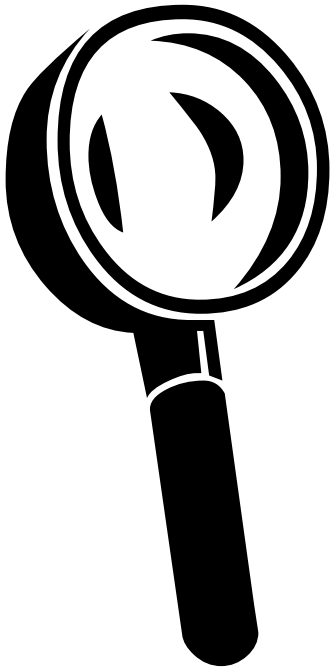


Institute of Microbiology shows:



TRACING THE CULPRIT

Part one: Staphylococcus

Contents of the slideshow

Clinical characteristics: *Staphylococcus aureus*

Clinical characteristics: CONS (coagulase-negative staphylococci)

Diagnostics of staphylococci

Differential diagnostics of staphylococci I

Differential diagnostics of staphylococci II

Antibiotic susceptibility testing and antibiotic treatment

Clinical
characteristics:
Staphylococcus
aureus

Story One



- **Mrs. J. K.**, cook in students canteen, has a **blister on her hand, full of white-yellow pus**. She is not aware. She takes dumplings by her hand, although the dumplings are already cooked (and now they will be only slightly heated, not cooked).
- **Student Rashid** and his girl-friend eat the dumplings. In the afternoon, they should have a rendez-vous ... BUT... half an hour before the rendez vous, Rashid started to have **abdominal pain, vomiting and diarrhoea**. The girl-friend, called, says she has the same problems... So, no romantic afternoon...

Who was guilty?

- It was the not careful cook, but also ***Staphylococcus aureus***
name from Greek σταφυλή (staphylé) = grape
- This „golden staphylococcus“ often causes pyogenic infections of skin, hairs, nails etc.
- Some (very rare!) strains produce **enterotoxins**, that act as so named **superantigens**
- Intoxication by a bacterial toxin usually starts quickly and finishes quickly, unlike a bacterial infection (e. g. salmonellosis). It is necessary to understand the difference:
 - **Enterotoxigenesis** = situation where the disease is caused by ingested toxin produced **outside** the intestine, not directly by the microbe. The microbe is often even absent in the intestine
 - **Intestinal infection** = situation where the microbe multiplies in the gut (and either invades the wall of the intestine, or produces toxins **inside** the intestine)

Story Two

- **Student P. Z.** is nervous: again, she has „her days“. Luckily, she has the cheap tampons, that she bought several days ago...
- Suddenly, she started to have **shaking, faintness, fever**. The room-mate found her lying on the floor, and called 155 (or maybe 112?). A **rash** emerged. The student is hospitalized on emergency unit of infectology clinic...

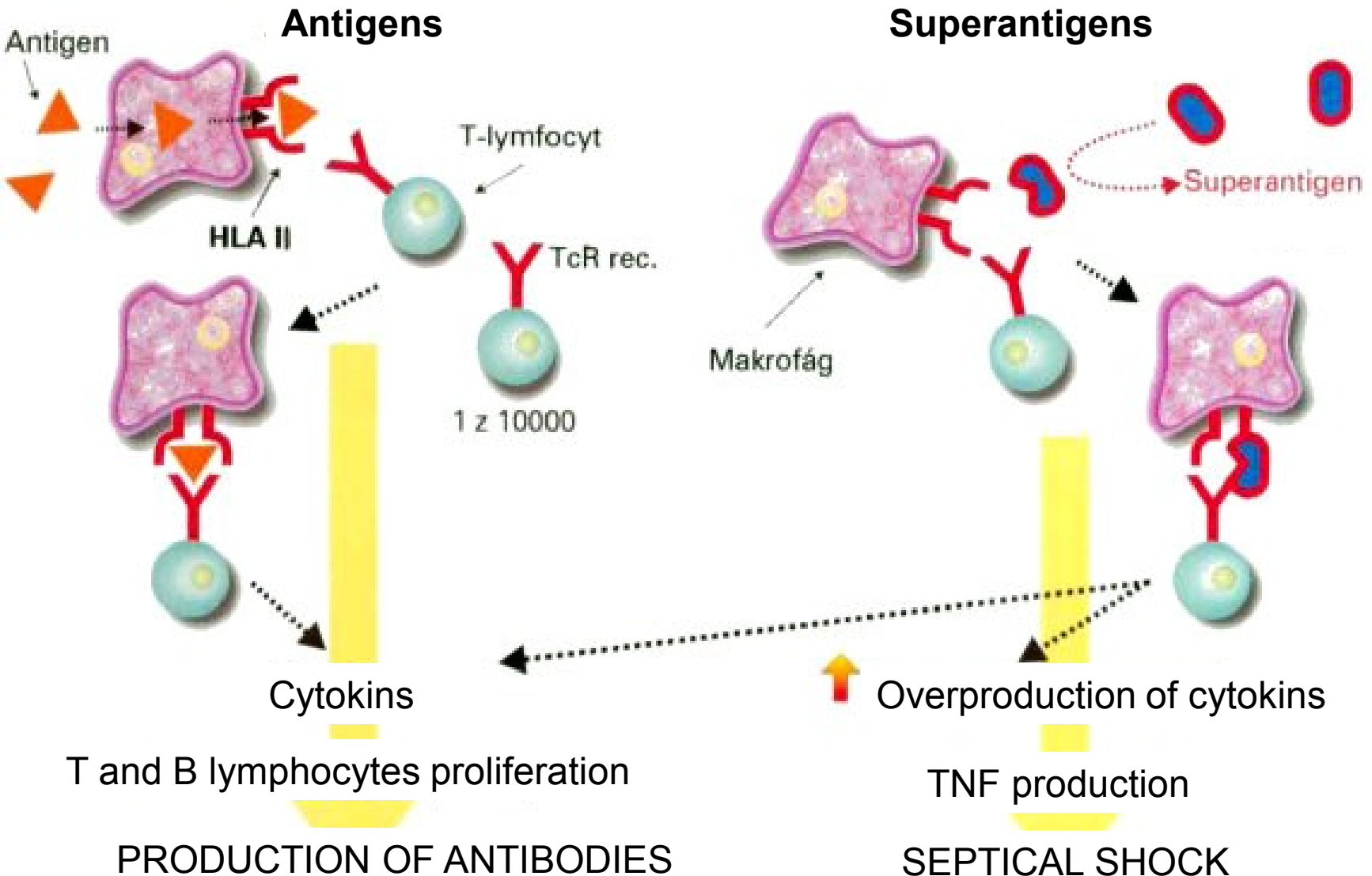
Who is guilty?

- Again, it is ***Staphylococcus aureus***, now a strain called **TSST-1** (toxic shock syndrome toxin)
- This toxin, too, is a **superantigen**
- It causes toxic shock, typically

