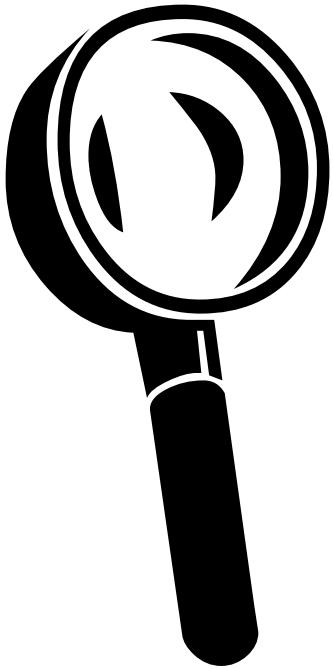


Institute of Microbiology shows:



TRACING THE CRIMINAL

Part one: Straphylococcus

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 is guilty?

- It is *Staphylococcus aureus*
name from Greek staphylé = grape
- This „golden staphylococcus“ often causes pyogenic infections of skin, hairs, nails etc.
- Some 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)

Of course, the cook, that prepared meals despite her blister, is guilty, too!

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 infectological clinic...

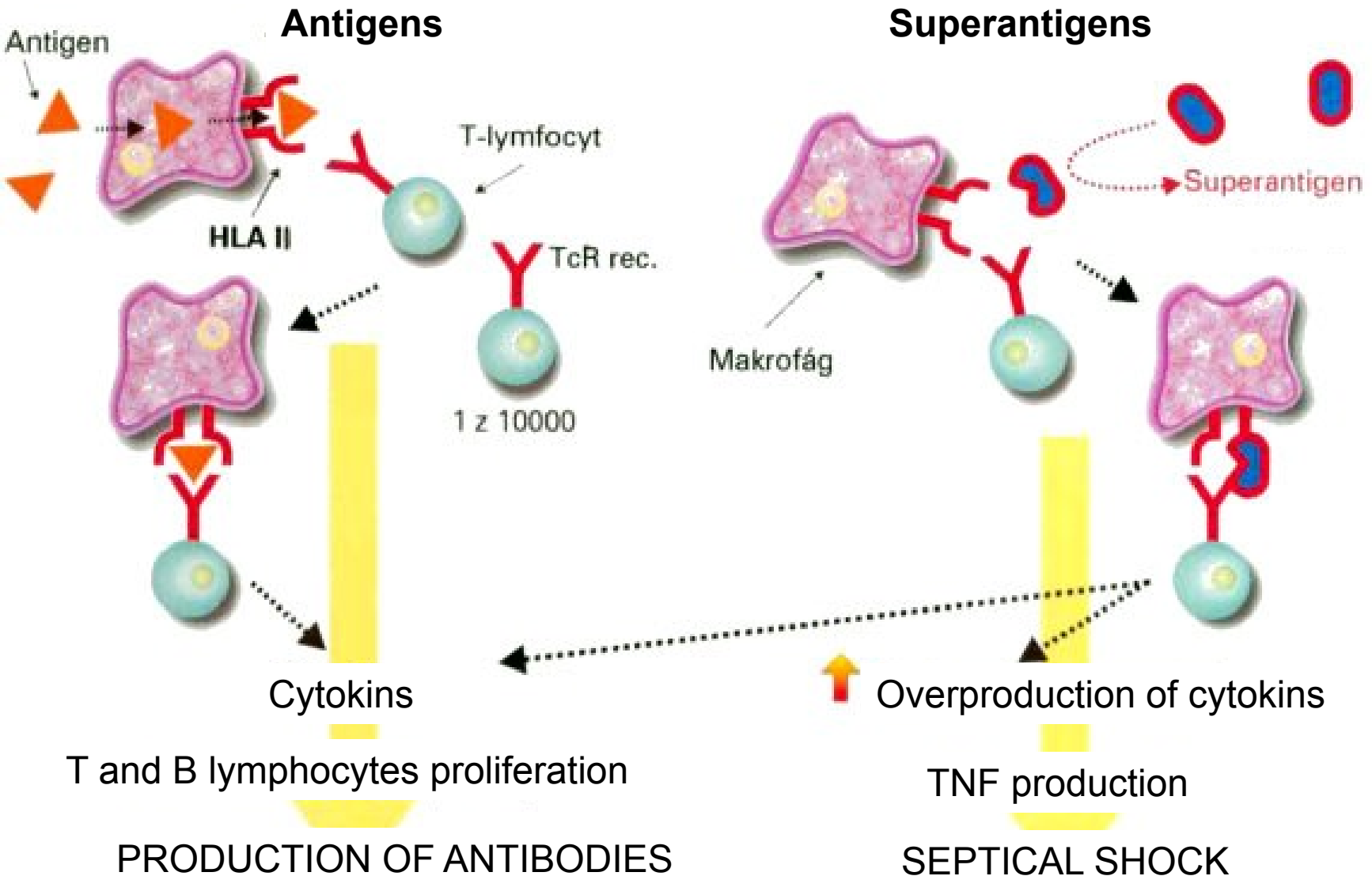
Who is guilty?

