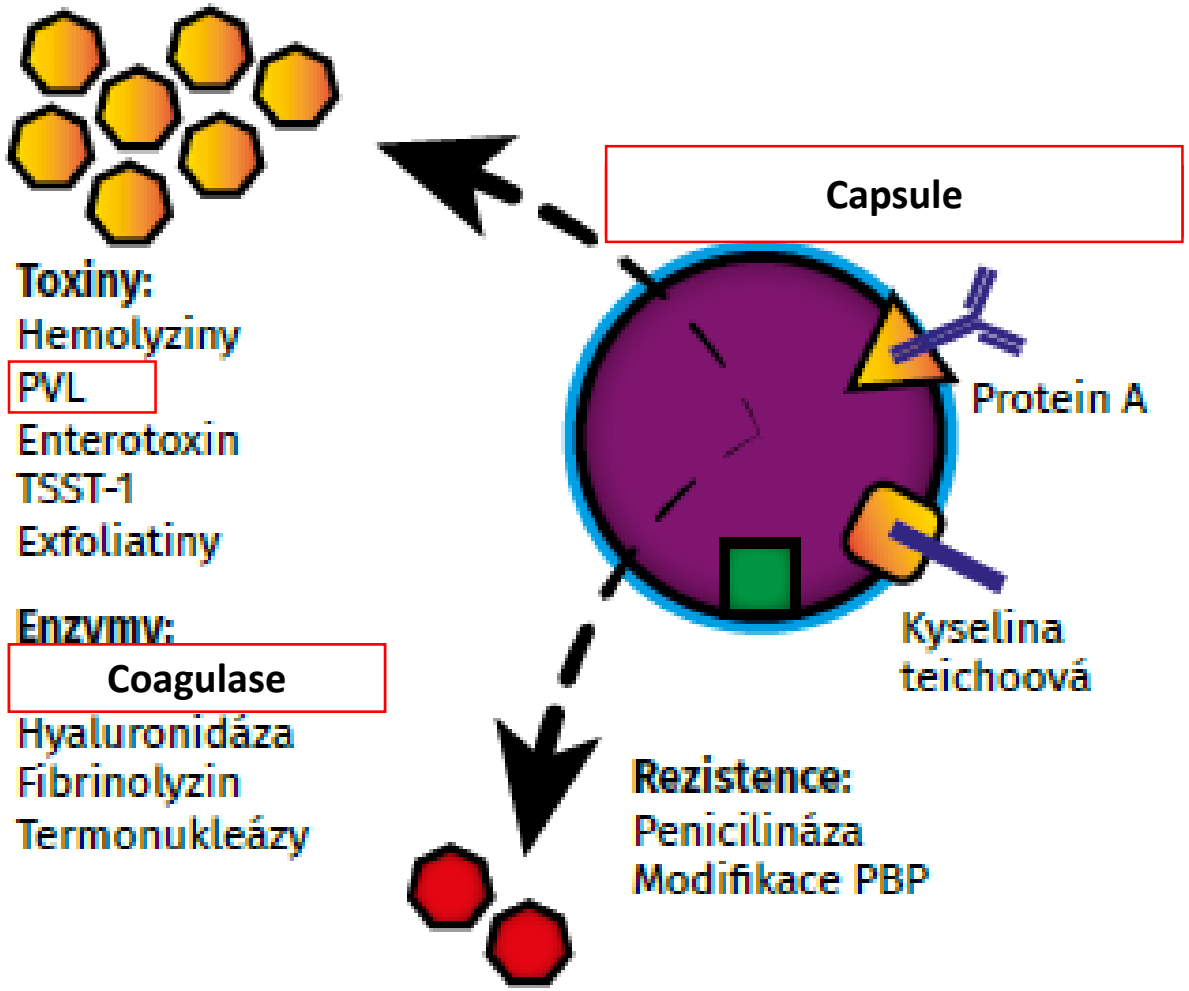


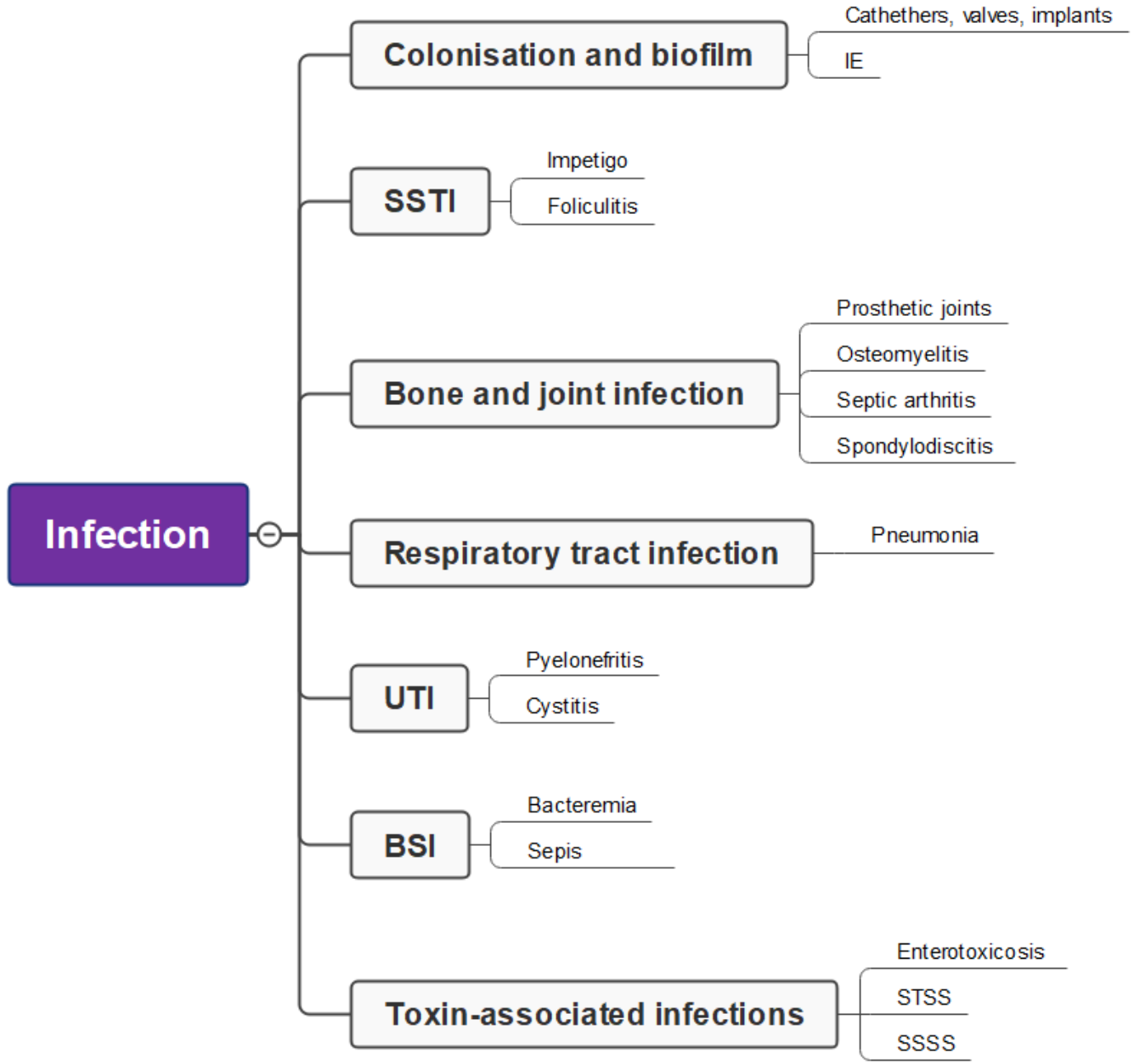
Name	Hemolysis	Patogenity	
S. aureus	Yes	+++	**Physiologically in the nose** (about 20%); Pathogenic potential: SSTI, orthopaedic infections, pneumonia (! PVL+), UTI, BCI, Enterotoxicosis, STSS, SSSS
S. capitis	Yes	+	**Physiologically on skin**; Colonization of catheters and valves
S. epidermidis	No	+	
S. hominis	No	+	
S. haemolyticus	Yes	+	
S. lugdunensis	Yes	++	**Physiologically on skin;** SSTI, endocarditis, orthopedic infections, BSI
S. saphrophticus	No	++	**Physiologically on skin;** UTI

* SSTI = skin and soft tissue infections; BSI = bloodstream infection; UTI = urinary tract infection; STSS = staphyl. toxic shock syndrome; SSSS = staphyl scalded skin syndrome; PVL = Panton-valentine's leucocidin)

