



2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA




**Univerzita
Karlova**

**FN
M+H** Fakultní
nemocnice
Motol a Homolka



Národní institut
virologie a bakteriologie

Diagnostics of significant Gram-positive cocci II (streptococci and enterococci)

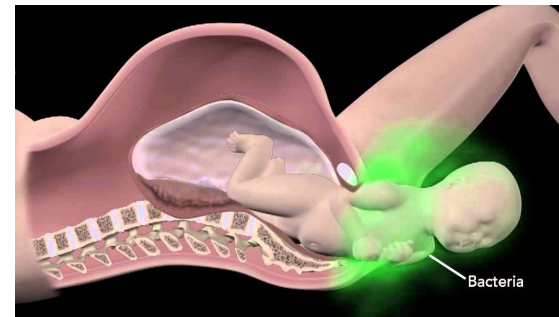
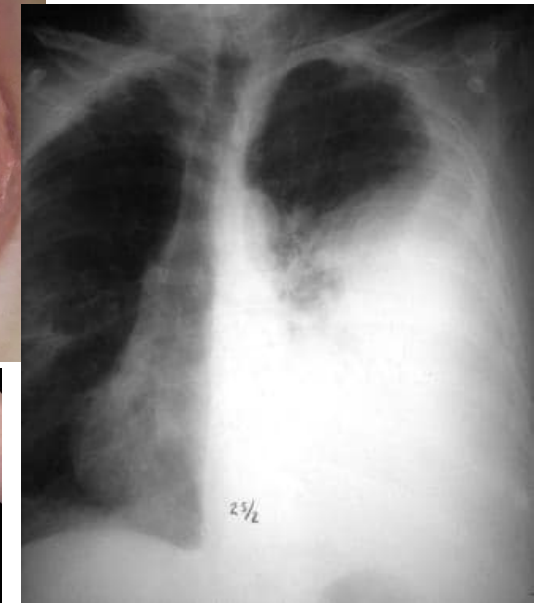
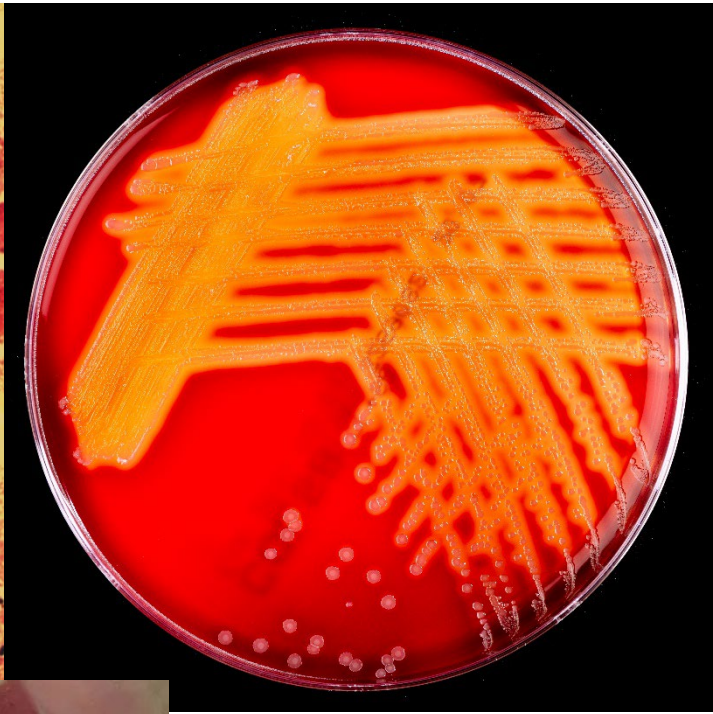
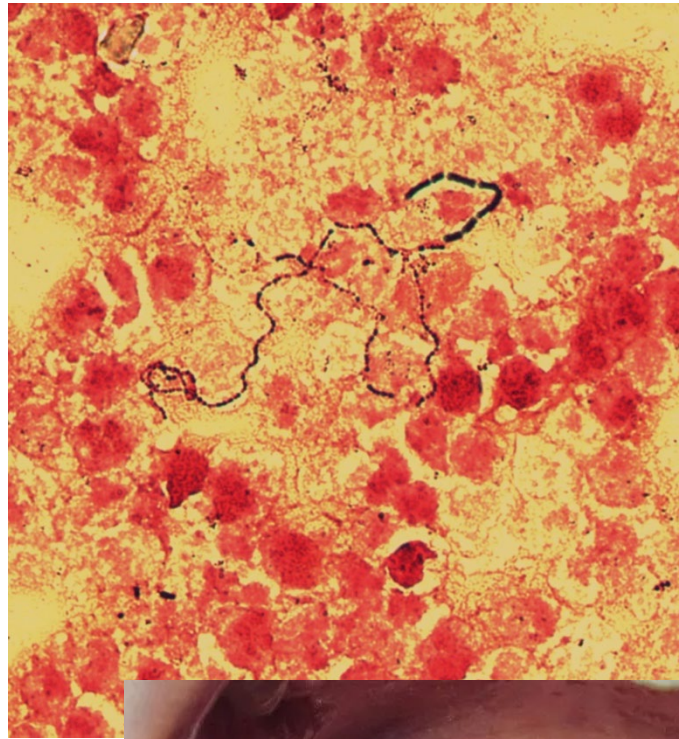
Jakub Hurych

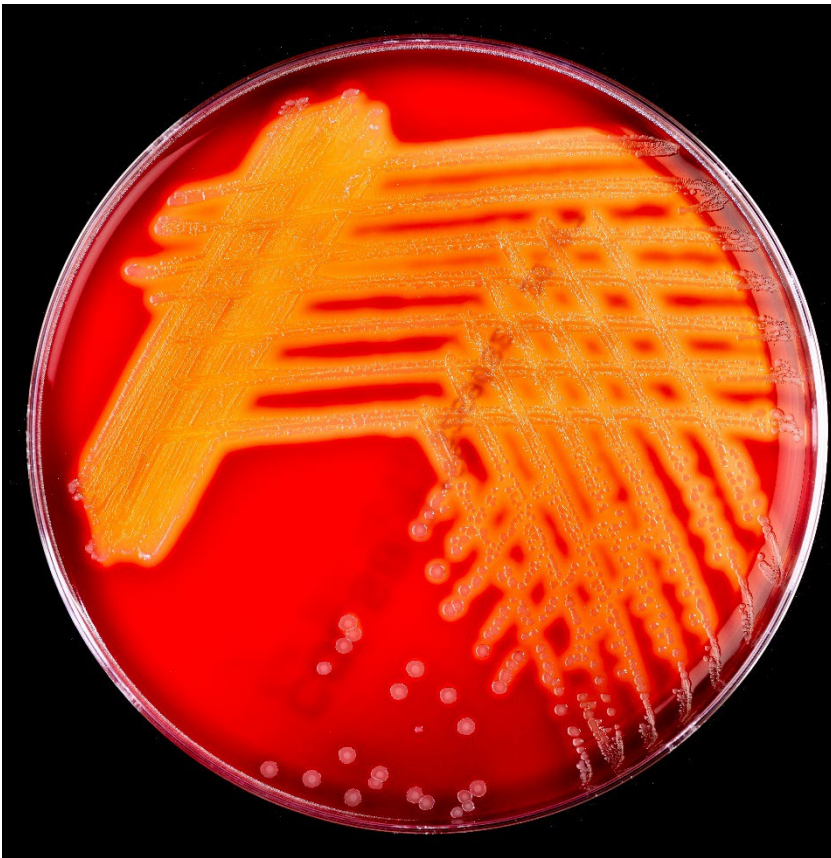
Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University &
Motol and Homolka University Hospitals

Microbiology I
Practical sessions

Who are they?