- 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

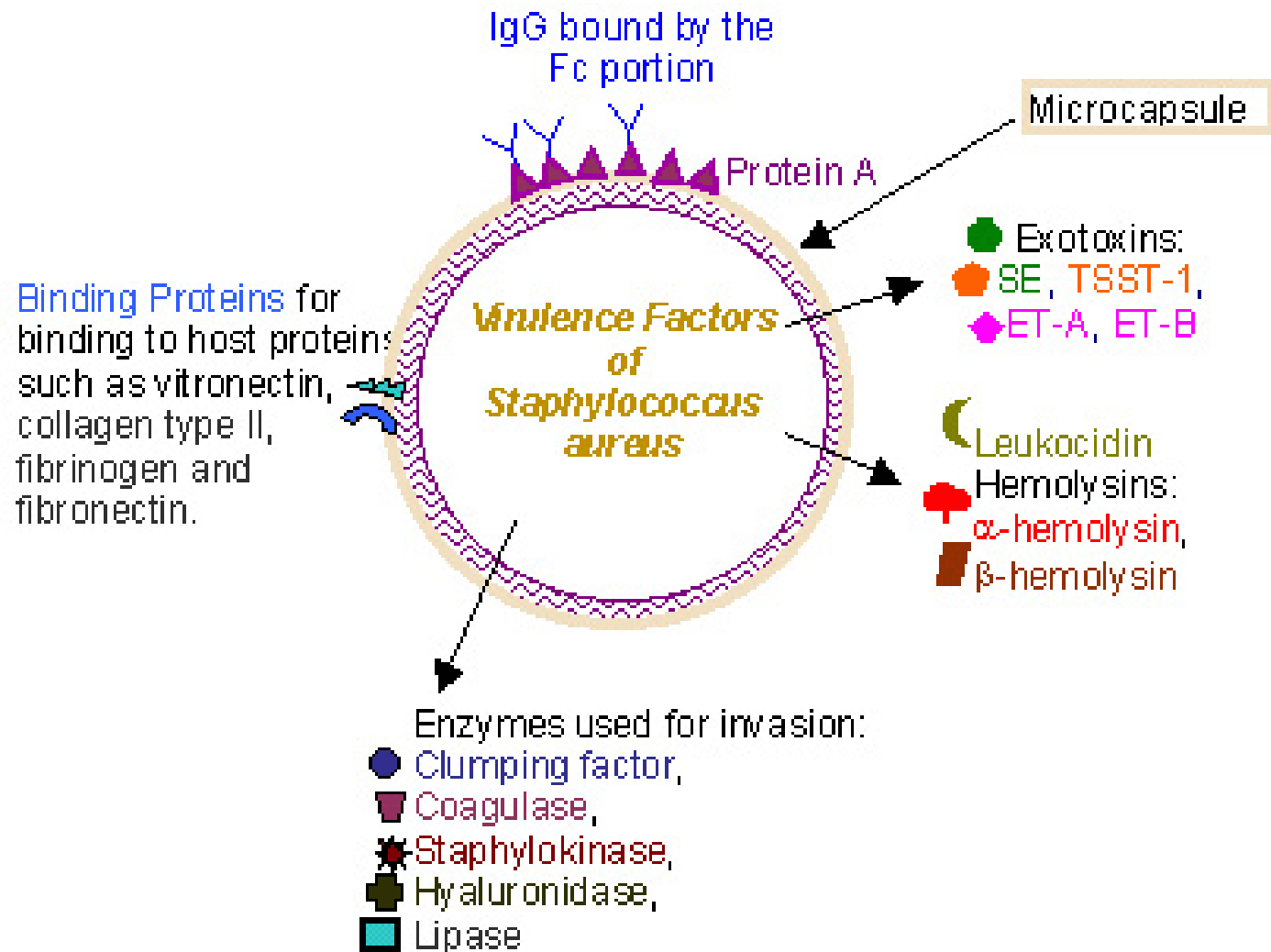


Staphylococcus aureus

(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...



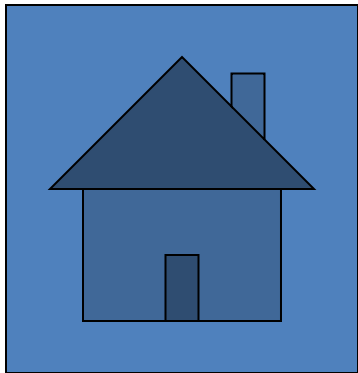
...or skin infection with crusts...

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



...but also brain abscesses

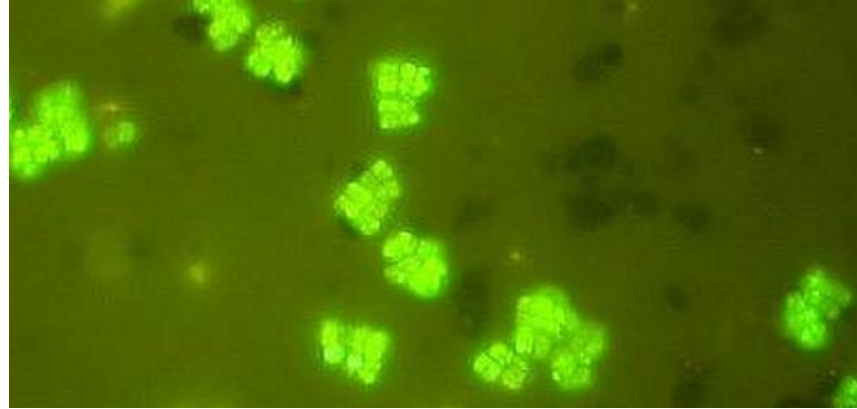
http://www.mja.com.au/public/issues/176_12_170602/got10354_fm.html



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.

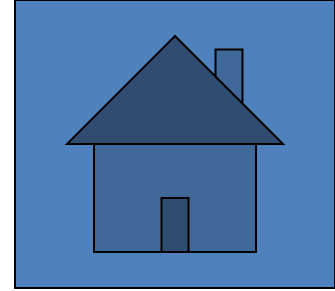
Author: Prof. MVDr. Boris Skalka, DrSc.



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.

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*

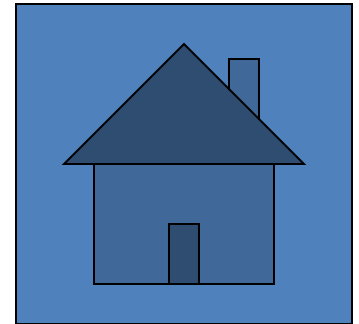
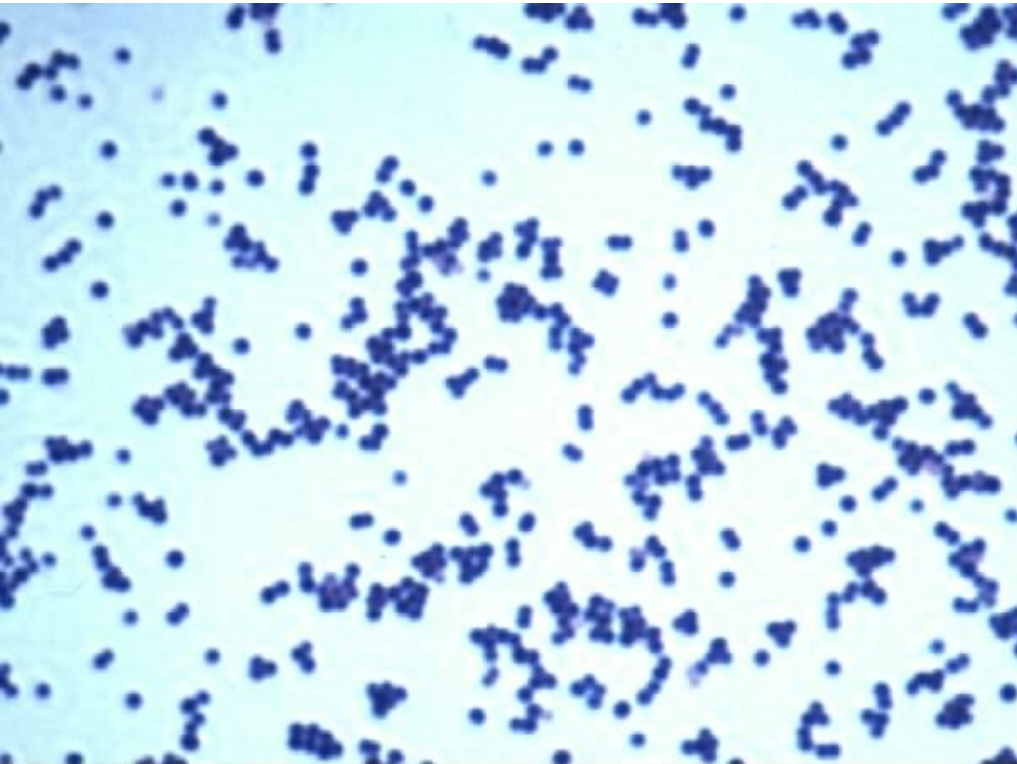
S. simiae (AT727531)