Staphylococcus aureus



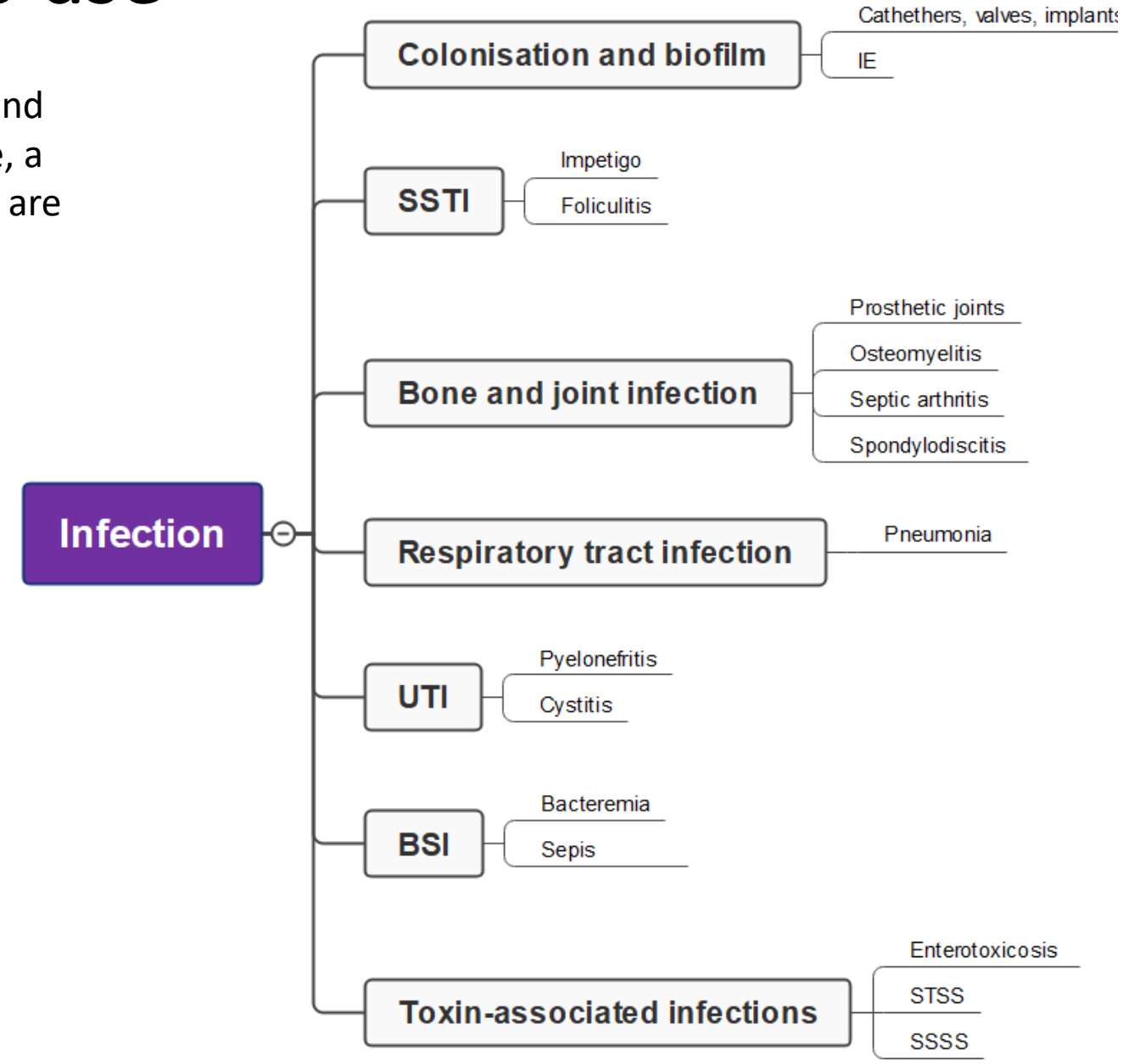



Infection
Colonisation and biofilm
Cathethers, valves, implants
IE
SSTI
Impetigo
Foliculitis
Bone and joint infection
Prosthetic joints
Osteomyelitis
Septic arthritis
Spondylodiscitis
Respiratory tract infection
Pneumonia
UTI
Pyelonefritis
Cystitis
BSI
Bacteremia
Sepis
Toxin-associated infections
Enterotoxicosis
STSS
SSSS