- **G+ cocci in pairs and chains**
 - Facultatively anaerobic
 - **Catalase-negative**
- Culture conditions
 - Undemanding: at 37°C, 18–24 h
 - No special atmosphere required
 - Pathogenic species are more difficult to culture
- Occurrence and significance
 - Opportunistic pathogens
 - Physiological microbiota





Beta haemolysis

Representatives

S. pyogenes

S. dysgalactiae, *S. constellatus*,
S. anginosus, *S. intermedius*

S. agalactiae



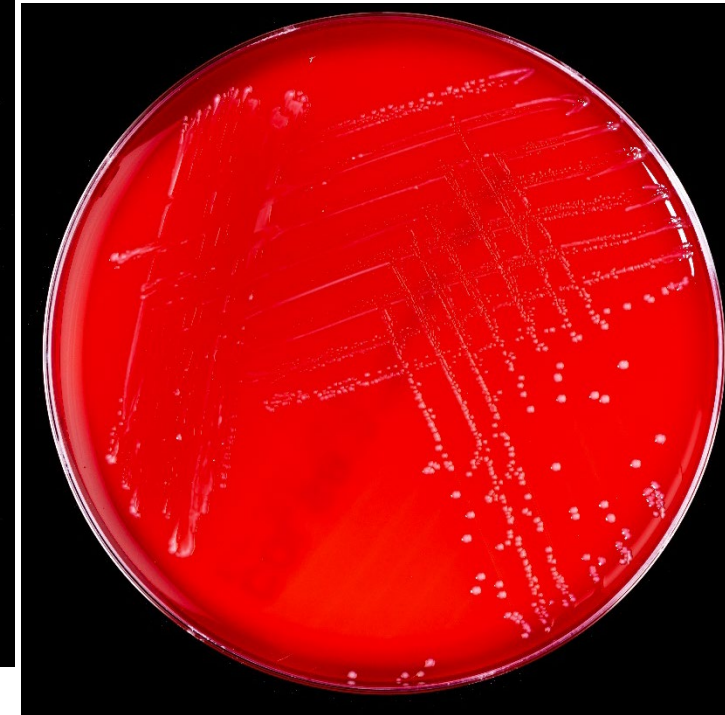
Alpha haemolysis

Representatives

S. pneumoniae

S. mitis, *S. mutans*, *S. salivarius*,
S. sanguinis

Sometimes *E. faecium*

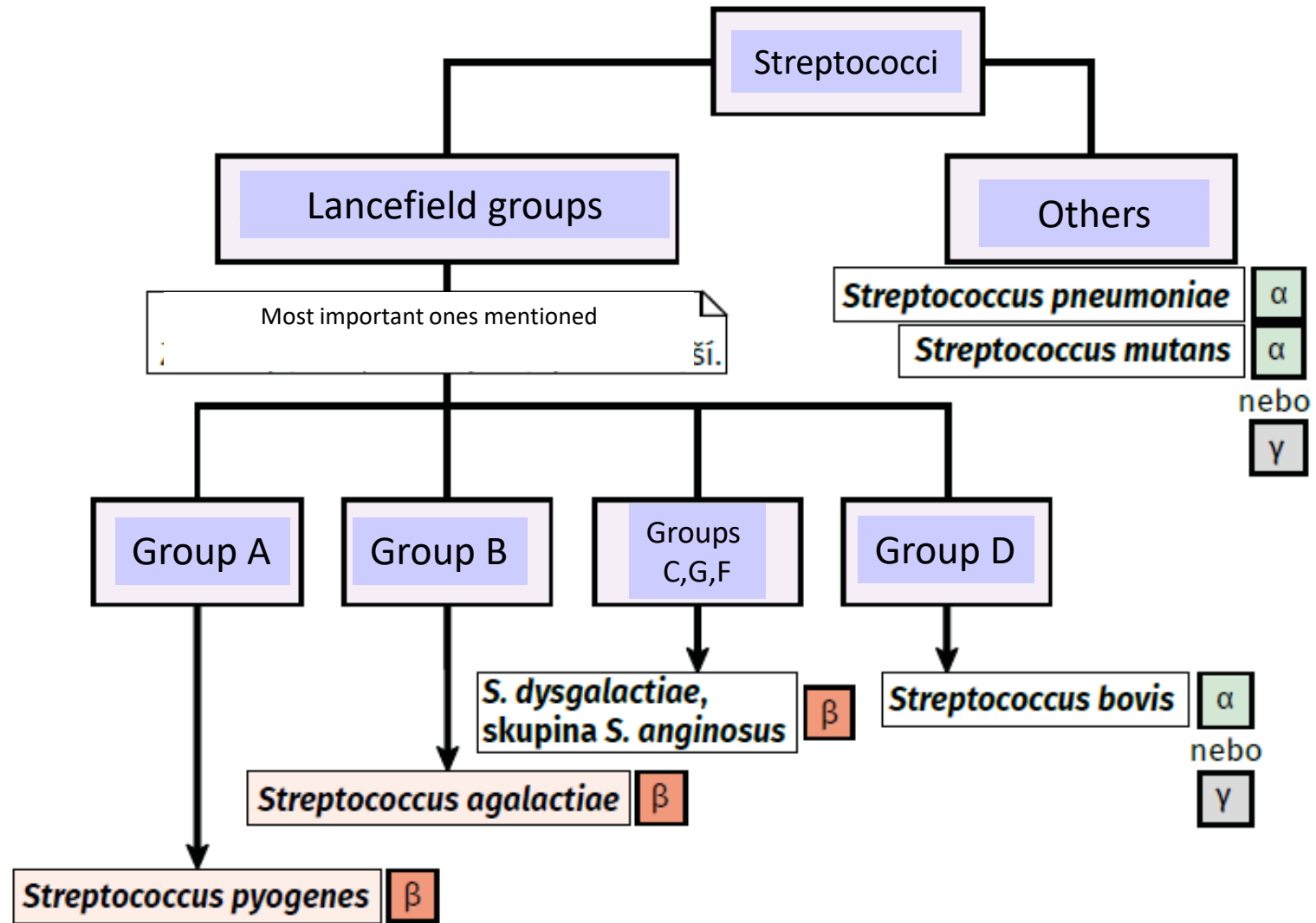


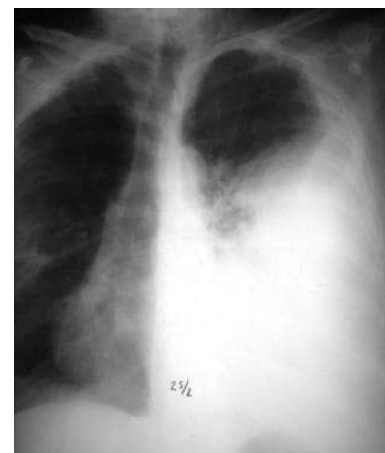
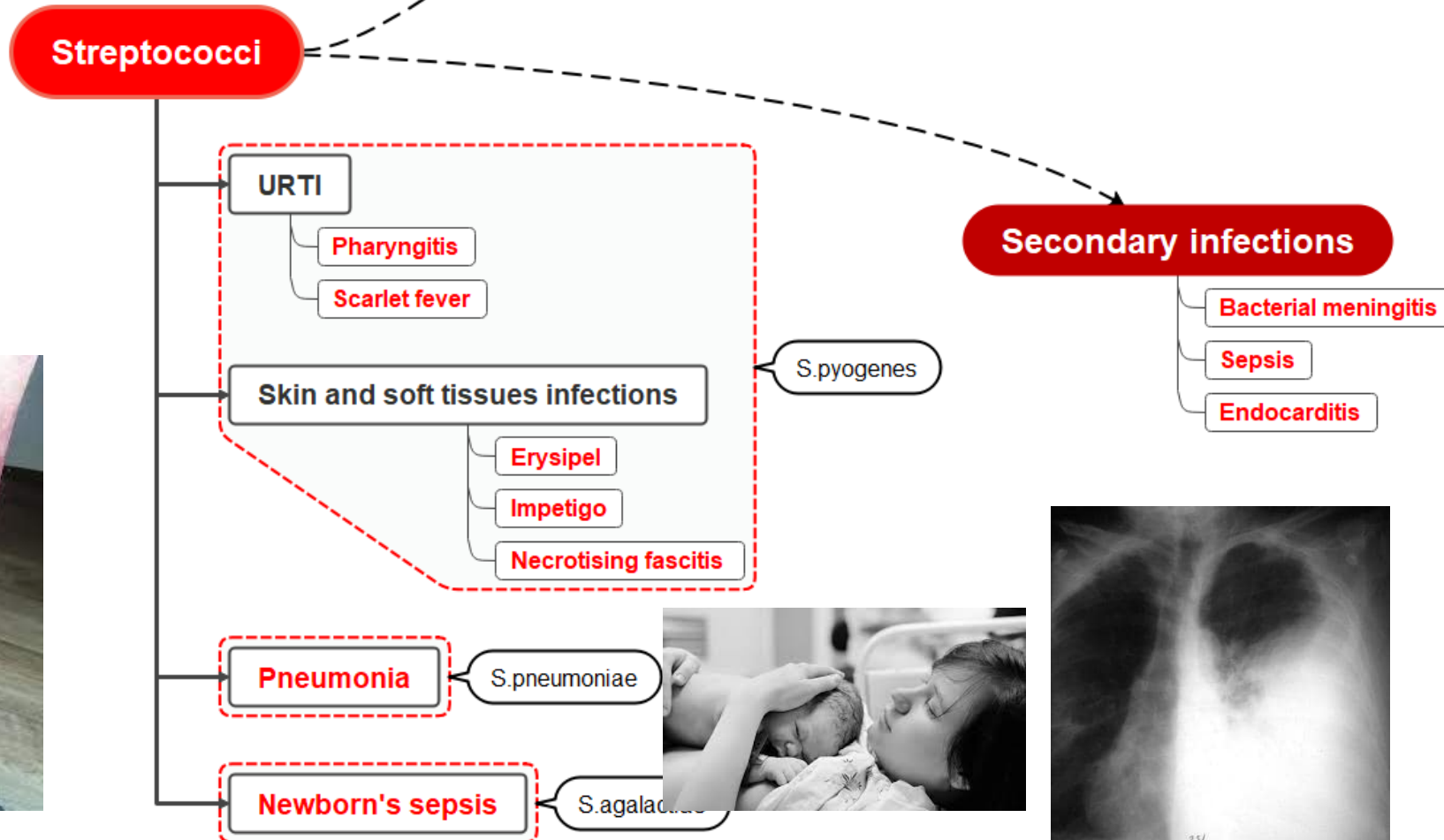
Gamma haemolysis