Diagnosatics of staphylococci

Description of criminals (diagnostics) 1

- **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** maybe very helpful at the diagnostics

Photos from Criminal Database



Author: Prof. MVDr. Boris Skalka, DrSc.

Author: Prof. MVDr. Boris Skalka, DrSc.



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

Survey of microbiological diagnostics of a „staph“ infection

- *(Microscopy of SPECIMEN (e. g. sputum))*
- Microscopy of isolated STRAINS

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

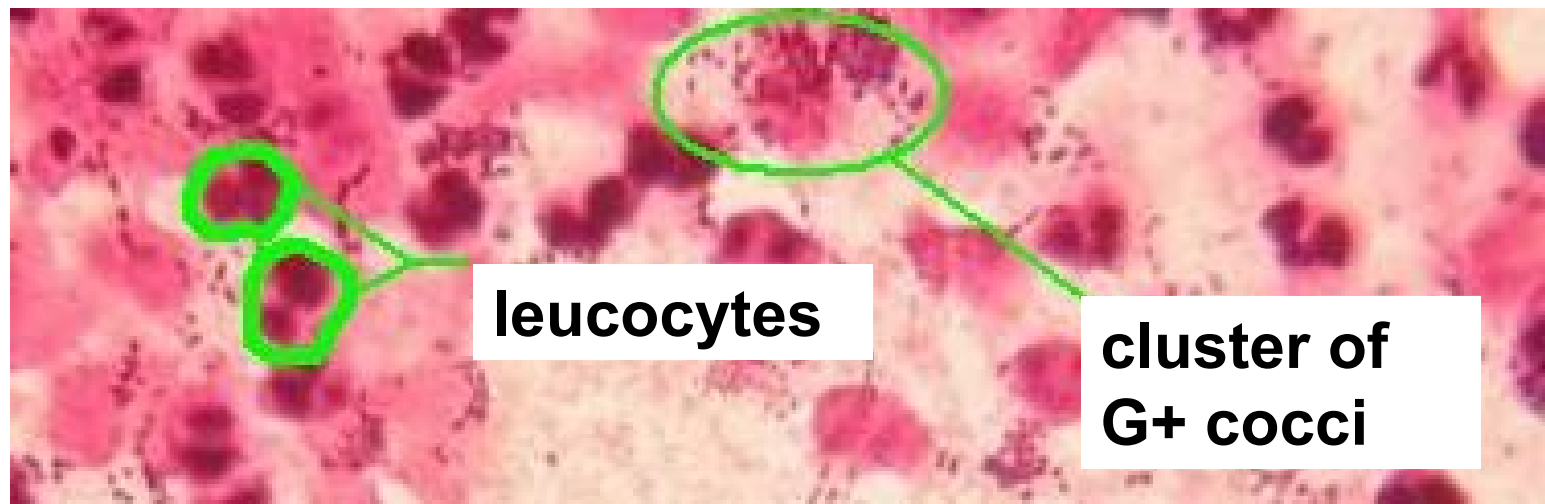
- (Description of colonies on blood agar)
- Catalase test (Staphylococci × other G+ cocci)
- Growth on BA with 10 % NaCl

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

- Differentiation of "golden" *Staphylococcus* from coagulase negative species
- Species diagnostics of *Staphylococcus*
- *Atb susceptibility testing (when Staph is a pathogen)*

Searching for criminal microscopically in the specimen

- We observe a Gram stained **microscopic sputum preparation**
- We search for **Gram-positive cocci** in clusters, but also for **leucocytes** (polymorphonuclears mainly), typical for bacterial inflammation)