Superantigens

Immunity response

www.zuova.cz/informace/nrlpab16.php

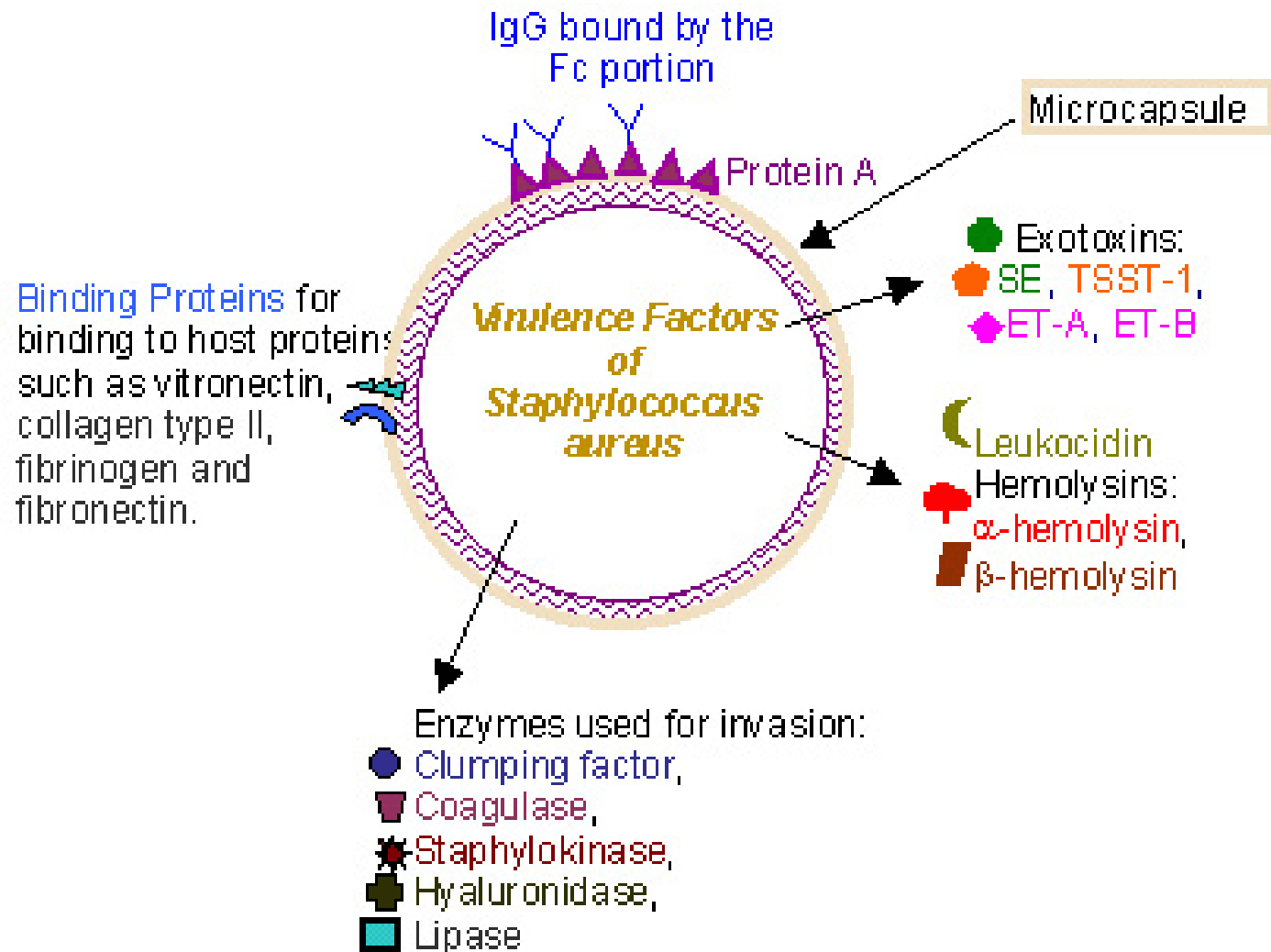


Staphylococcus aureus

(golden staphylococcus)

- The only one routinely important for humans among so named **coagulase positive staphylococci**
- Causes skin, hair, nail infections, otitis externa, conjunctivitis, respiratory infections
- Sometimes also causes abscesses in tissues
- Some strains with **uncommon** virulence factors cause serious, but rare, diseases
- On the other hand, the microbe may be often found even on skin of healthy persons

There exist plenty of virulence factors found in *S. aureus*...



- ...but only some of them are present in nearly 100% strains; other are produced just by one strain among one thousand!

Abscesses

- Unlike streptococci, producing in tissues mostly uncoated **phlegmonas**, staphylococci form mostly coated **abscesses**.
- *Formation of an abscess (using so named clumping factor and plasmacoagulase – see further!) is in a scheme, from a German website – see here:*



Examples of infections caused by staphylococci: Impetigo...



...bulous impetigo...



...otitis externa
with a furuncle...



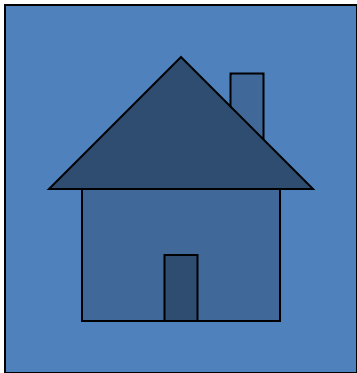
...or skin infection with crusts...

http://www.dermatology.co.uk/media/images/Infection_staphylococcus_crusting_chin_closeup.jpg



...but also brain abscesses

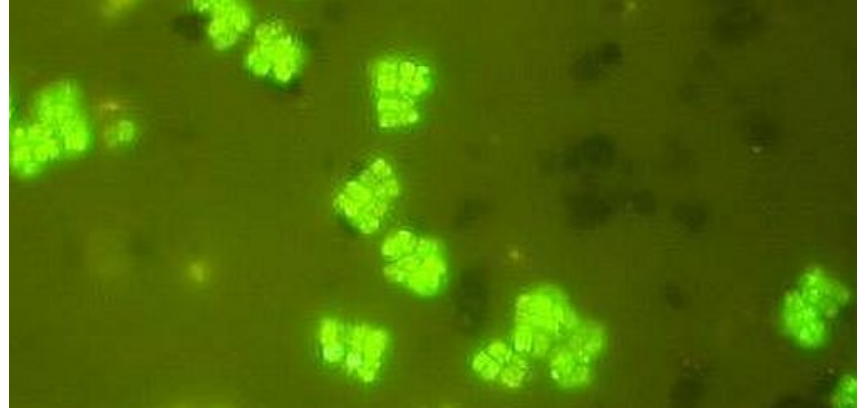
http://www.mja.com.au/public/issues/176_12_170602/got10354_fm.html



Clinical
characteristics:
Staphylococcus
sp. (coag.-neg.)

Story Three

<http://www.zuova.cz/informace/pic/ompovabac20b.jpg>



- **Young man F. B.** recovers after a severe traffic accident. He has two venous catheters for infusion nutrition and blood taking.
- Suddenly, his status **worsened actually**, high and quickly changing fevers – the ward doctor has suspicion for septicemia and takes blood for blood culture
- After **catheter change** and **antibiotic treatment** the status improved again

And who is guilty now?

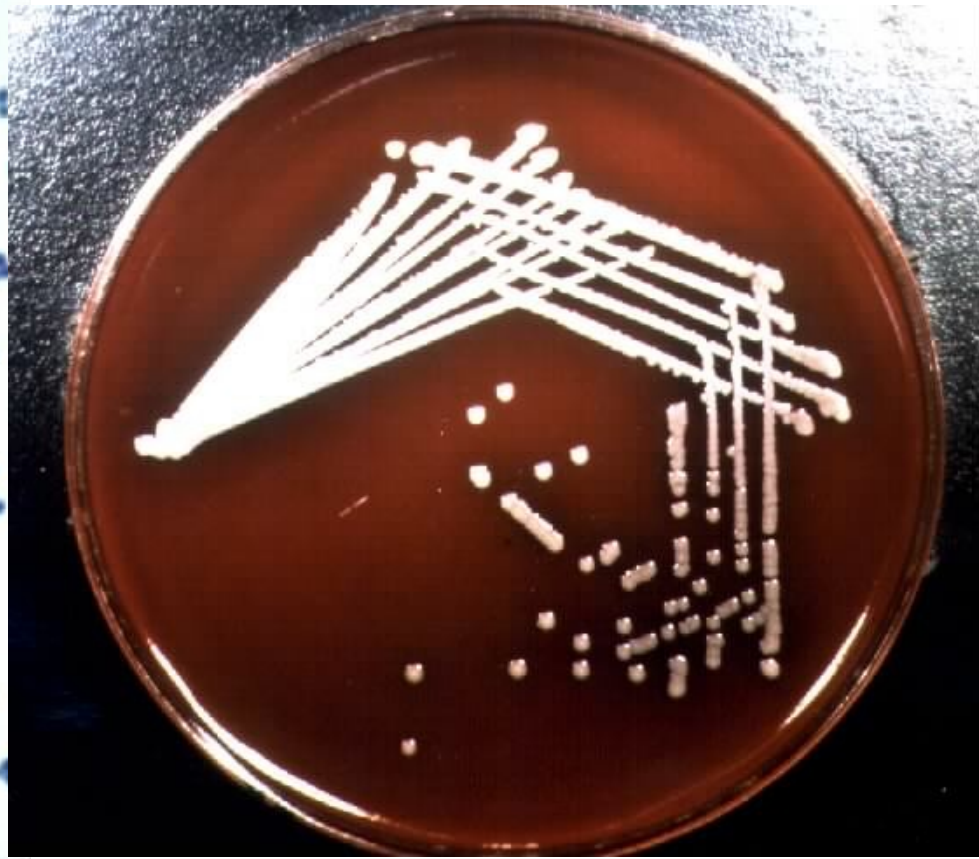
- The guilty is *Staphylococcus epidermidis*, the most common among **coagulase negative staphylococci**
- Coagulase negative staphylococci belong to **the same genus as „golden staphylococcus“**
- They are **much less pathogenic**
- In last decades, they started to be very important causative agents of infections in **weakened persons**, mostly as hospital infections
- Often forms **biofilm** on venous catheters

Why „coagulase negative staphylococci“? See later...