Representatives

E. faecalis, *E. faecium*

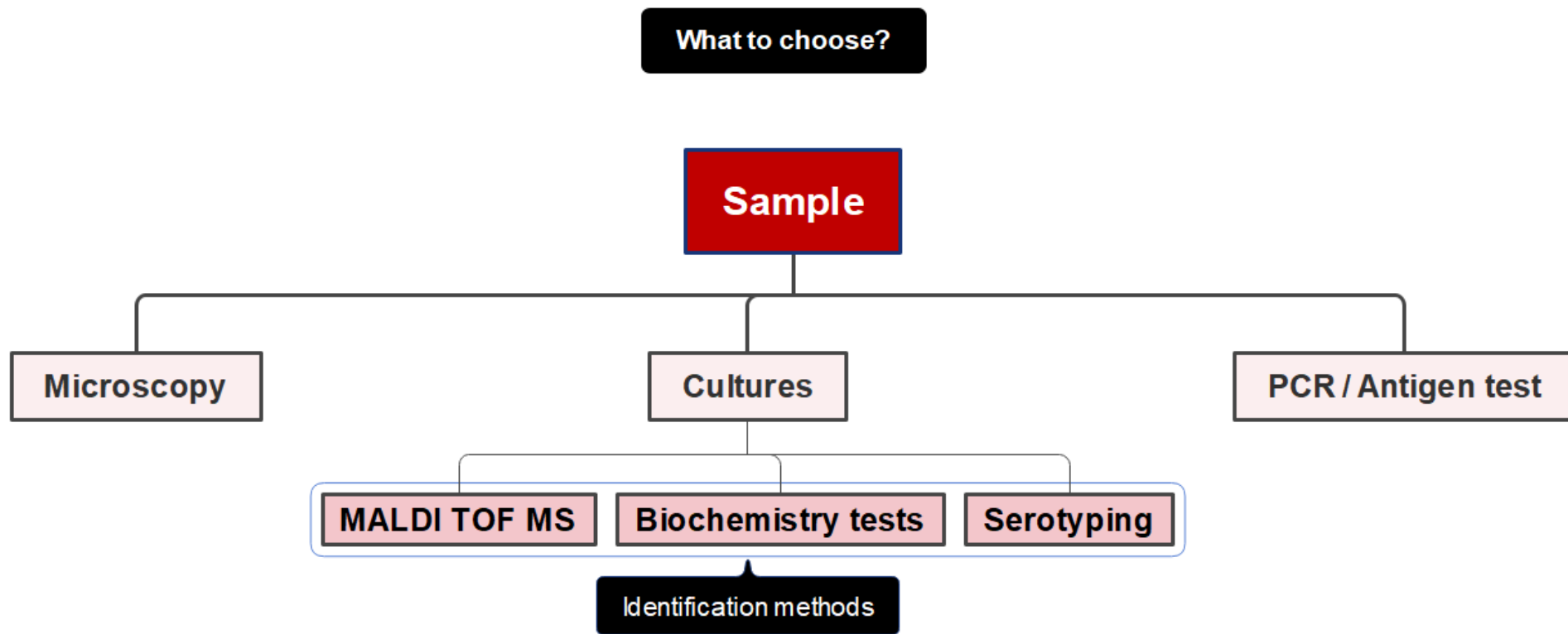
Many streptococci





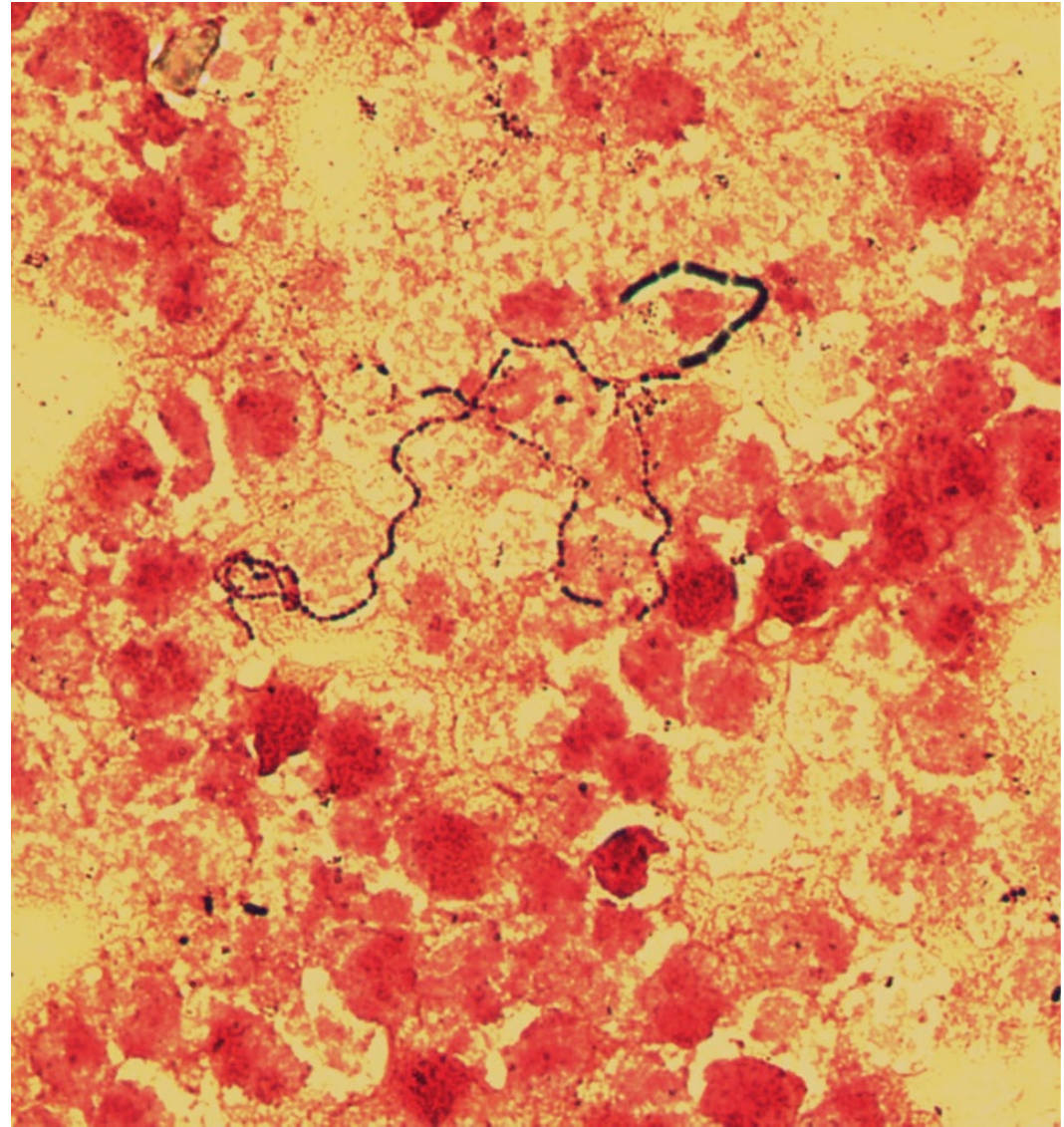
Agent	Hemolysis	Pathogenicity	Diseases
<i>S. pyogenes</i>	β	+++	- Respiratory infections (tonsillopharyngitis, scarlet fever) - Pyoderma (impetigo, erysipelas, cellulitis) - SSTI (necrotising fasciitis to myonecrosis) - BSI - Toxic shock syndrome - Sterile complications (rheumatic fever, acute post-glomerulonephritis)
<i>S. dysgalactiae</i> , <i>S. constellatus</i> , <i>S. anginosus</i> , <i>S. intermedius</i>	β	++	Physiologically in URT - Respiratory infection (tonsillopharyngitis)
<i>S. agalactiae</i>	β	+++	- Neonatal meningitis - Neonatal sepsis - SSTI (chronic wounds, joints)
<i>S. pneumoniae</i>	α	+++	- Pneumonia - Meningitis - Otitis The no. 1 agent of bacterial pneumonia, OMA and sinusitis
<i>S. mitis</i> , <i>S. mutans</i> , <i>S. salivarius</i> , <i>S. sanguinis</i>	α	+	Physiologically in the oral cavity * SSTI = skin and soft tissue infection; BSI = bloodstream infection

Diagnosis of streptococci



Microscopy

- G+ cocci in chains





Cultures

Blood agar (visible changes), chocolate agar

18–24 hours

37°C, elevated CO₂ tension

Assessment: viridation, complete haemolysis, no change on BA

Confirmation – biochemical tests (commercial kit, e.g. Streptest), mass spectrometer, individual tests (see below)