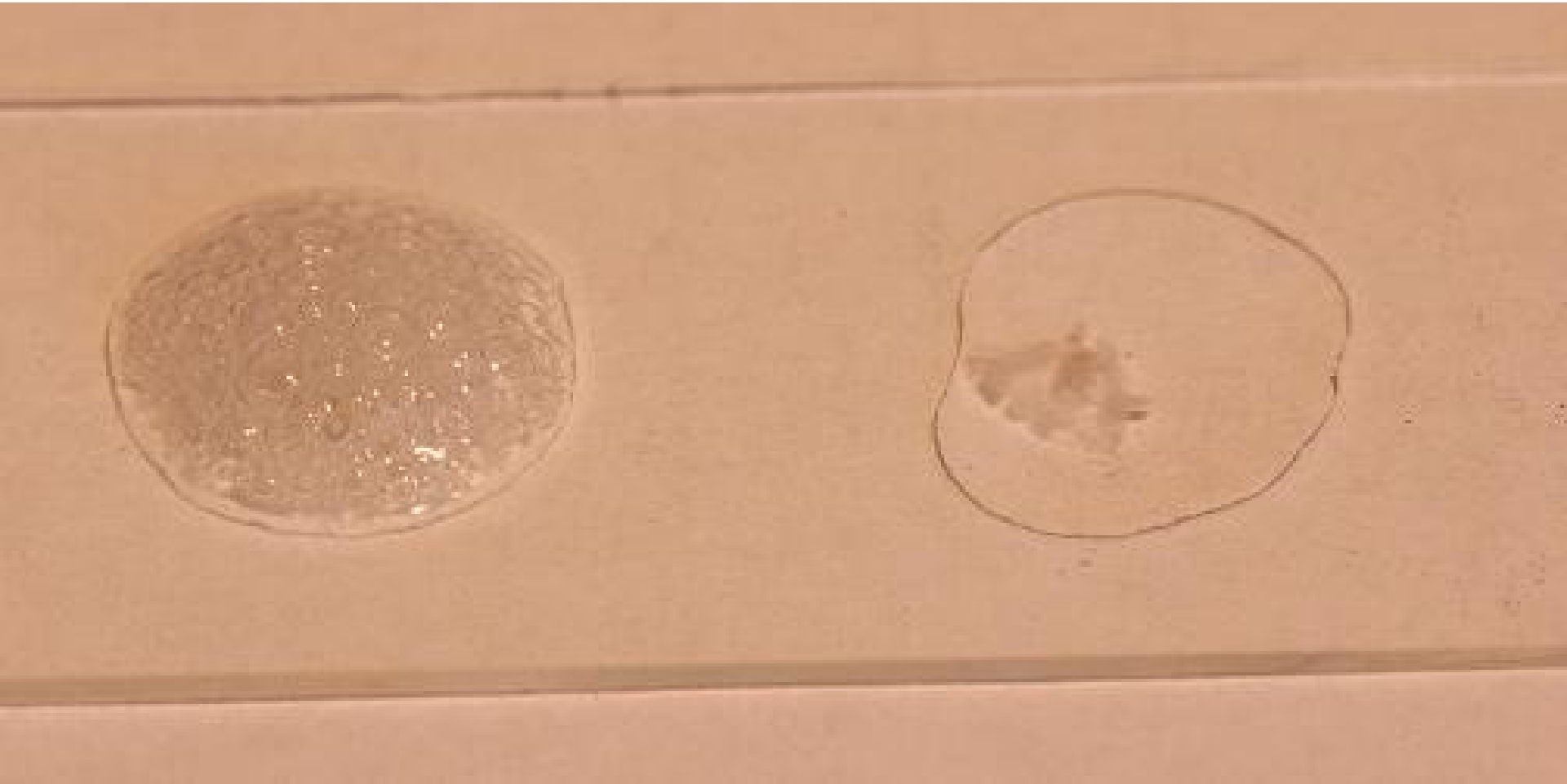
Singling out of other suspects (differential diagnostics 1)

- 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, they 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, Lugol 30 s, 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 -

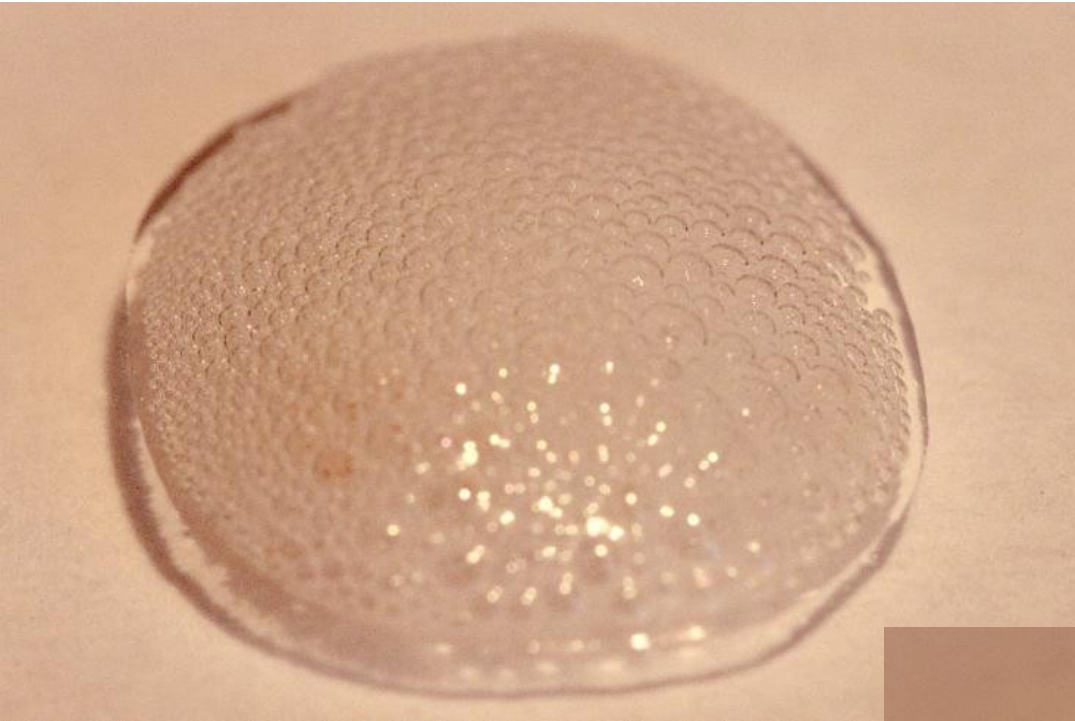
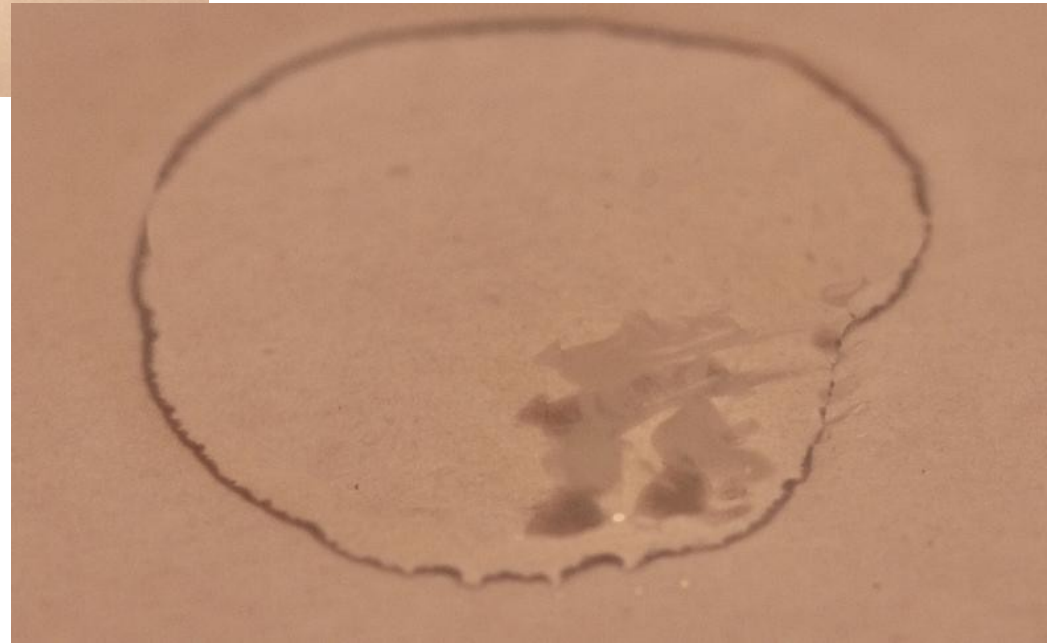
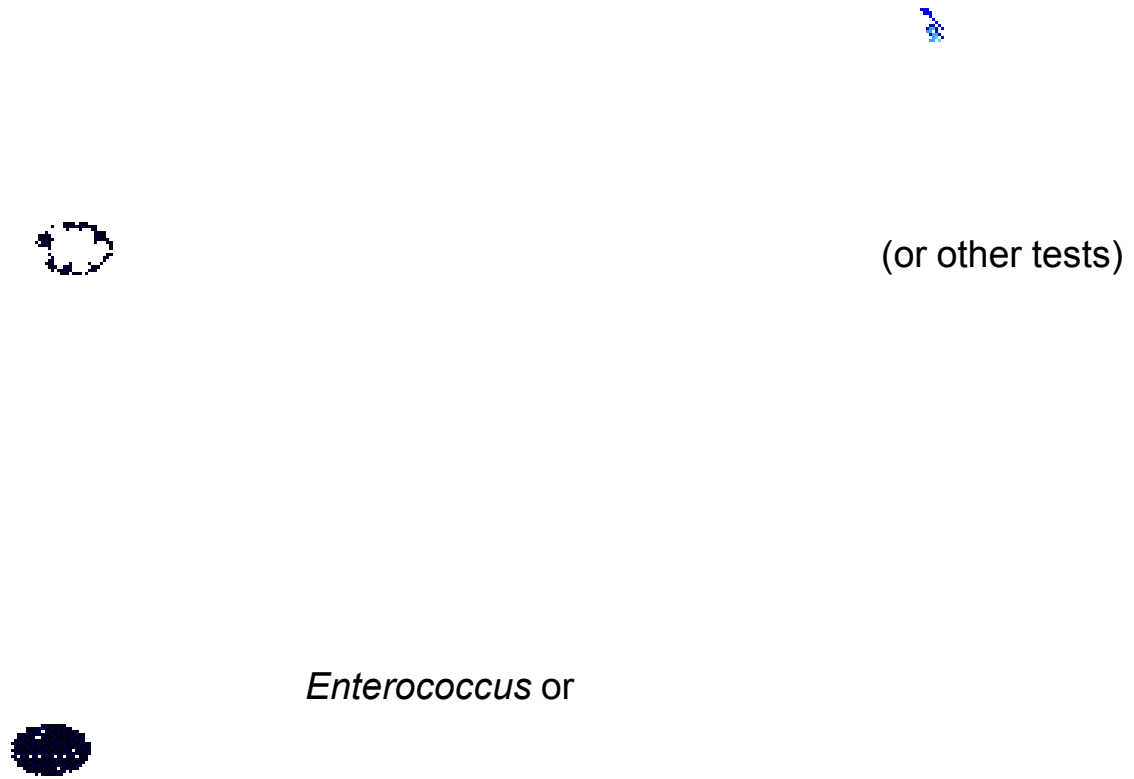