Diagnostics 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

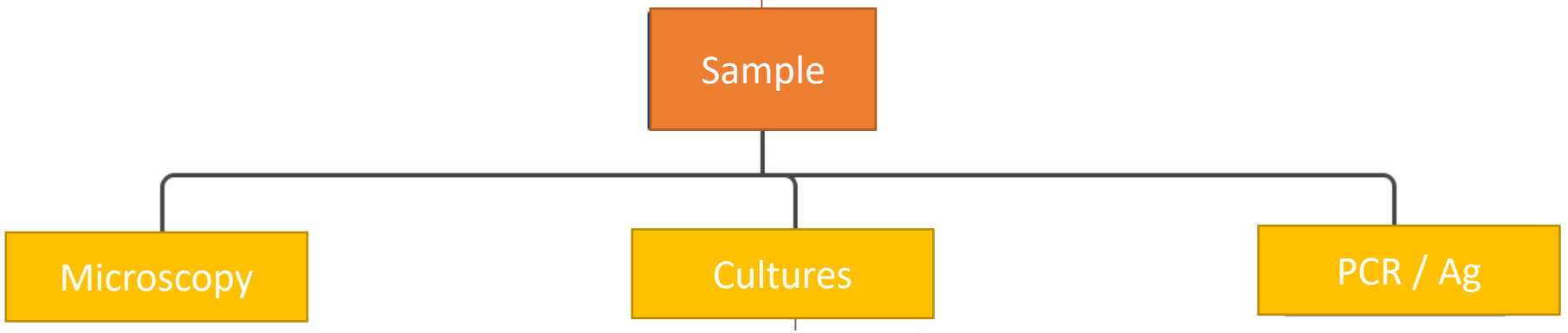
Skin swab / abscess contents

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

Tissue / catheter / valve

Sputum / BAL / PCR for PVL toxin

BSI



Microscopy

- **G+ cocci in clusters**
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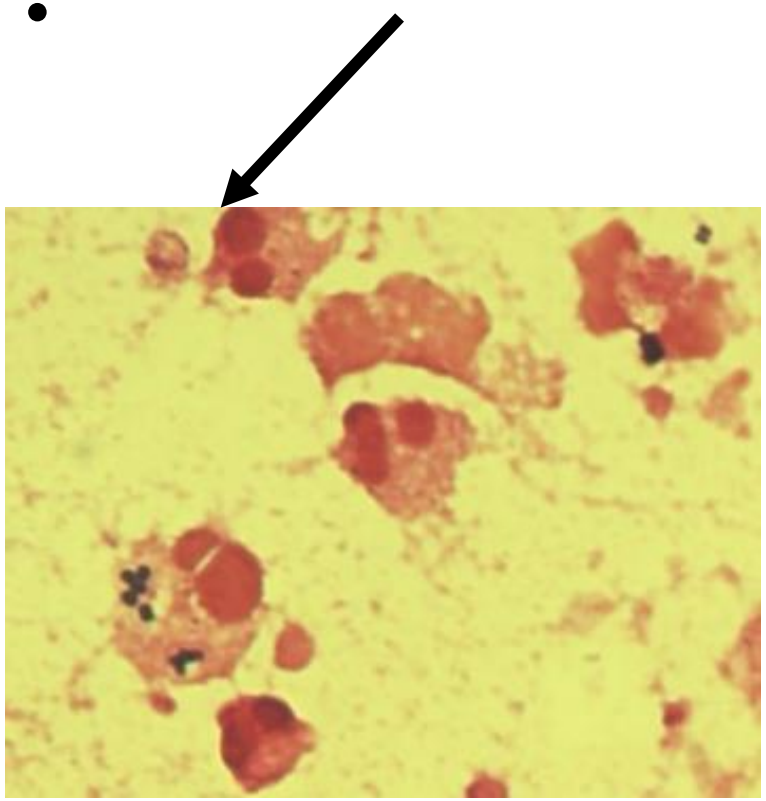
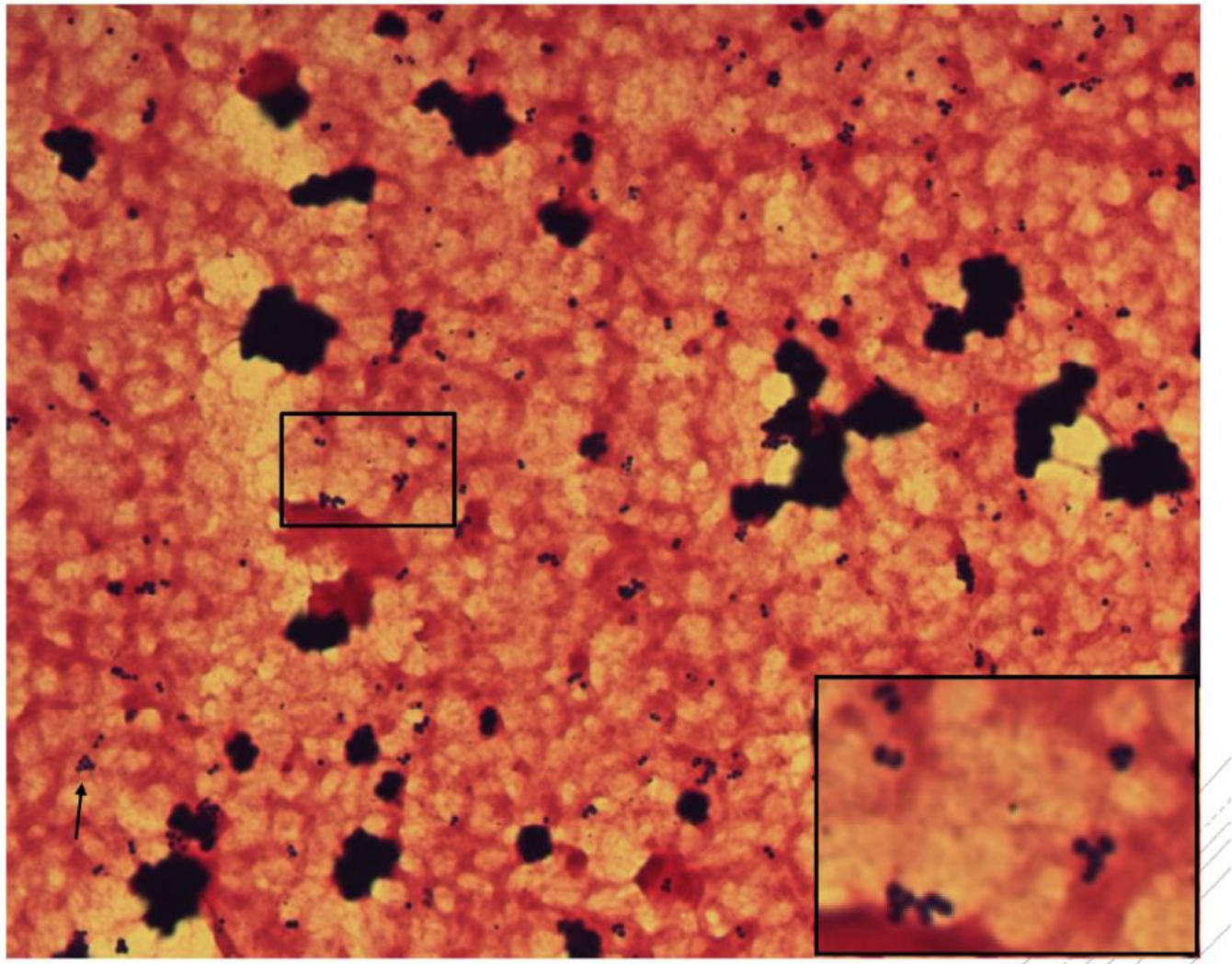




Cultures

Solid medium :

- blood agar
- chocolate agar

How long?