Biochemical tests

- **PYR** test (pyrrolidone-l-arylamidase) – strip, colour change positive for *S. pyogenes* (but also for enterococci)

L-Pyrrolidonyl Arylamidase (PYR) Test



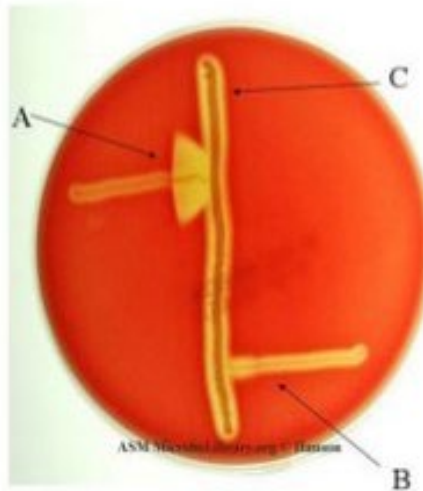
Biochemical tests

- **CAMP** test – type of haemolysis near *S. aureus* – typical butterfly- or bow-shaped haemolysis → positive for *S. agalactiae* (group B)

(A) *Streptococcus* (group B)
B) positive test
(enhanced hemolysis)

(B) *Streptococcus pyogenes* (group A)
negative test

(C) *Staphylococcus aureus* – replaced by
Clostridium perfringens
in reverse CAMP test
(see next slide)



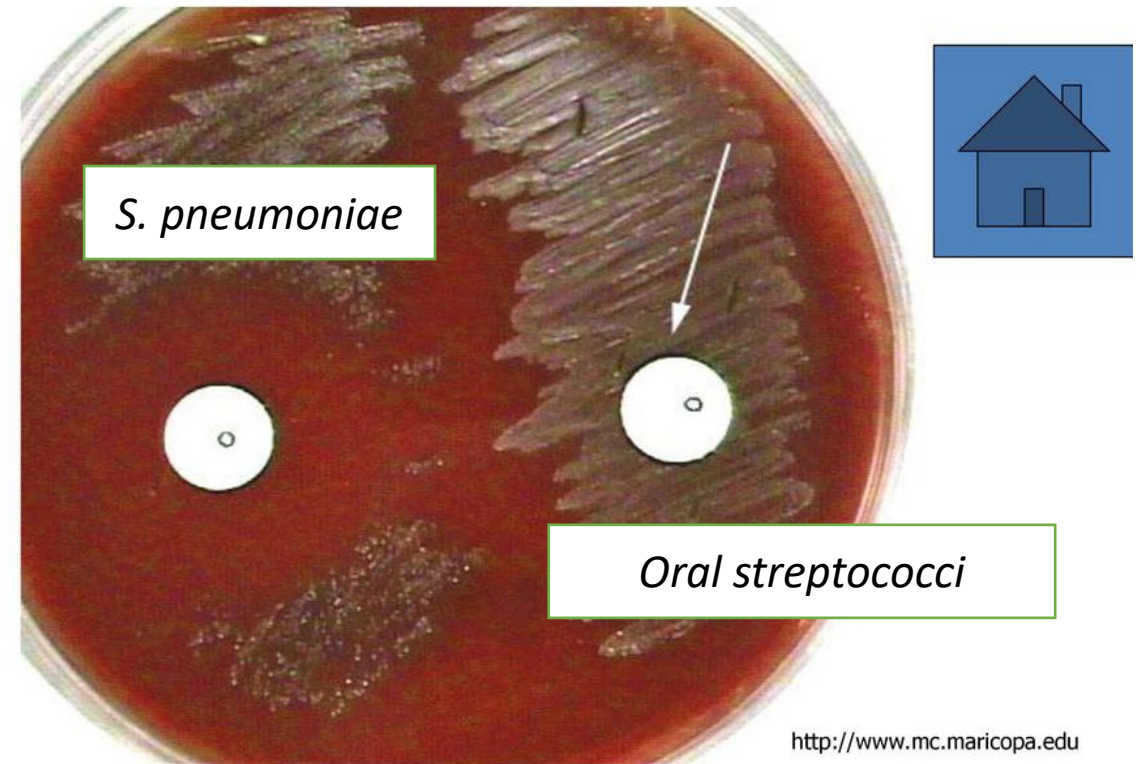
If the PYR and CAMP tests are negative, we can rule out *S. pyogenes* and *S. agalactiae*



Biochemical tests

Optochin test and bile solubility test

- Result:
 - Both positive for *S. pneumoniae*
 - Negative – **oral viridans streptococci**



Selective media

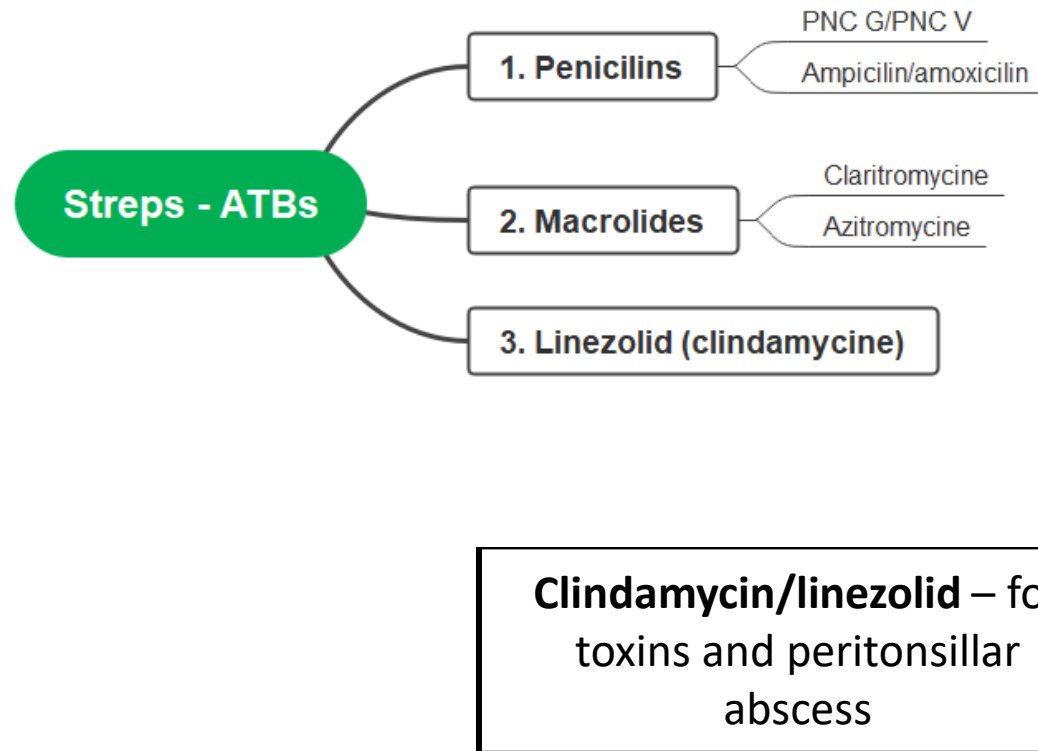
- **Chromogenic media** – presence of *S. agalactiae* in vaginal swabs from pregnant women (covered by law)