Foto: Veronika Holá



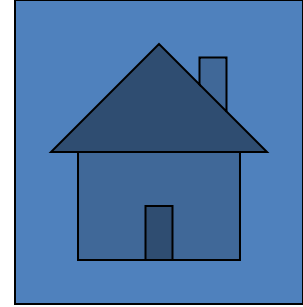
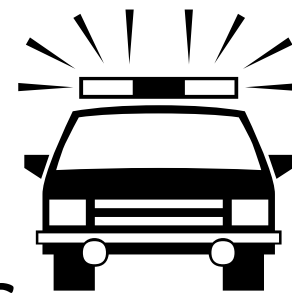
Survey of diagnostics (simplified)



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

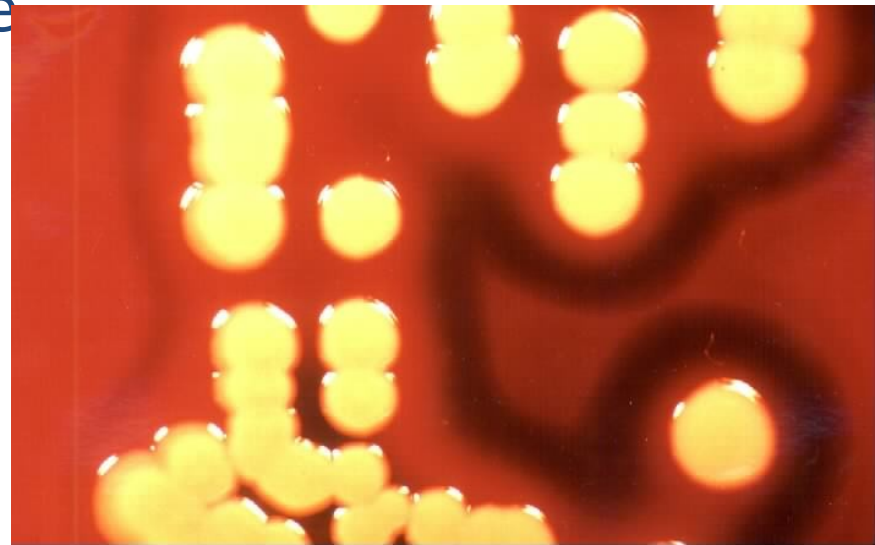
Singling out of other suspects (differential diagnostics 2)

- **Free plasminogen activator** is positive in „golden staphylococcus“, negative in coagulase negative ones (here the origin of their name)
- **Clumping factor or bound plasminogen activator** 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