- 18-24 hours

What conditions?

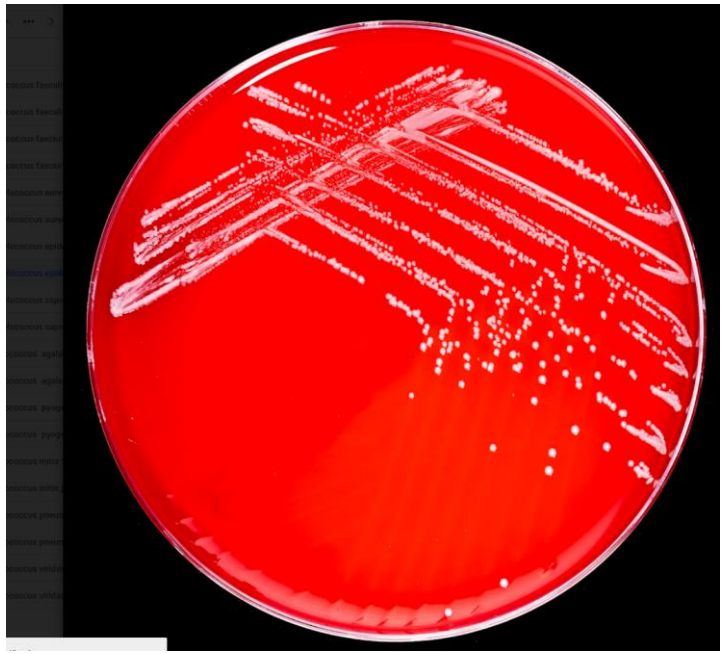
- 37°C, in normal atmosphere

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)

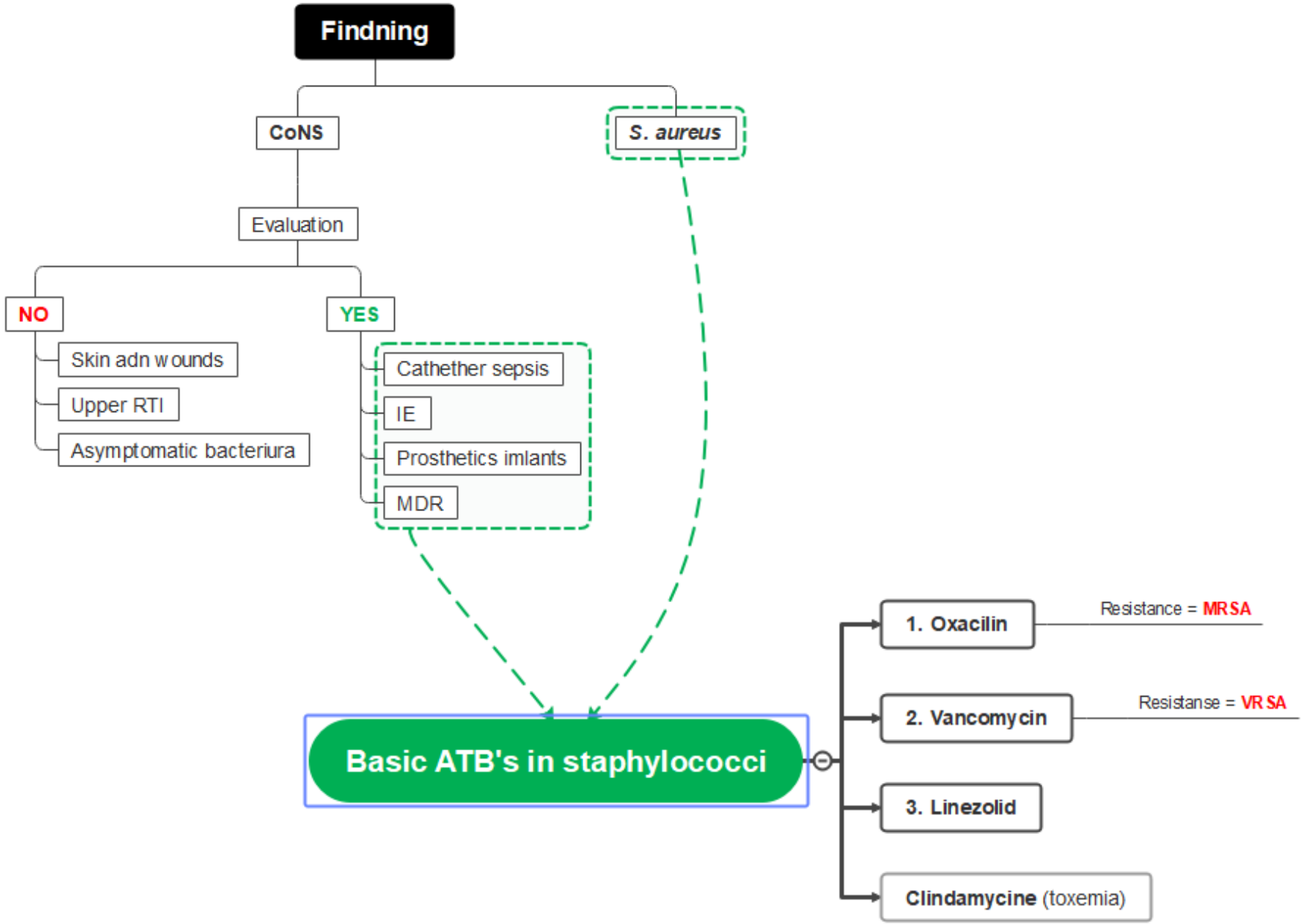
- **Plasmacoagulase**
  - On the surface of staphylococcus – agglutination – on the slide (video)
  - Sensitized plasma + staphylococcus → evaluated within 30s → we observe agglutinates