Staphylococcus epidermidis

Author: Prof. MVDr. Boris Skalka, DrSc.

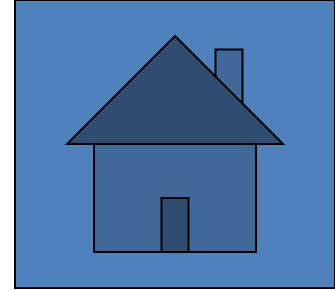
Author: Prof. MVDr. Boris Skalka, DrSc.



Coagulase negative staphylococci

- **Coagulase negative staphylococci** (*Staphylococcus epidermidis*, *S. hominis*, *S. haemolyticus* and about forty other species and subspecies) are the main parts of the common skin microflora.
- Nevertheless, they may cause UTI (mostly *S. saprophyticus*), wound infections, catheter septicaemias etc.
- So, the finding has a different meaning e. g. in nasal cavity (or in stool), in the urine, and of course, in blood culture.
- *Besides S. aureus and coagulase negative staphylococci, there also exist category of „coagulase positive staphs other than S. aureus. Nevertheless, this category has minimal importance in human clinical microbiology. Therefore we often simplify the situation and only speak about S. aureus and coagulase-negative staphylococci.*

There are many species of staphylococci today



E. g. S. simiae
was found by
Moravian
scientists in
rectal swabs
of Saimiri
sciureus
monkey in a
ZOO on Saint
Hill at
Olomouc

S. simiae (AT727531)

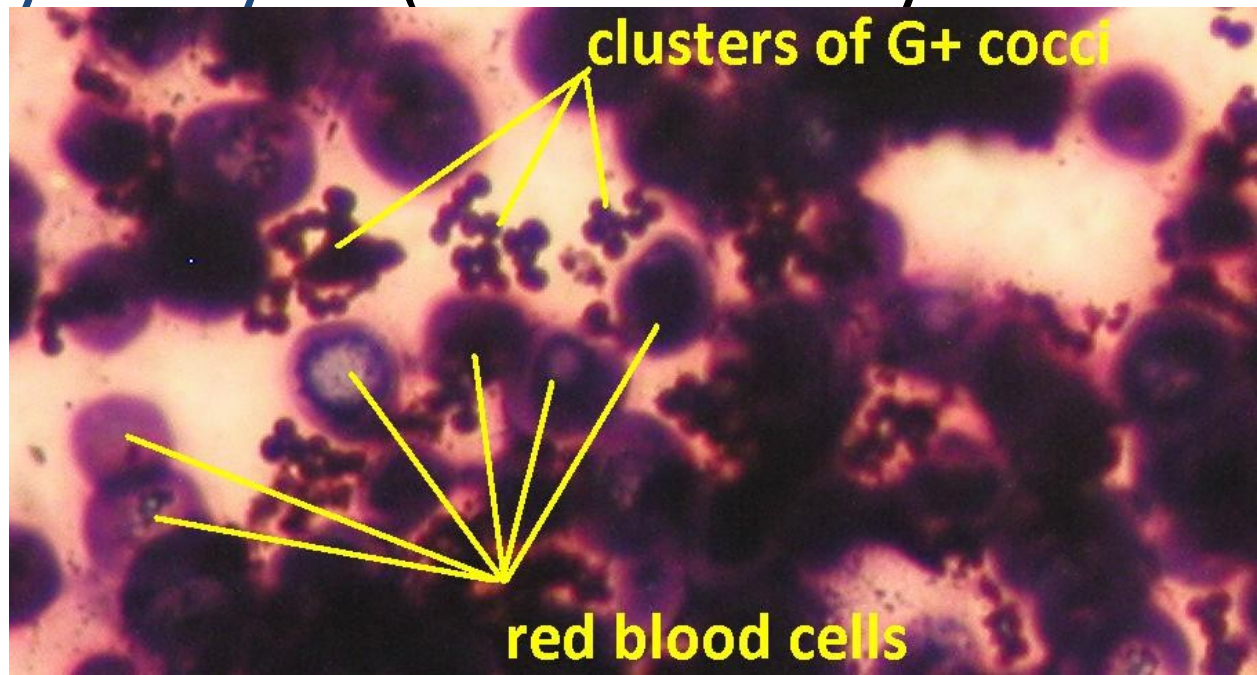
Diagnosatics of staphylococci

Description of culprits (diagnostics)

- **Microscopy:** gram-positive cocci
- **Cultivation:** on BA colonies 1–2 mm, slightly convex, butter consistence, white, or (mostly in golden staphylococcus) goldish
- **Biochemical tests:** catalase positive, oxidase negative, it is possible to differentiate individual species biochemically
- **Antigen analysis and special tests** may be considered very helpful at the diagnostics

When we try to find the culprit microscopically in the specimen

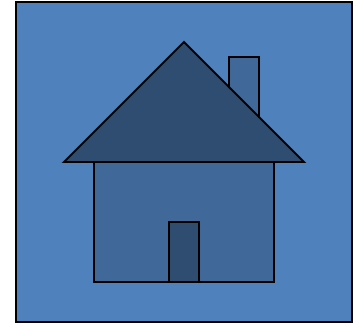
- We observe the Gram stained **microscopic blood culture preparation**
- We search for **Gram-positive cocci** in clusters, and also for **erythrocytes** (and eventually also other objects)



Photos from culprit Database



Author: Prof. MVDr. Boris Skalka, DrSc.



Author: Prof. MVDr. Boris Skalka, DrSc.



Differential diagnostics
of staphylococci I: from
„unknown bacterium“
to „*Staphylococcus*“

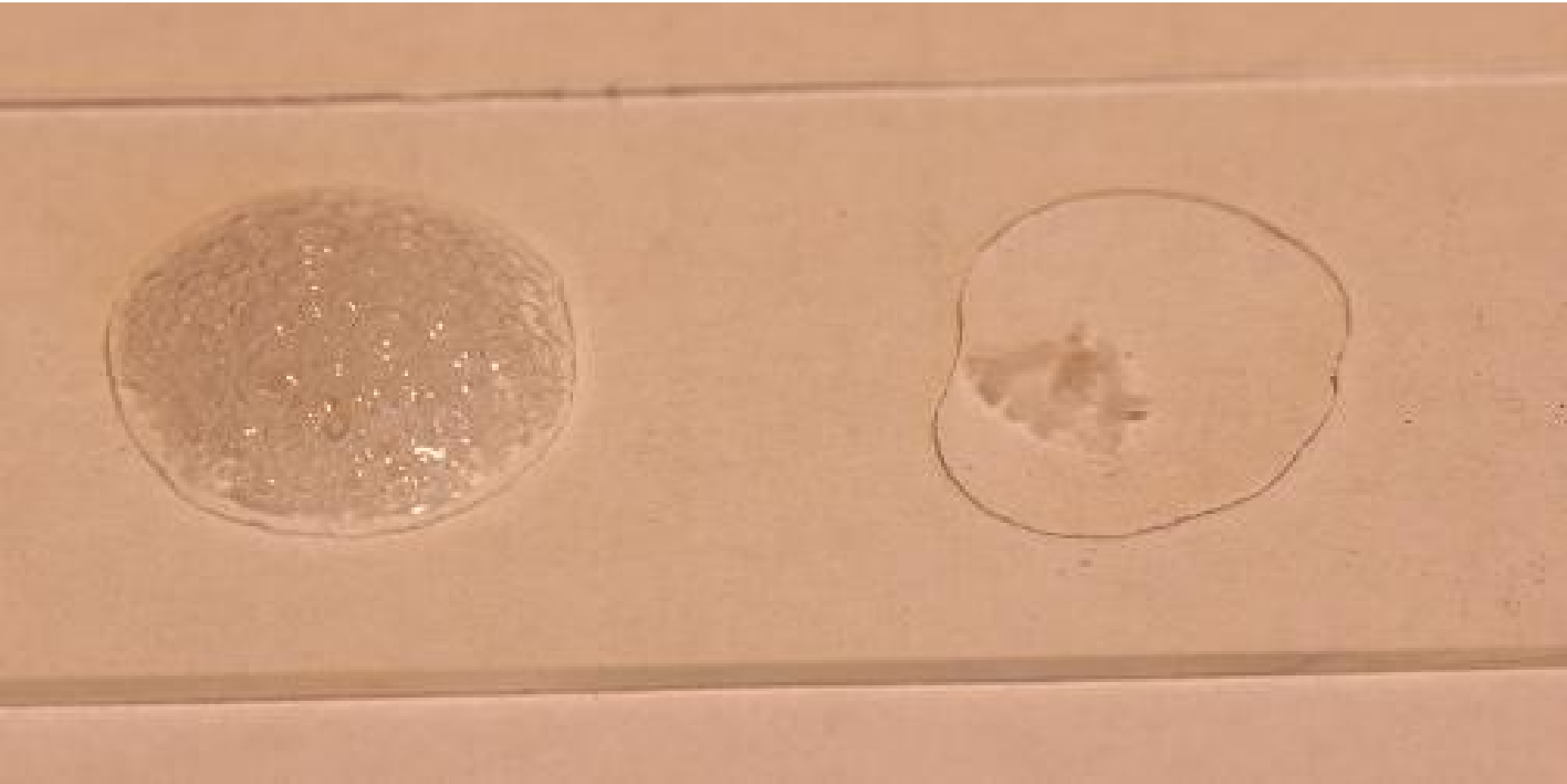
Singling out of other suspects (differential diagnostics 1)