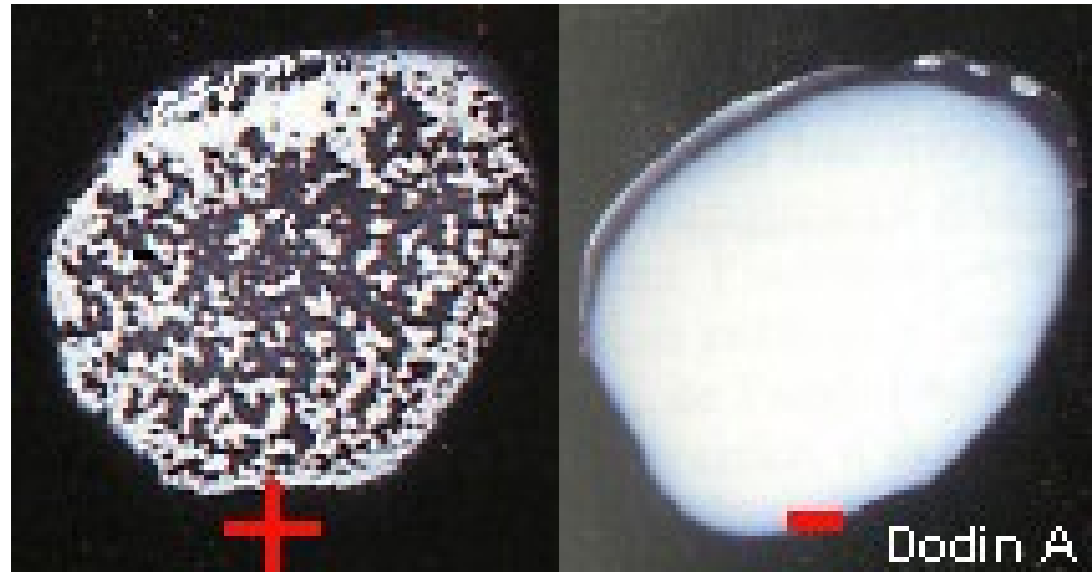
- **First test-tube = positive** (gel, does not change shape when reclined)
- **Second and third test tube = negative** (liquid, horizontal level is always horizontal)