AST testing

- In CoNS, evaluate the importance – always in catheter sepsis, endocarditis, infections of artificial matherials, etc., multidrug-resistant strains

- Key *in S.aureus* – occurrence of resistant strains

- MRSA – resistance to methicillin (oxacillin variant in the Czech Republic), also screening → susceptibility to oxacillin and cefoxitin
  - Resistance indicates reduced sensitivity to other preparations
  - Dispensarization


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)

Toxiny

- Some strains produce significant toxins:
- Superantigens
    - Exfoliatins
    - TST
    - Enterotoxins
    - **PVL**
- **Gene detection by PCR**
- **Add clindamycin to the teraphy**
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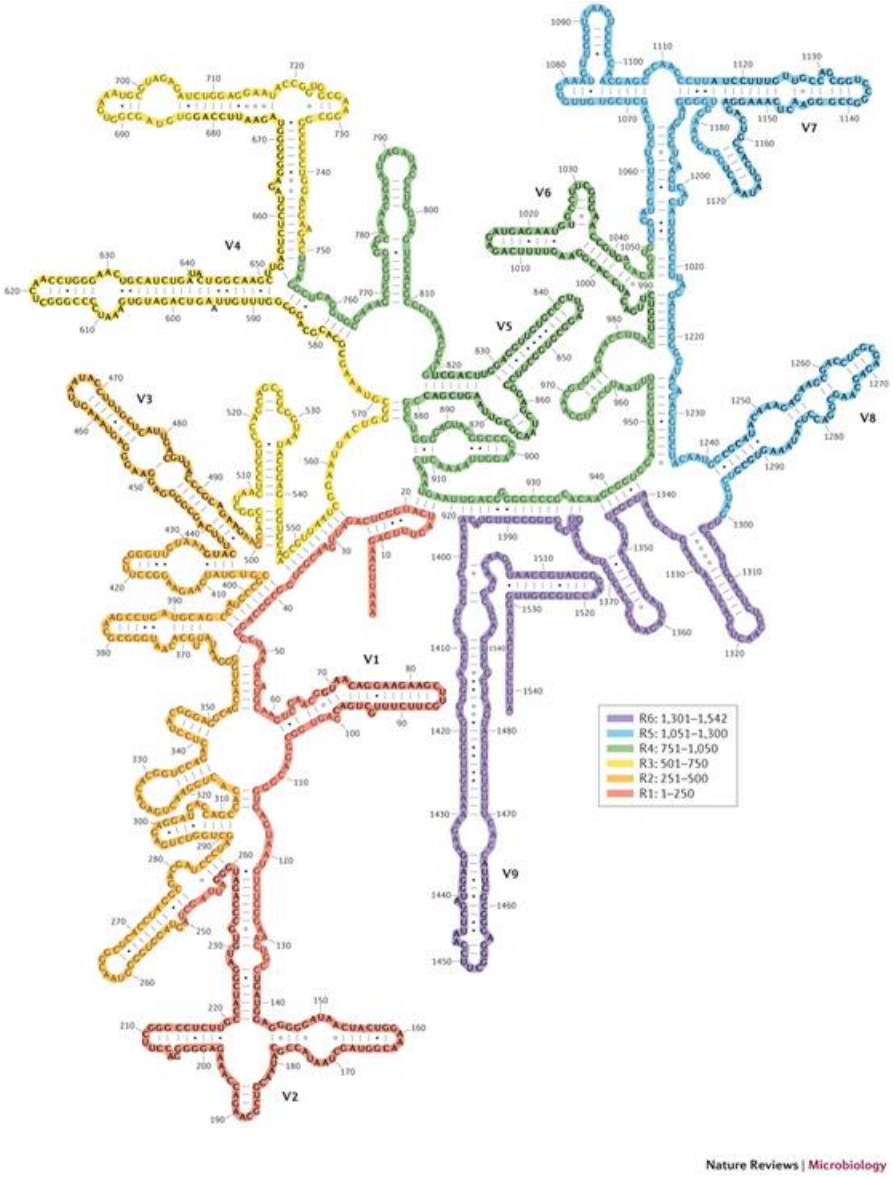
What if cultivation fails?