- **Gram stain** differentiates **gram-positive cocci** from other shape/cell wall type bacteria
- **Positive catalase** differentiates staphylococci from streptococci and enterococci
- The same (and even better in a mixture) is **cultivation on BA with 10 % NaCl**
- *For orientation we can also use the fact that colonies of other G+ cocci are neither white nor goldish, and in microscopy, there do not have clusters*

Gram stain (repeating)

- **Gram stain:** we make a smear (using a small drop of saline), we let it dry, we fixate by a flame, then we stain: Gram 30 s, water, Lugol 30 s, water, alcohol 15 s, water, safranin 60 s, water, dry, immersion object lens 100× magnifying)
- **Now, we can exclude** all object that are gram negative and/or rods, e. g. that do not belong into group of „G+ cocci“

Catalase test (for remembering)



Catalase test + and -

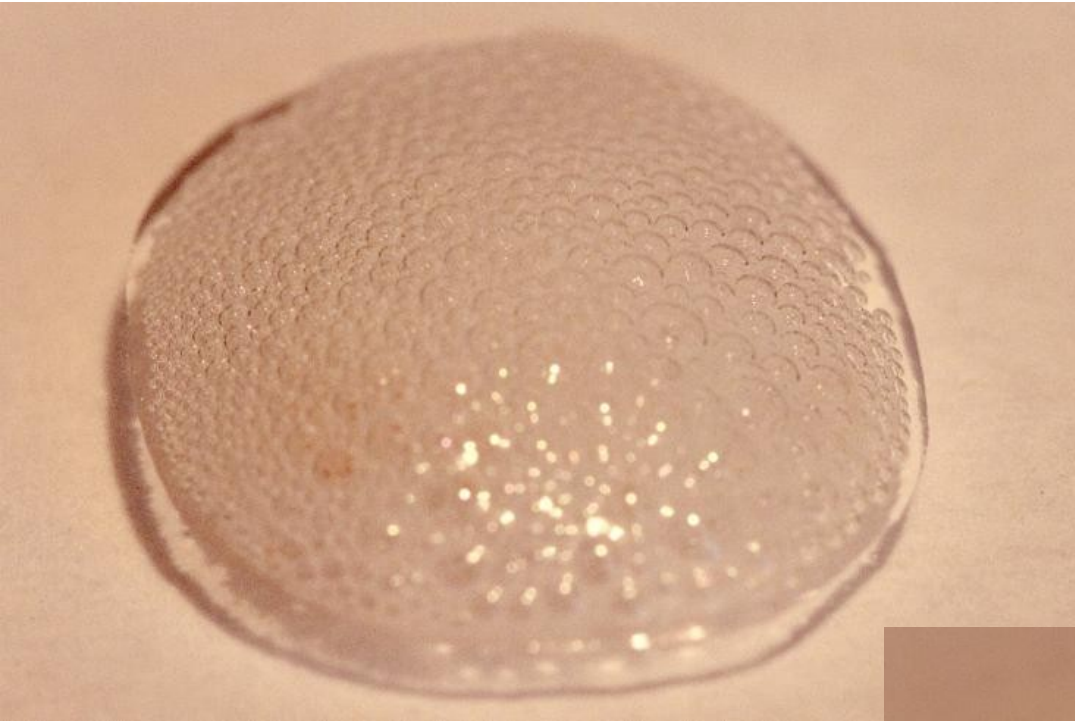
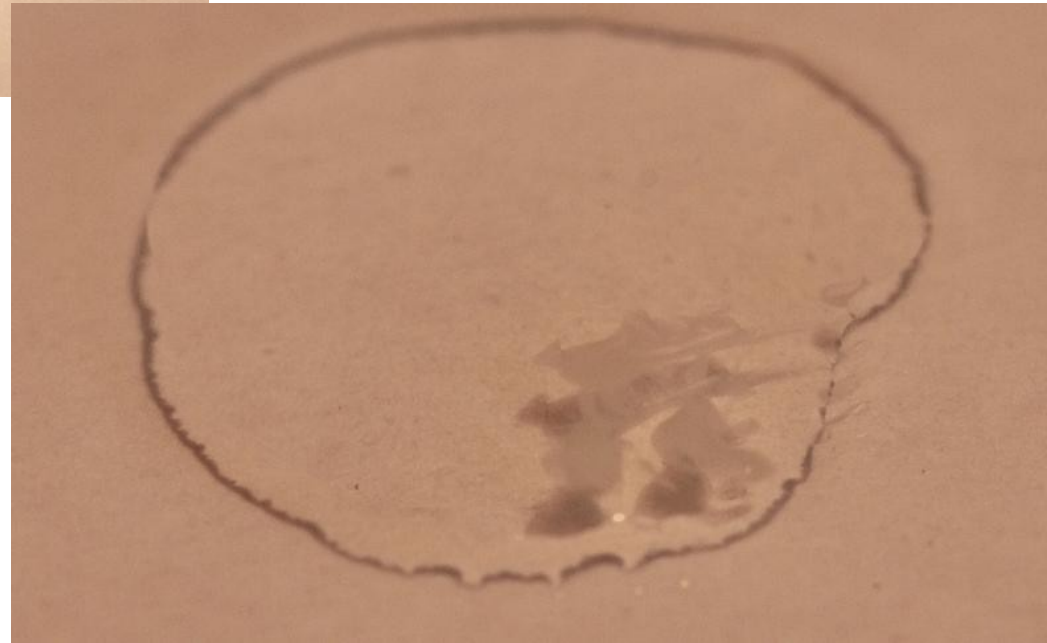
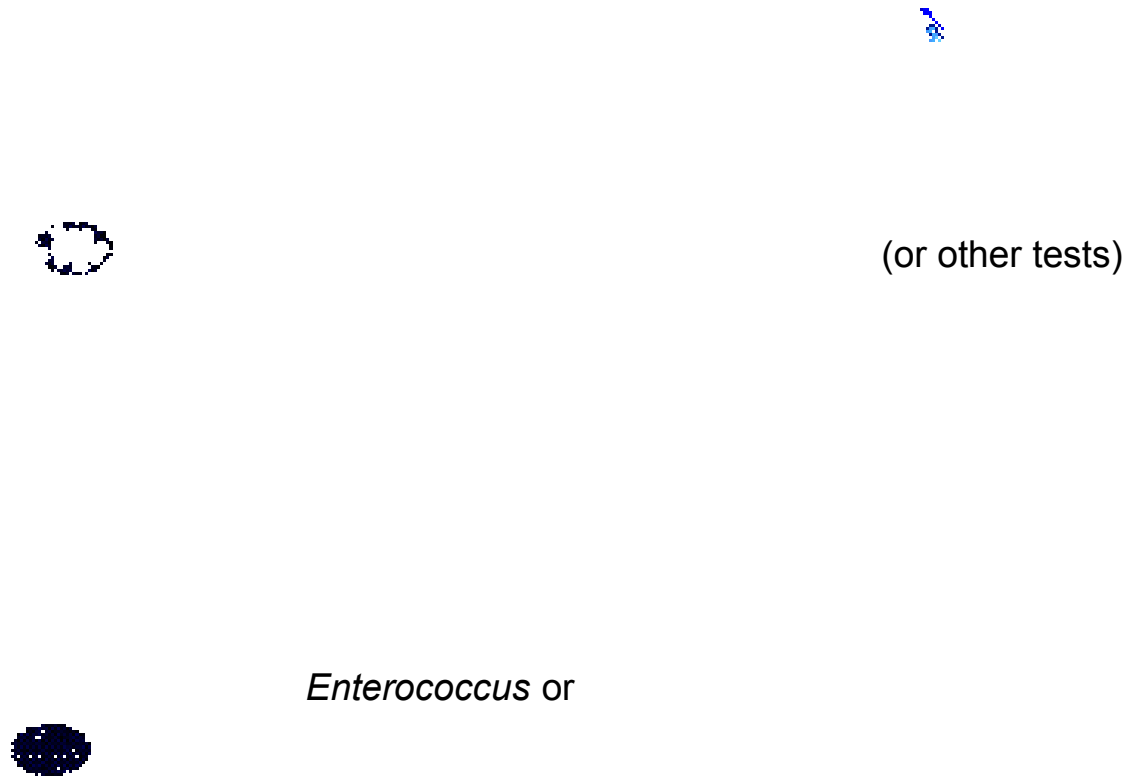