GBS screening

- in all pregnant women between 35 and 37 weeks of pregnancy;
- culture from a swab of the lower third of the vagina and the rectum
- In positive cases – indication for intrapartum antibiotic prophylaxis

Antibiotic susceptibility testing

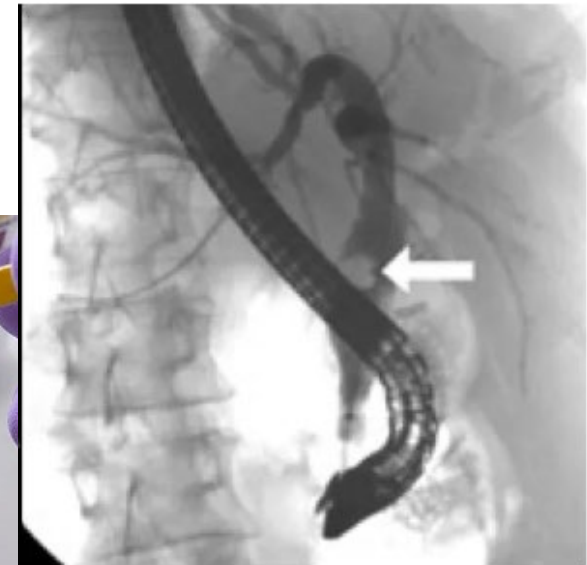
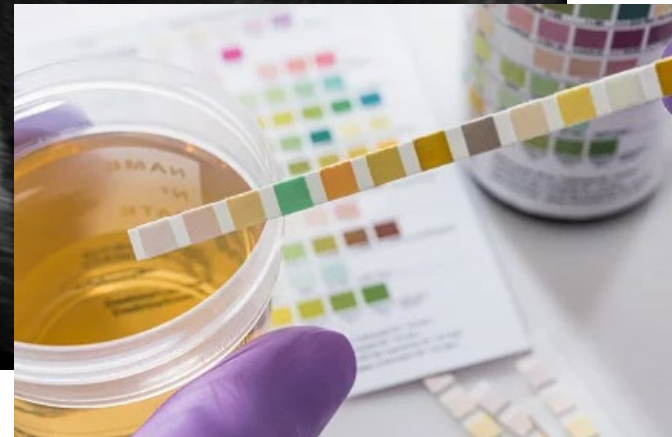
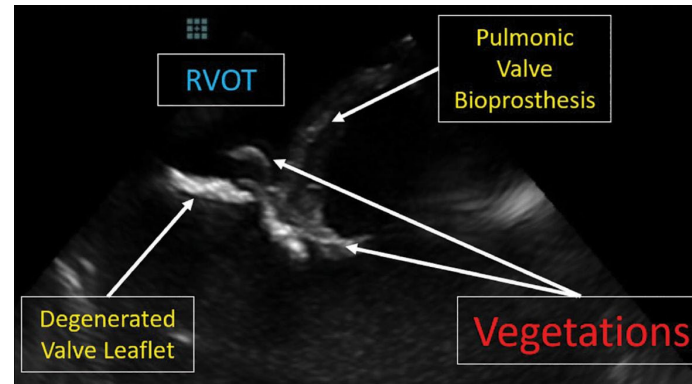
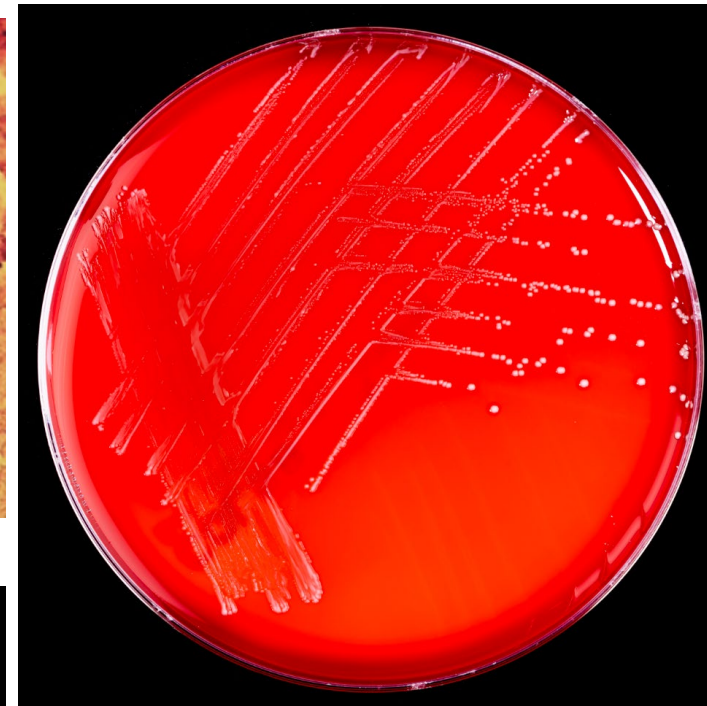
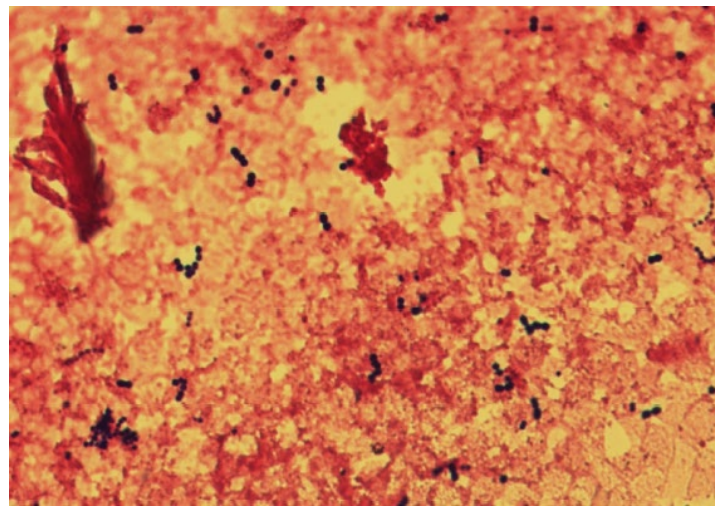


- **MLSb** – cross-resistance to macrolides, lincosamides and streptogramins (quinupristin/dalfopristin) – **high resistance rates (up to 20%)**
- *S. pyogenes* – sensitive to penicillin
- *S. pneumoniae* – penicillin resistance approx. 5%

Enterococci

Who are they?