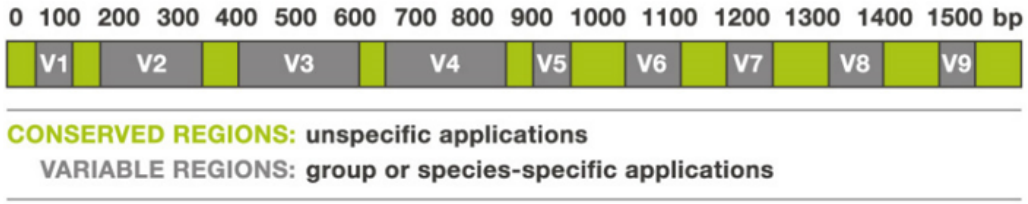
- Clear suspicion of infection, but microscopically negative and culture as well.
- How to help yourself?



Panbacterial PCR

- **Gene for 16S rRNA**
  - Gen size: 1542 bp
  - Structure: conservative regions (where the primers attach) and nine hypervariabile regions V1-V9 (mostly V3-V4)
- **Methods**
  - 1. PCR 16S rDNA
  - 2. Sequenicng the amplicons
  - 3. Compring with the reference database (BLAST, GreenGenes, RDP, Silva)

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



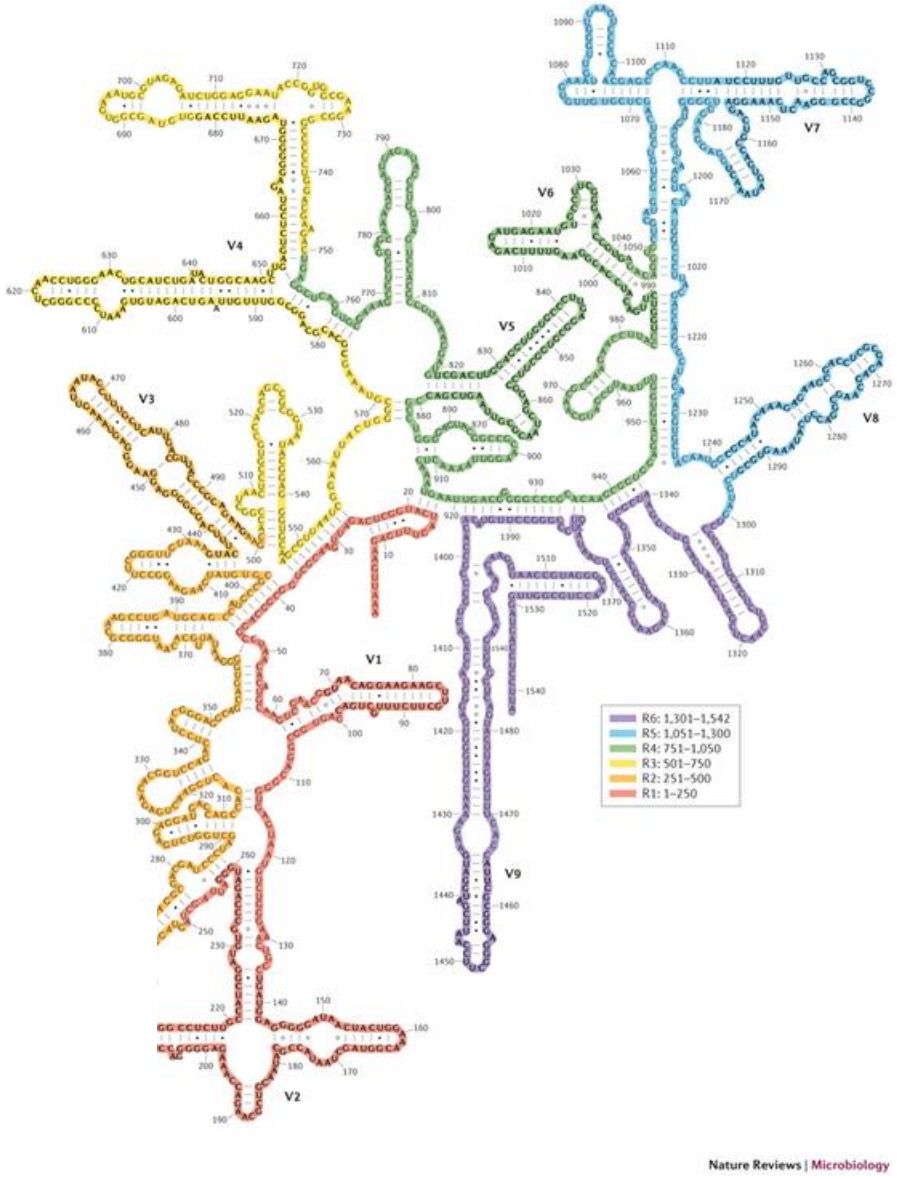
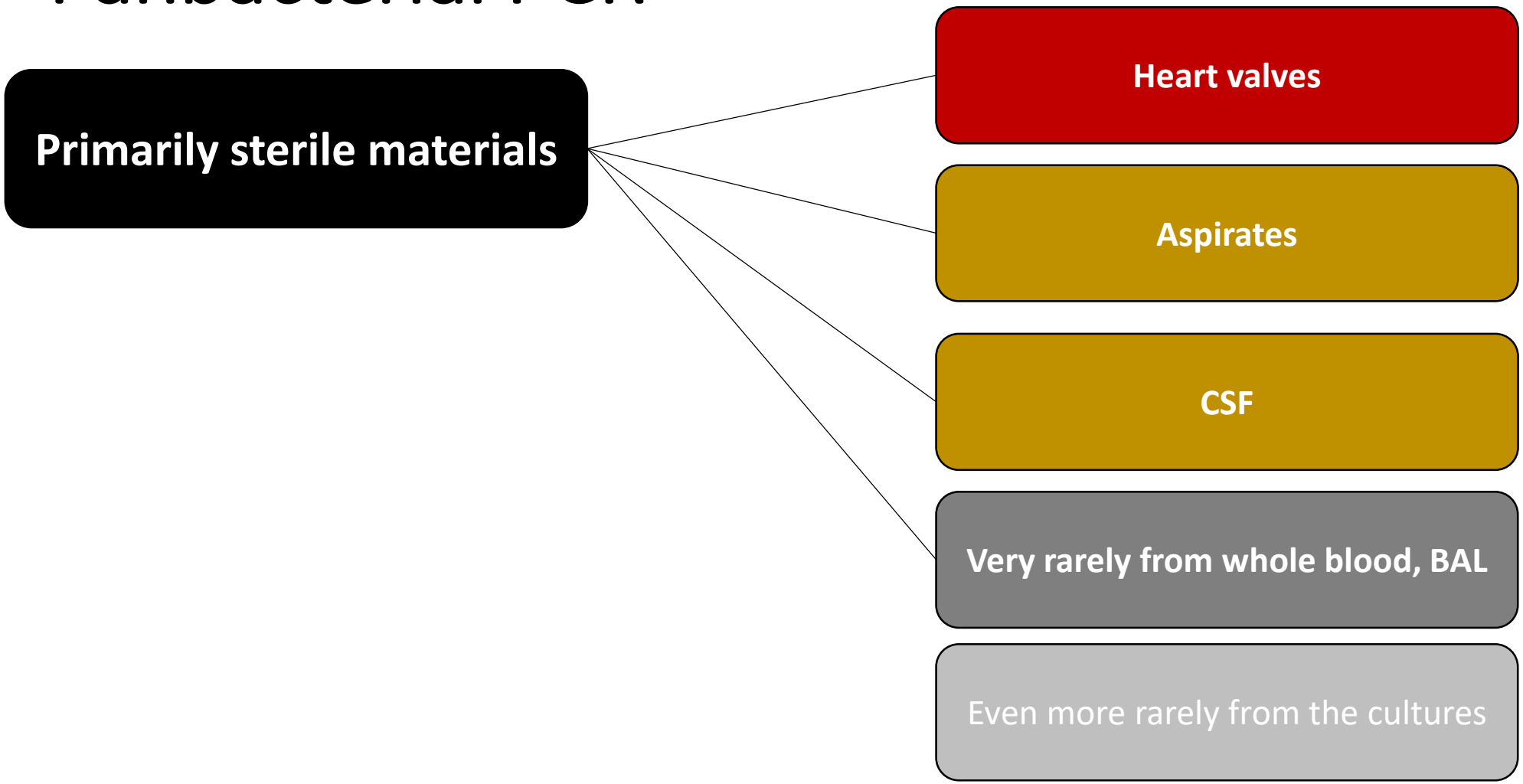