Foto: Veronika Holá



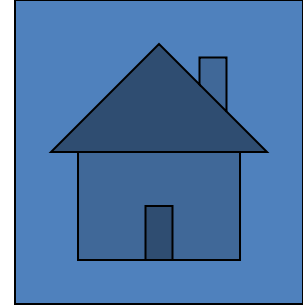
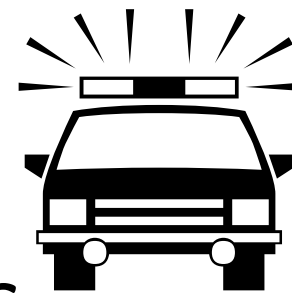
Survey of diagnostics (simplified)



Description of colonies on BA

- Description of colonies on blood agar does not have a specific place in differential diagnostics of staphylococci. Nevertheless, it is useful, as it can lead us to certain suspicion (e. g. staphylococci have rather whitish/yellowish colonies, unlike grey/colourless streptococci)

Discrimination between *Staphylococcus* and *Streptococcus/Enterococcus*



- In a bacterial mixture, a *Staphylococcus* may be selected using growth on BA with 10 % NaCl; other G+ cocci do not grow.
- If a pure strain is available and we require a quick diagnostics, catalase test catalase test may be used (a colony is mixed with a drop of hydrogen peroxide, bubbles = positive).

Attention! By jumping over the previous steps, we would do a mistake. Positive catalase test is common in many bacteria. Only in a known G+ coccus it is possible to use it for diagnostics!

Differential diagnostics
of staphylococci II:
steps inside genus
Staphylococcus

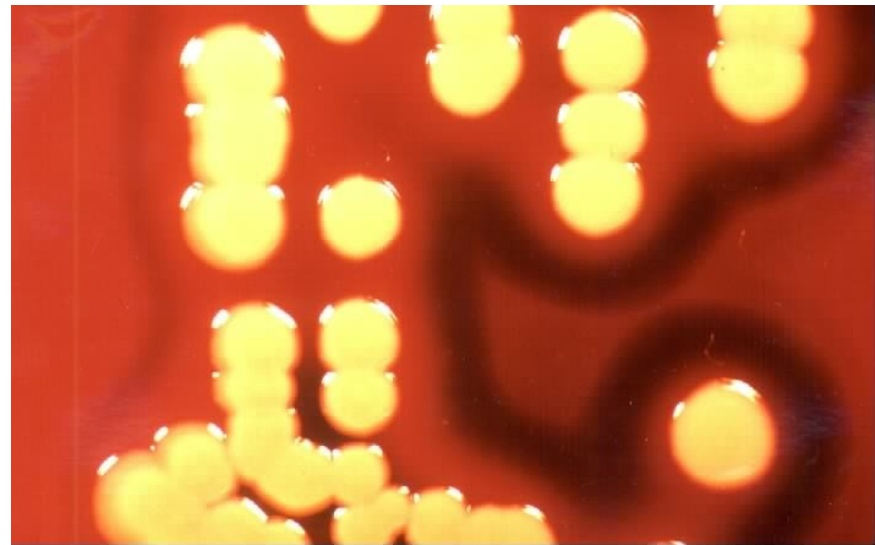
Singling out of other suspects (differential diagnostics 2)

- **Free plasmacoagulase** is positive in „golden staphylococcus“, negative in coagulase negative ones (here the origin of their name)
- **Clumping factor or bound plasmacoagulase** is used in the same situations, but is worse
- **Commercial tests** based on antigen analysis are very good on the other hand (but expensive)
- **Hyaluronidase** is not only good, but cheap, too

Less sure tests: useful in searching, but cannot be used as a „proof for court“!

- **Haemolysis:** Coagulase negative staphylococci may produce delta haemolysin, „Golden“ staphylococci may produce alpha, beta and delta haemolysin, so their haemolysis uses to be stronger.
- **Goldish colour of colonies** and their larger diameter may be useful, too.
- **Larger clusters in microscope** are also typical for „golden“ staphylococci

Photo: Archive of
Institute of
Microbiology



Clumping factor or bound plasmacoagulase – quick

- Colonies are mixed with a drop of rabbit plasma on a slide
- Positive reaction = formation of „clusters“ in plasma drop (see next screen)
- In fact, it is not a COAGULATION, but AGLUTINATION of plasma
- The test is not very sure

Clumping-Factor positiv



<http://memiserf.medmikro.ruhr-uni-bochum.de>

Clumping-Factor negativ



Free coagulase – classic

- The most classical among differentiation test for "golden" *Staphylococcus* (the coagulase positive *Staphylococcus*)
- Colonies, taken by a loop, are mixed with rabbit plasma in a test-tube
- When the plasma coagulates (gel consistence), the strain is coagulase positive

Positive and negative plasmacoagulase

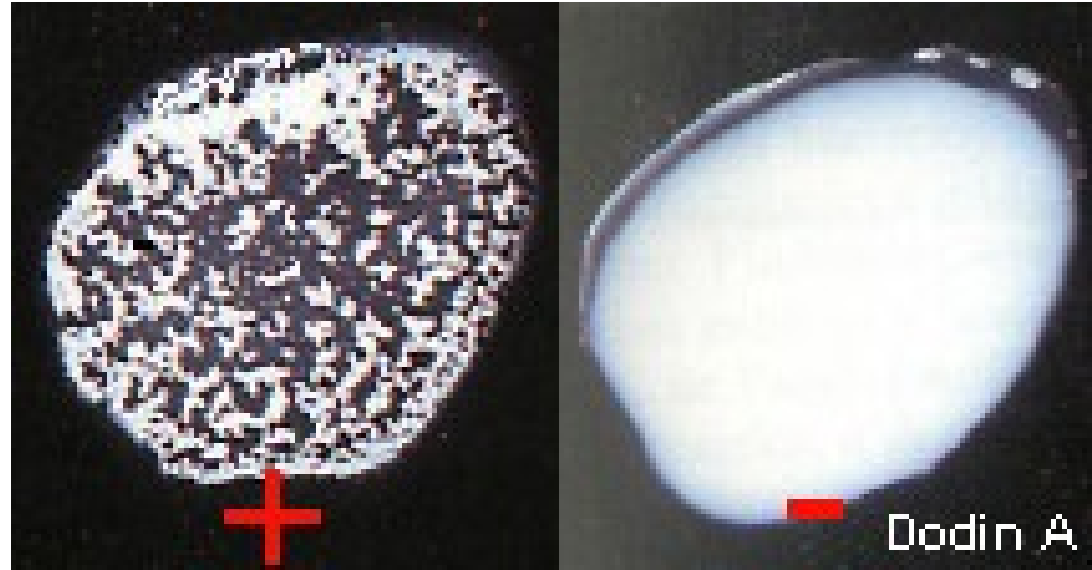
- **First test-tube = positive** (gel, does not change shape when reclined)
- **Second and third test tube = negative** (liquid, horizontal level is always horizontal)

<http://microbiology.scu.edu.tw>



Commercial tests, e. g. Staphaurex (not in the practical)

- The way of using them is the same as in the clumping factor test, but they are even more sure than free coagulase
- **Unfortunately, they are relatively more expensive than previous ones**