- **G+ cocci in pairs**
 - Facultatively anaerobic
 - Catalase-negative
 - PYR positive
- Culture conditions
 - Undemanding: at 37°C, 18–24 h
 - No special atmosphere required
- Occurrence and significance
 - Opportunistic pathogens
 - Physiological microbiota of the gut and vagina

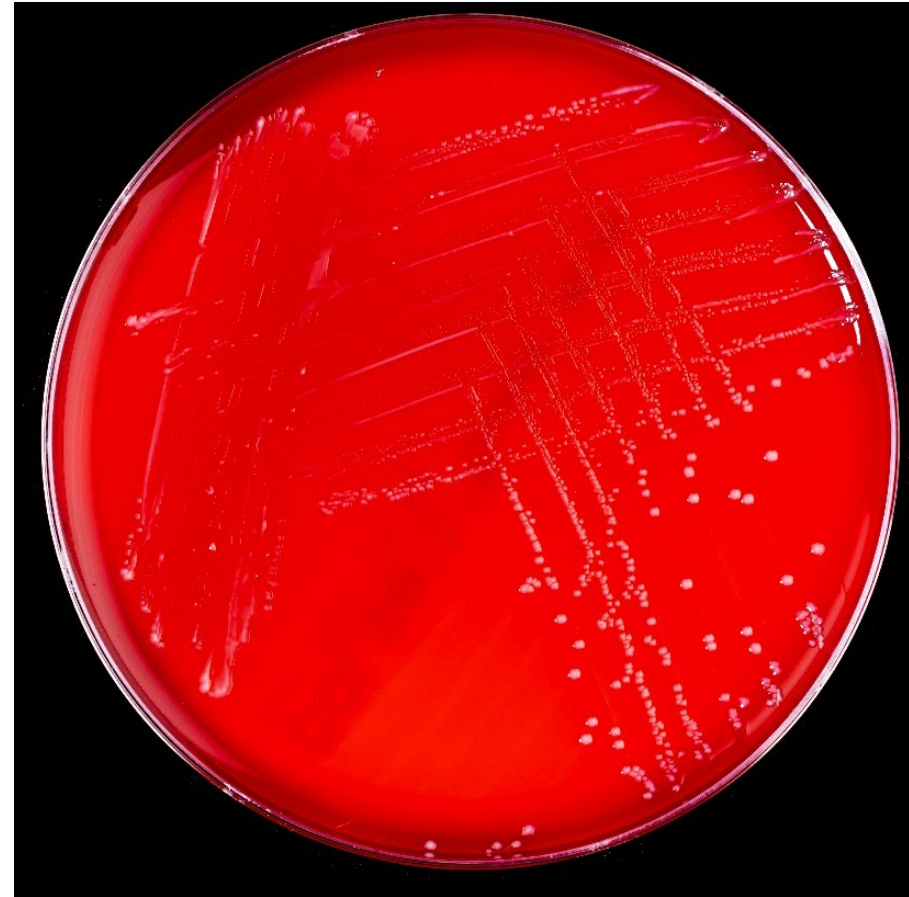




Alpha haemolysis

Representatives

E. faecium – occasionally

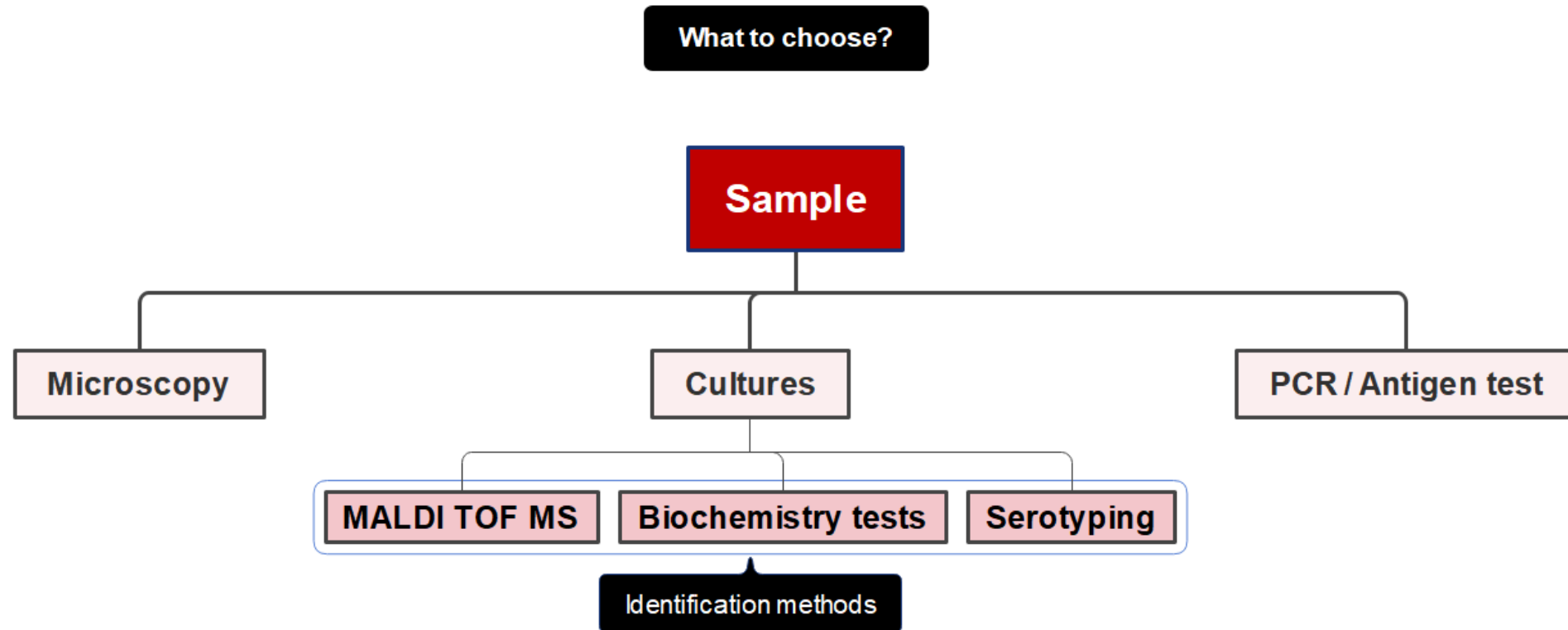


Gamma haemolysis

Representatives

E. faecalis, *E. faecium*

Diagnosis of enterococci



Microscopy

- G+ cocci in pairs or short chains



CHARLES UNIVERSITY
Second Faculty of Medicine

Preparát č. 6

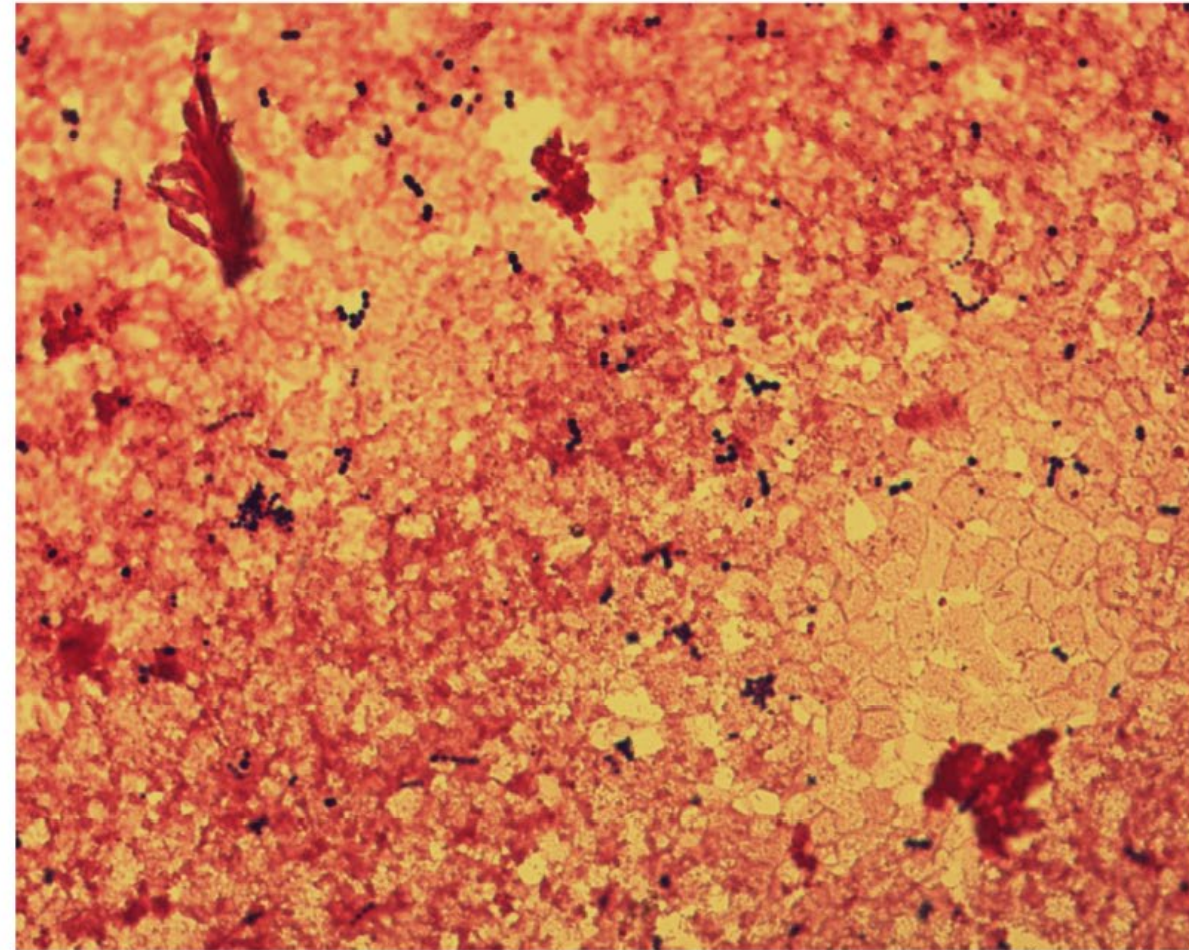
*Enterococcus
faecalis*

Materiál: hemokultura

Pacient: žena, 71 let

Dg.: cholangiogenní seps

Mikroskopie: grampozitivní koky
v řetízcích



Autor: MUDr. Petra Kabelíková

Cultures



Blood agar (visible changes)

18–24 hours

37°C

Assessment: viridation, no change on BA

Identification – mass spectrometer

Biochemical tests

- **PYR** test (pyrrolidone carboxylic acid lyase) – strip, colour change positive for enterococci