<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

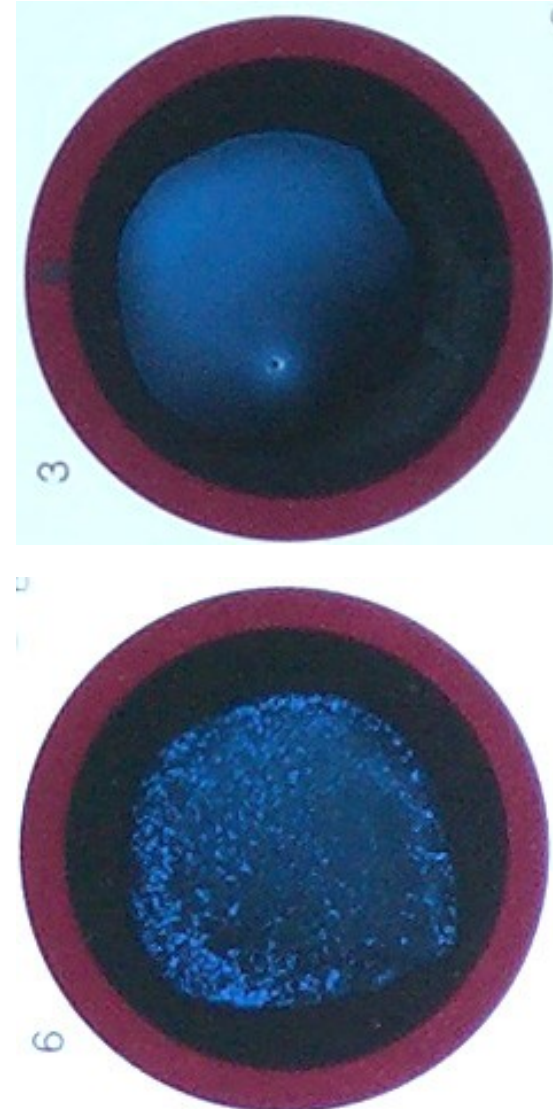


Staphaurex kit and results

<http://www.pathologyinpractice.com>



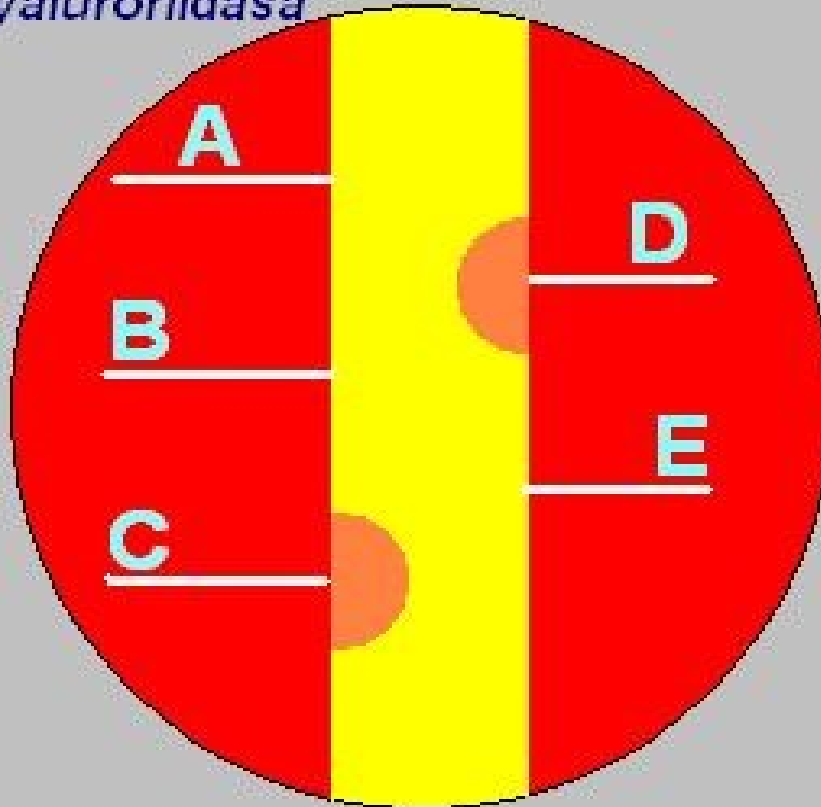
www.microbes-edu.org



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



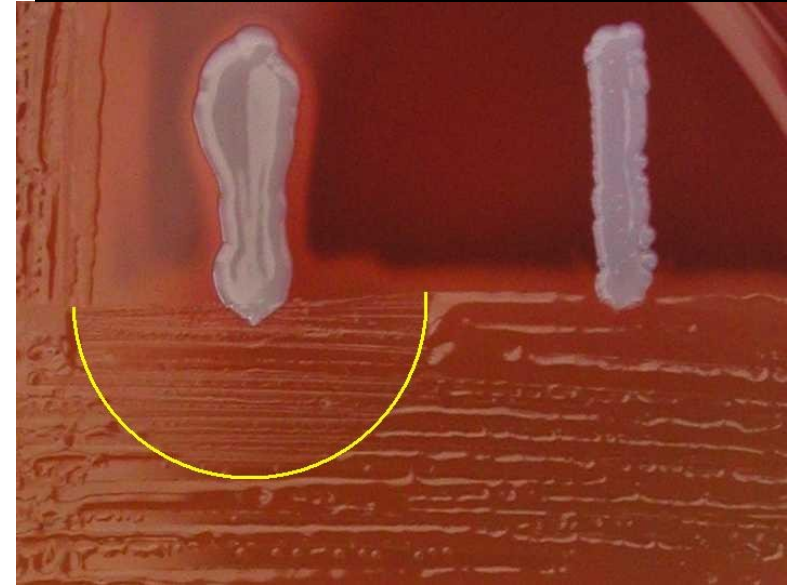
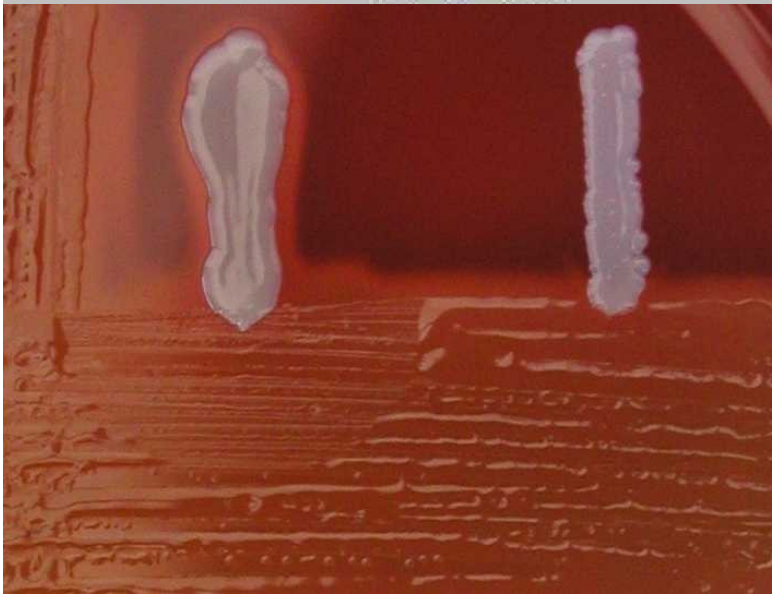
Yellow – *Streptococcus equi*
(mucous)

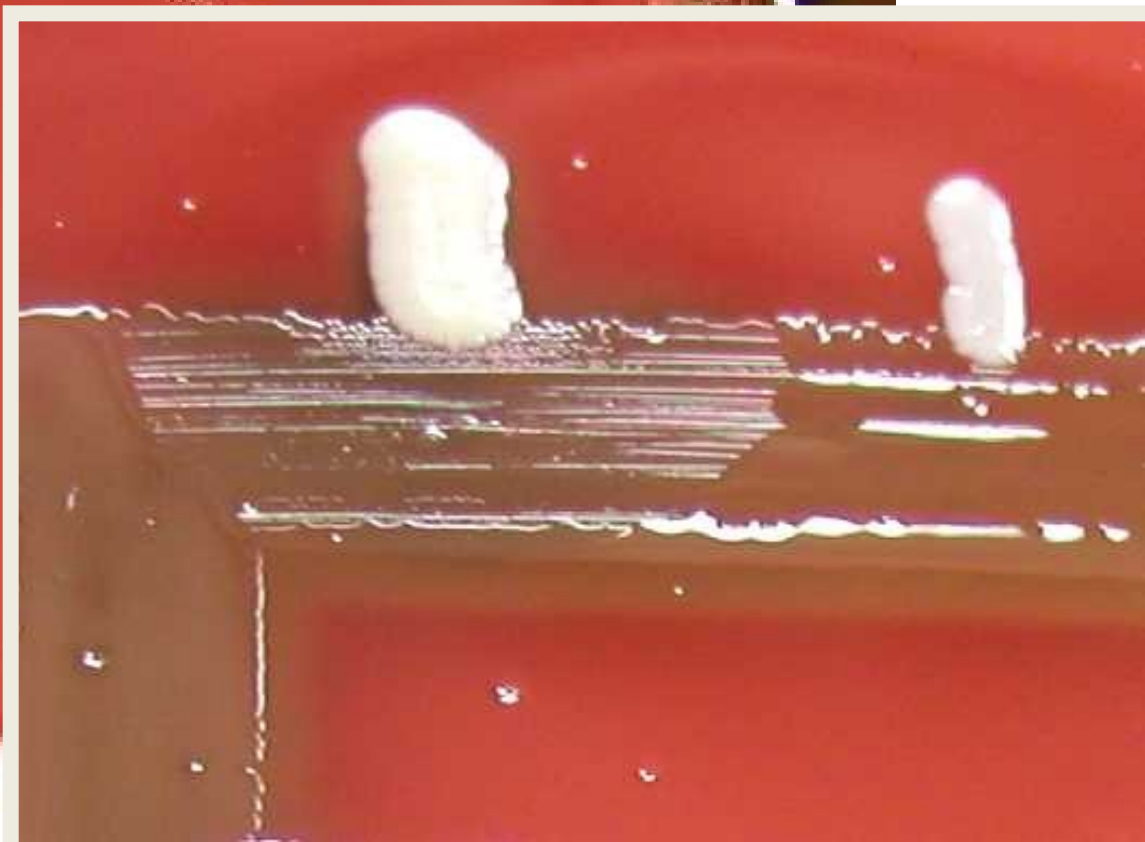
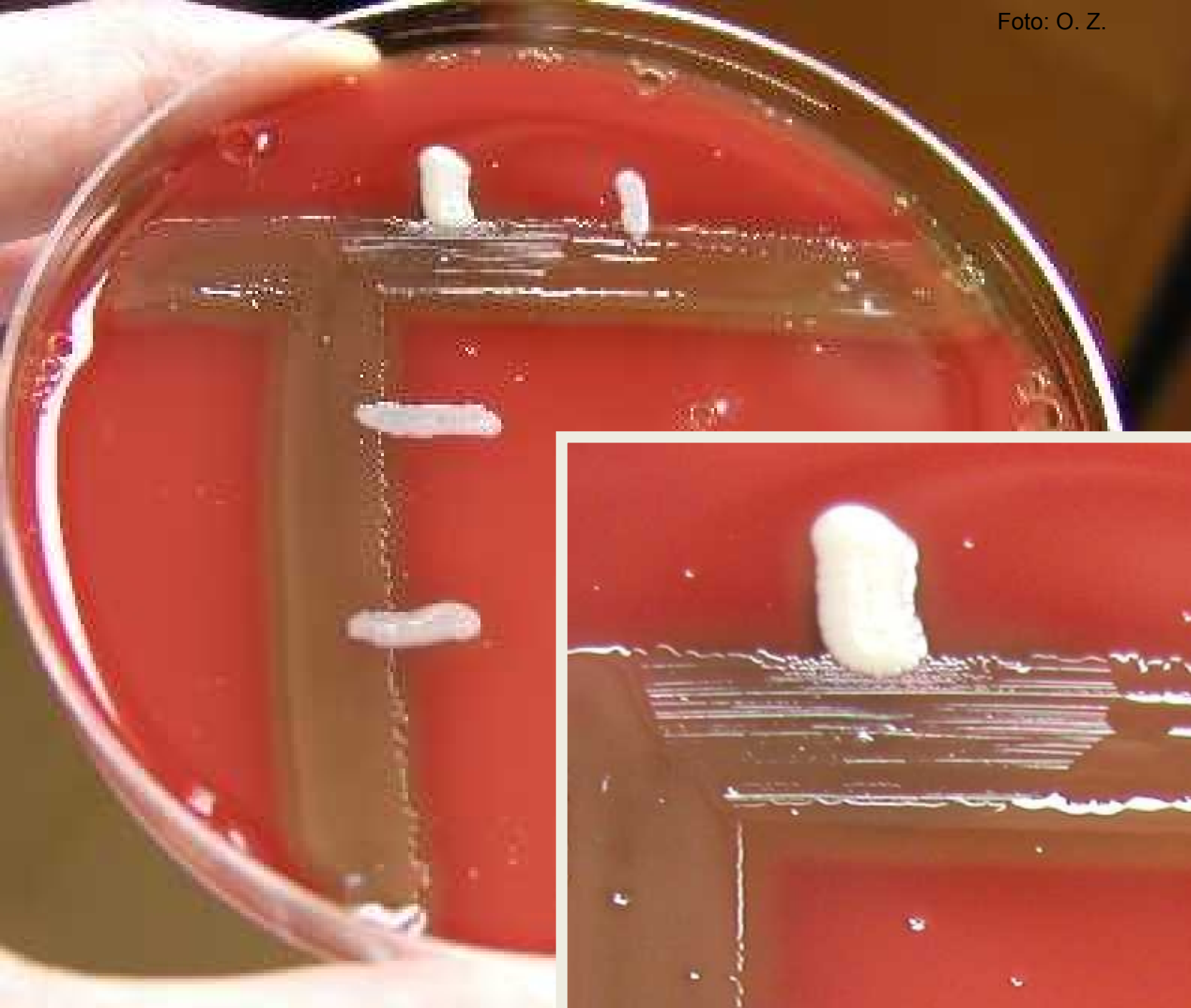
White – tested staphylococci