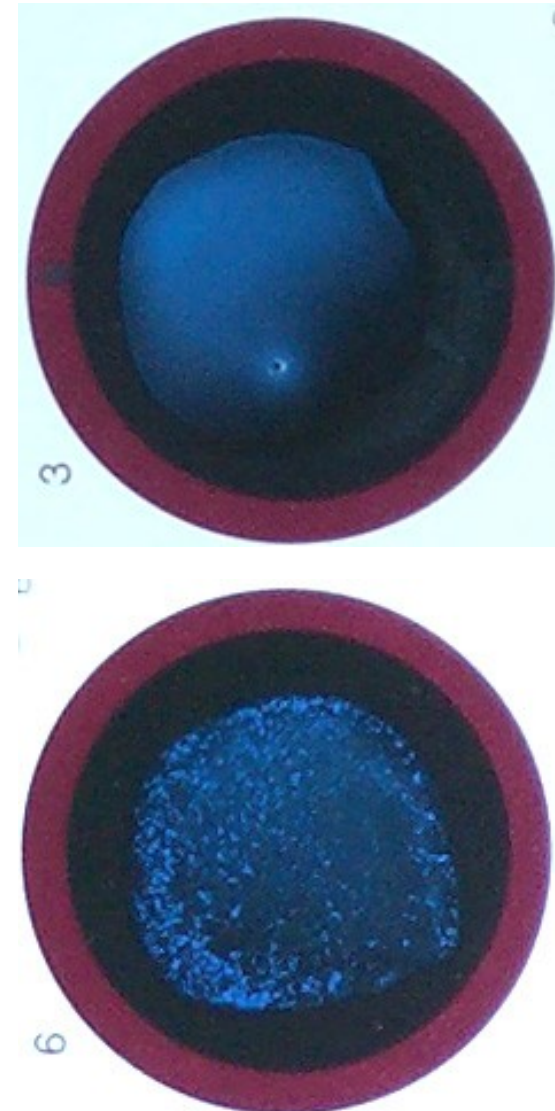


Staphaurex kit and results

<http://www.pathologyinpractice.com>



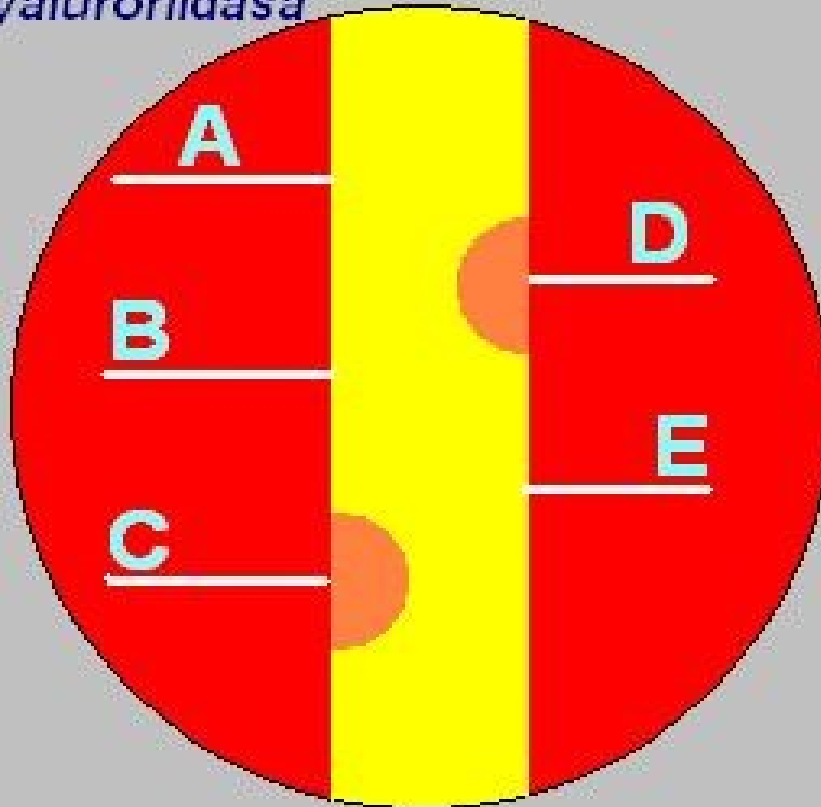
www.microbes-edu.org



Hyaluronidase (decapsulation)

- **An elegant test**, its principle is the fact, that the **hyaluronidase, produced by *S. aureus*** (but not coagulase negative staphylococci) breaks the capsule of encapsulated bacteria. We use ***Streptococcus equii***, a streptococcus that is not pathogenic for humans
- **Lack of a capsule** is seen as **change of feature of streptococcus** (no „mucosity“)

Hyaluronidasa



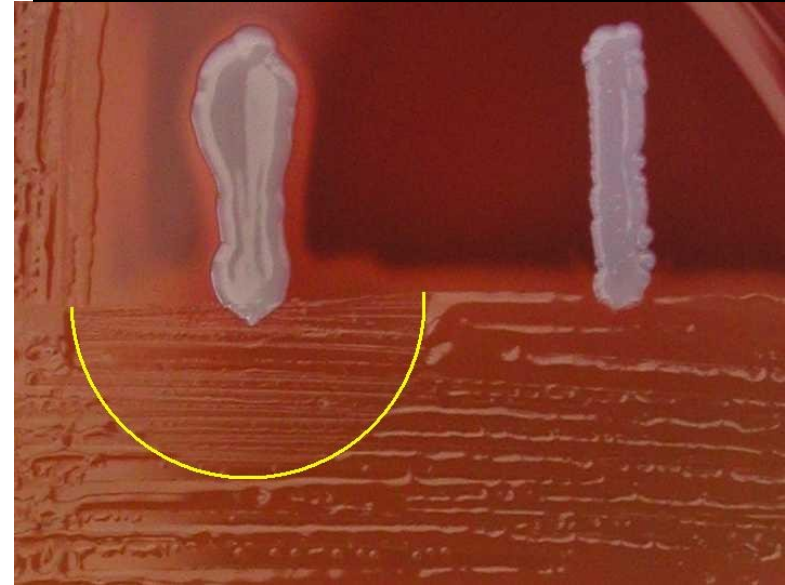
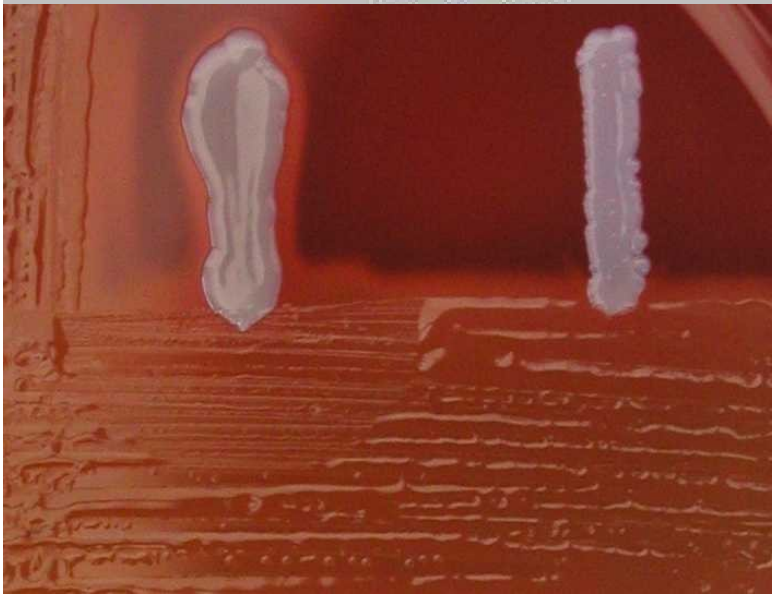
Yellow – *Streptococcus equi*
(mucous)