L-Pyrrolidonyl Arylamidase (PYR) Test



Selective media

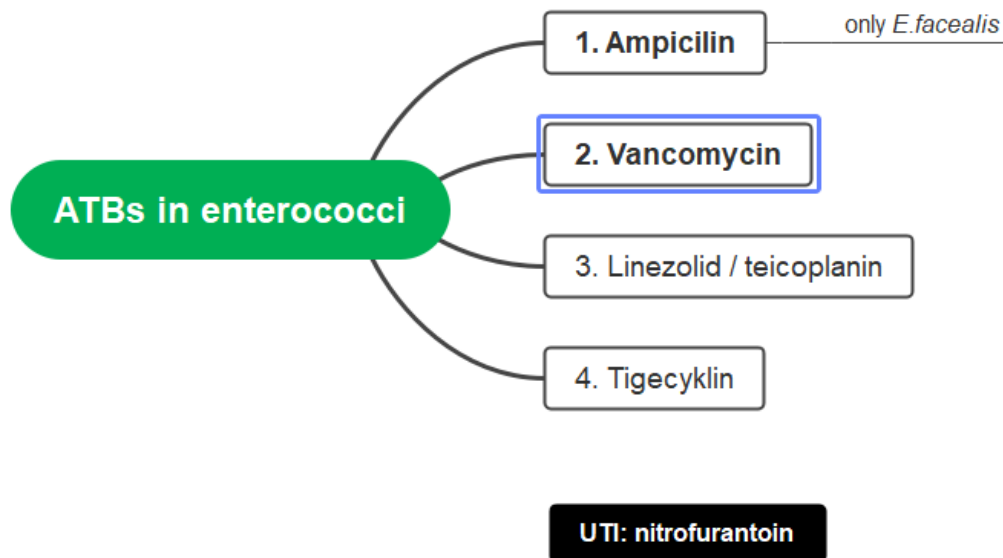
- **CAP agar** – blood agar with added colistin and aztreonam – only G+ bacteria grow

VRE screening

- In immunocompromised patients
- Culture from rectal swab
- Culture from swabs
- In positive cases – it is essential to know the antibiotic susceptibility profile and consider administering antibiotics in the event of a sudden deterioration in condition (linezolid, teicoplanin)



Antibiotic susceptibility testing



- **VRE** – screening required
- Resistance to ampicillin in *E. faecium* is acquired, but is now predominantly prevalent; it is therefore considered PR

Primary resistance
to cephalosporins!

Antibiotics tested in practice

425 Zona (ENFE)	
ATB	Mez
ampicilin	8-10
vankomycin	12-12
teikoplanin	18-18
linezolid	20-20
tigecyklin	20-20

Systemic infections



412 Zona (ENFE)	
ATB	Mez
ampicilin	8-10
nitrofurantoin	15-15
ofloxacin	12-12
vankomycin	12-12
teikoplanin	18-18

Urinary tract infections