Figure 1: An example of a 16S rRNA gene. The regions in green are conserved in all microorganisms. These are the sites that are targeted by primers for PCR amplification so that all the 16S rRNA genes in a sample are amplified. The grey regions are the species-specific regions that-- when sequenced-- 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

Panbacterial PCR



Case reports

Case 3 – use special instructions

Case 1

- Beware of bacterial infections (incl. S.aureus) after viral pneumonia
- In severe courses, always think about PVL and adapt to it terpaii – add ATB inhibiting protein synthesis (Clindamycin / linezolid)
-

Case 2

- Coagulase-negative staphylococci (CoNS) are not always contaminated! A relevant find wherever it can form a biofilm.
- There are more than two CoNS - not just S. epidermidis and S. saphrophyticus (S. hominis, S. capitis, S. warneri, S. haemolyticus, S. lugdunensis and 100 others).
-

Case 3

- A culture-negative finding does not necessarily mean sterility and termination of the investigation.
- With clear clinical suspicion and prompting the laboratory, it is possible to investigate panbacterial PCR from primarily sterile materials.
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Case 4

- Not all CoNS are contamination of urine collection. S.saphrophyticus is a relevant pathogen.)
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- Specific sensitivities are examined according to the microbe and material (urinary staphylo, sensitivity)
-

Case 5

- *S. aureus* is a common cause of IE in IV drug addicts.
  - Treatment according to antibiogram
  - Empirically vancomycin – due to possible MRSA, depending on susceptibility - de-escalation to oxacillin
  - Always in combination, usually gentamicin (synergistic effect) and rifampicin (penetration into biofilm)

When you cann‘t keep going, go faster!

YouTube od 2:07


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