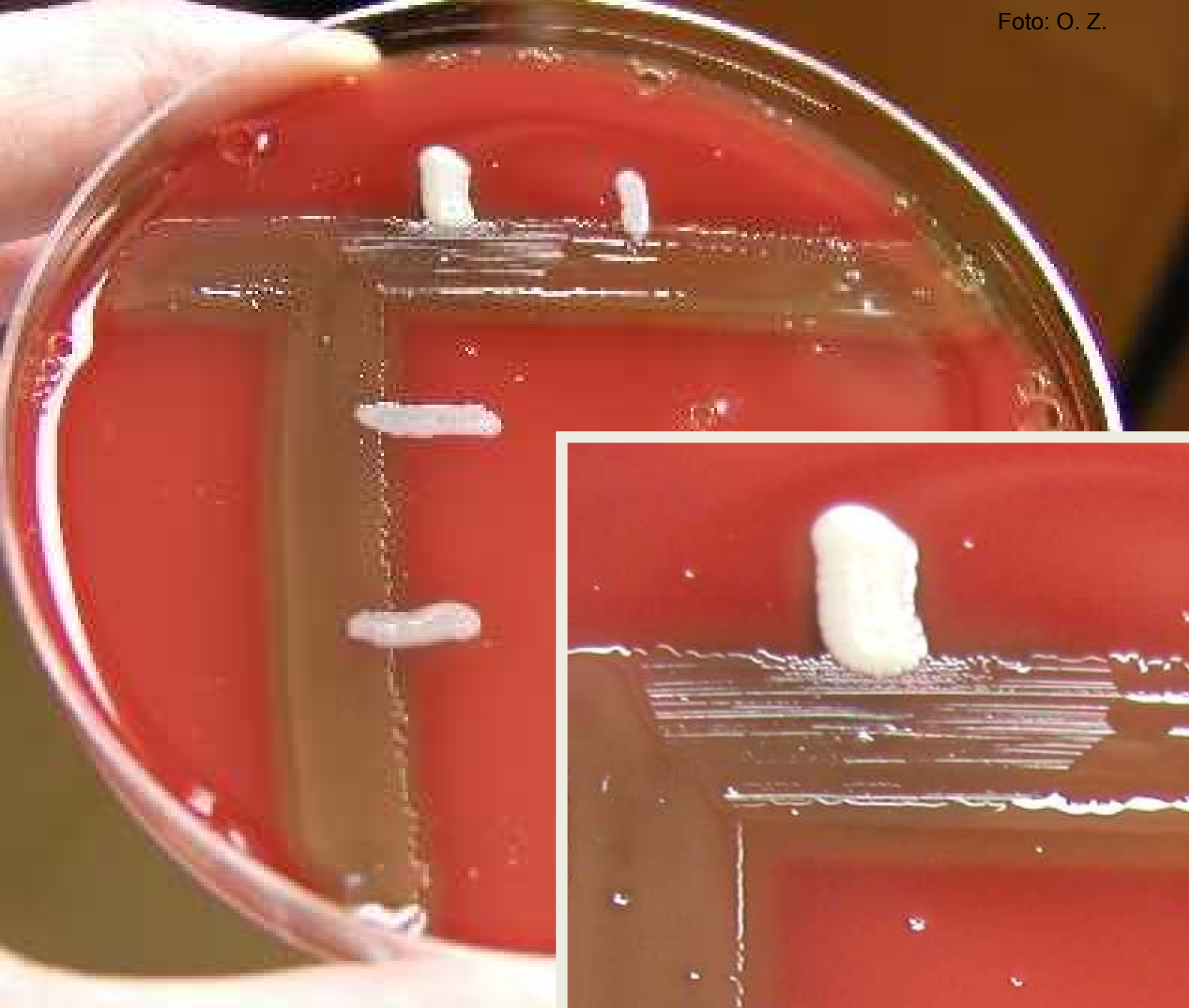
White – tested staphylococci

Results for this example:

C and D are positive
(*S. aureus*)

A, B and E are negative
(coagulase negative staphyl.)





Survey of methods distinguishing *S. aureus* from CONS (coagulase negative staphylococci)

- **Clumping factor test:** a drop of plasma is mixed with a tested strain on a slide
- **Plasmacoagulase test:** strain is mixed with rabbit plasma in a test tube. Preliminary reading is done after 4 h and definitive reading after 24 h. Coagulated liquid = positive
- **Hyaluronidase test:** Positive strain dissolves the mucosity of an encapsulated strain (a horse streptococcus *Streptococcus equi* is used mostly for this test)

It is not „The Golden“. What now?

- Usually we simply say „it is a coagulase negative one“ **and we do not insist on species diagnostics**
- When species would be important (e. g. in blood cultures), it can be performed **biochemically**
- In Czech conditions, mostly **STAPHYtest 16** (Erba-Lachema) is used

Mutual differentiation of staphylococci


































- STAPHYtest 16 is the most typical Czech variant of a biochemical testing system of staphylococci. It should be done according to guidelines. It certifies the identity of "golden" Staphylococcus and it identifies the other.
- **Normally it is useless to diagnose „golden staphylococcus“ by STAPHYtest 16**, tests of tasks 6a, b and c, or commercial tests are rather used for this
- So the test is used for **diagnostics of coagulase negative staphylococci**

STAPHYtest 16 – how to read it

- Attention – despite its name, there are 17 reactions in it. **We start by reading VPT test in a test tube.** Red fluid in the test tube = positive VPT, colourless fluid = negative
- **First row of the STAPHYtest = 2nd–9th reaction**
- **Second row of the STAPHYtest = 10th– 17th r.**
- Count the code and compare with the codebook
- The code consists of six numbers. Five of them are based on triplets of test, the sixth is based on the last two tests (16 + 17)











































An example of a result

(703 651 = *S. aureus*, 99.8 %, $T_{in}=1,00$)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tube | H | G | F | E | D | C | B | A | H | G | F | E | D | C | B | A | |
| | Tube | First row of panel | | | | | | | | Second row of panel | | | | | | | | |
| + |  |  |  |  |  |  |  |  |  |  |  | | | | | | | |
| - |  |  |  |  |  |  |  |  |  |  |  | | | | | | | |
| ? |  |  |  |  |  |  |  |  |  |  |  | | | | | | | |
| | + | + | + | - | - | - | + | + | - | - | + | + | + | - | + | + | - | |
| | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | |
| | 7 | | | 0 | | | 3 | | | 6 | | | 5 | | | 1 | | |

Another example of a result

(703 241 = *S. epidermidis*, 97.95 %, $T_{in}=1,00$)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|--|---|---|---|---|---|---|---|---|---|---|---|
| | Tube | H | G | F | E | D | C | B | A | H | G | F | E | D | C | B | A |
| | | First row of panel | | | | | | | | Second row of panel | | | | | | | |
| + |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | | |
| - |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | | |
| ? |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | | |
| | + | + | + | - | - | - | + | + | - | - | + | - | - | - | + | + | - |
| | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 |
| | 7 | | | 0 | | | 3 | | | 2 | | | 4 | | | 1 | |

Api Staph – in some countries used equivalent of STAPHYtest 16

- Not regarding the producer, the principle is the same – **combination of many enzymatic reactions**, that can be seen as **colour change**

<http://www.microbes-edu.org>

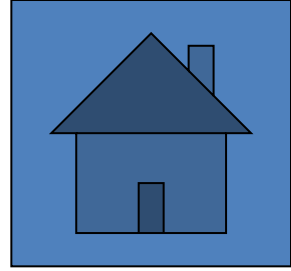


Another variant of a API-Staph

- The previous one was an API-Staph for automatic reading in a photometer. This one is for „ocular“ reading



Final survey of microbiological diagnostics of a „staph“ infection



- *(Microscopy of SPECIMEN – e. g. blood culture)*
- Microscopy of isolated STRAINS

Now, we are able to distinguish G+ cocci from others

- (Description of colonies on blood agar)
- Catalase test (Staphylococci × other G+ cocci)
- Growth on BA with 10 % NaCl

Now, we have differentiated staphylococci from the other G+ cocci

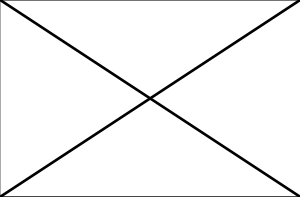
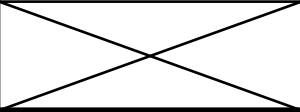
- Differentiation of "golden" *Staphylococcus* from coagulase negative species
- Species diagnostics of *Staphylococcus* (if needed)
- *Atb susceptibility testing (only when we consider the strain to act as a pathogen!)*

Antibiotic susceptibility
testing and antibiotic
treatment of
staphylococcal infection

Susceptibility testing

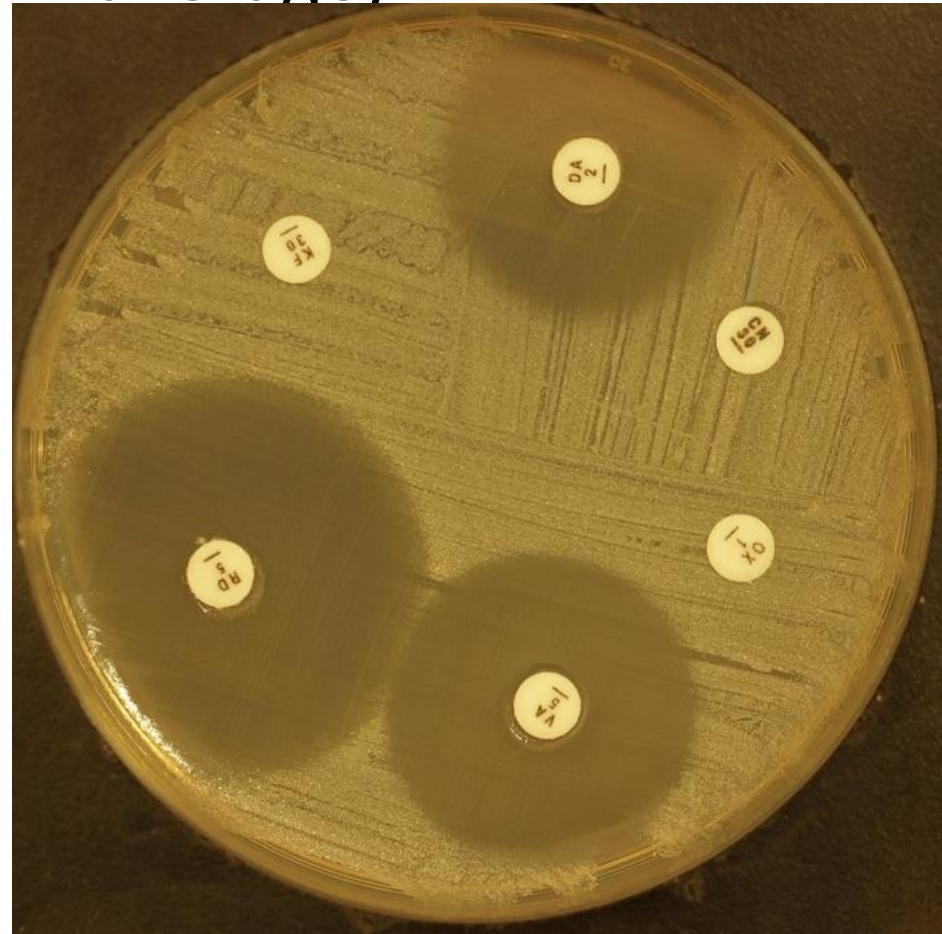
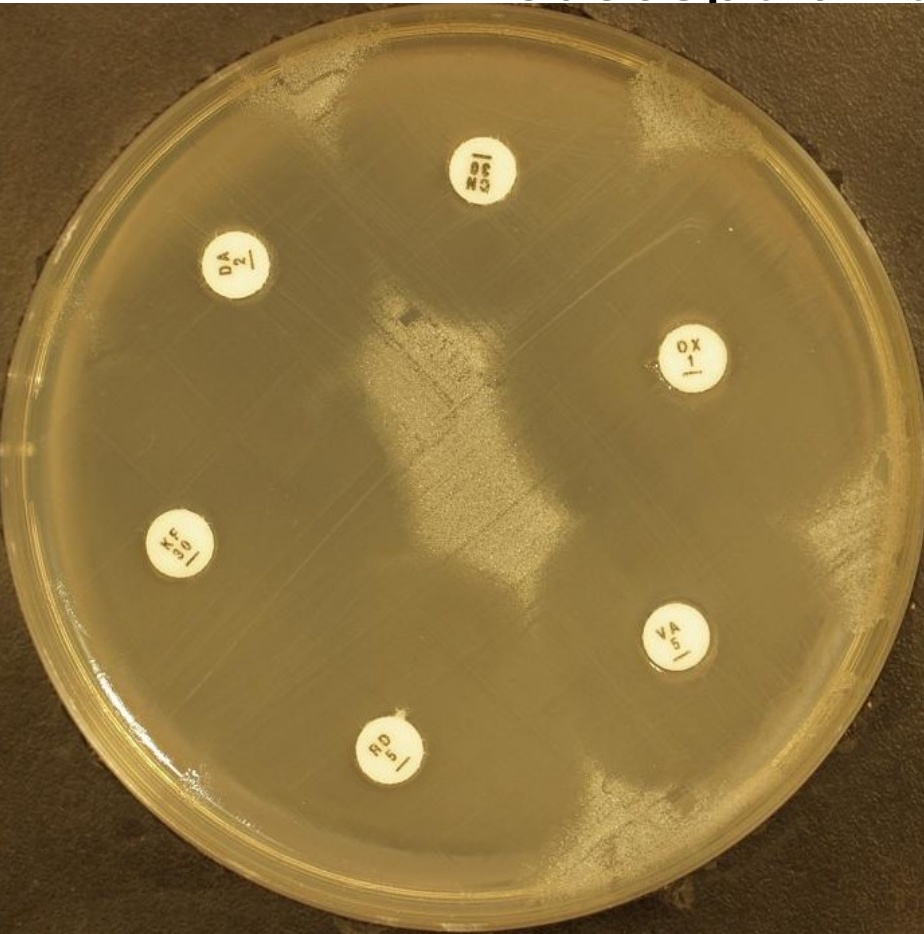
- Drug of choice for *S. aureus* infections is generally **oxacillin**, but there exist exceptions
- **Macrolids** and **tetracyclins** should be used as second choice (for allergic patients).
- **Cephalosporins** of 1st or 2nd generation recommended for UTI rather than oxacillin (that has poor access to the urinary system)
- **Lincosamids** are used for staphylococcal infection of locomotor infection (they have good access to the bone marrow)
- **Vancomycin** or **linezolid** are used for **MRSA** strains or for coagulase-negative staphylococci (that are resistant to oxacillin much more frequently than *S. aureus*)
- For **MRSA** = no betalactam antibiotics can be used, except 5th generation cephalosporin **ceftaroline**
- To check secondary resistances, we mostly use a **diffusion disc test** – we measure the inhibition zones and compare with reference zones
- Of course, antibiotic testing is only performed for staphylococci as pathogens (= not for staphylococci belonging to common microflora)

Reference zones for the most common antibiotics

| Antibiotic | Abb. | „S“ if ≥ than (mm) | „I“ if between (mm) | „R“ if < than (mm) |
|--|------|--------------------|---|--------------------|
| Cefoxitin (cephalosporin); interpreted as oxacillin etc. | CXT | ≥ 22/25* |  | 22/25* * |
| Erythromycin (macrolid) | E | ≥ 21 | 18–20 | < 18 |
| Clindamycin (lincosamid) | DA | ≥ 22 | 19–21 | < 19 |
| Co-trimoxazole (mixture) | SXT | ≥ 17 | 15–16 | < 15 |
| Tetracycline (tetracycline) | TE | ≥ 22 | 19–21 | < 19 |
| Chloramphenicol | C | ≥ 18 |  | < 18 |

*CXT: 22 mm *S. aureus*, 25 mm coagulase negative st.

Susceptibility test in *S. aureus* and a coagulase-negative staph (*S. aureus* uses to have better susceptibility in average)



Quantitative and qualitative tests

- As mentioned, usually we use a qualitative test (diffusion disc test). Nevertheless, it is also possible to use quantitative tests (microdilution test, E-test)



According to situation, we use either

← qualitative, or
quantitative tests →

<http://www.microbes-edu.org>



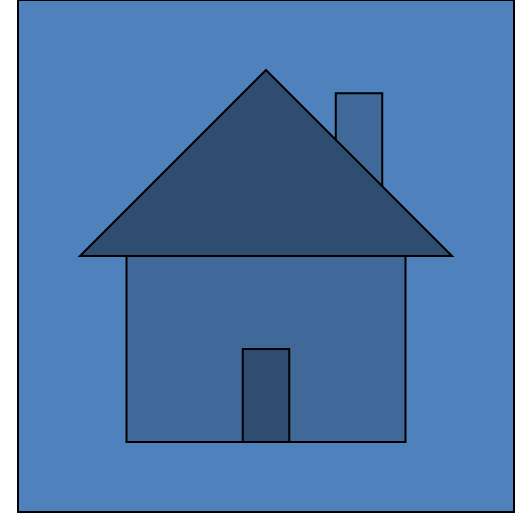
Usual rule: worse pathogen – better susceptibility

- You will probably see, that **a worse pathogen (*S. aureus*) uses to be more susceptible than the milder pathogen** (coagulase negative staphylococcus). It is logical: milder pathogenicity shows better adaptation, ability of a microbe to coexist without causing a disease → being used to common antibiotics
- **It is not absolute!** There are nicely susceptible *S. epidermidis* strains, and there exist MRSA strains that are resistant even to non- β -lactam antibiotics.

MRSA and their detection

- **Methicillin resistant staphylococci (MRSA)** are epidemiologically important strains, often causing serious hospital infections
- They are caused by change of so named membrane **penicillin binding proteins – PBP (= not production of a beta-lactamase!)**
- Problem is seen by a **small or absent zone at antibiotic discs of oxacillin or cefoxitin**
- It is also possible to use a **screening medium** (see topic J05) and **PCR for *mecA* gene coding the resistance** (see topic J09)

The End



A victim of a
staphylococcal
infection