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	
	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			2			4			1		

Api Staph – in some countries used equivalent of STAPHYtest 16

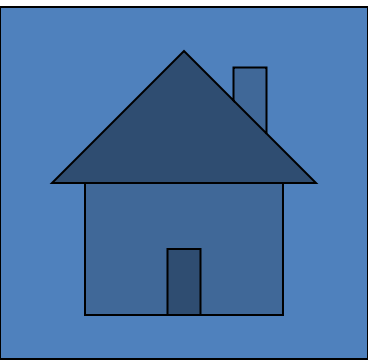
- Not regarding the producer, the principle is the same – combination of many enzymatic reactions, that can be seen as colour change

<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



Antibiotic susceptibility testing and antibiotic treatment of staphylococcal infection

Susceptibility testing

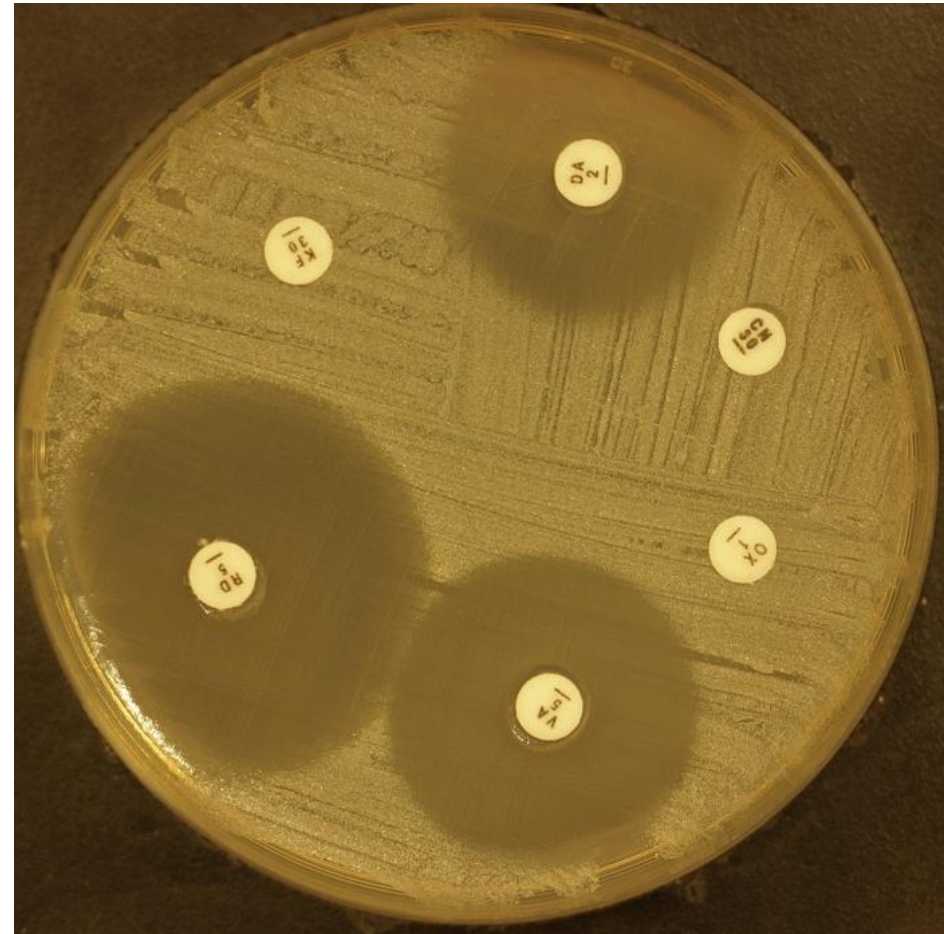
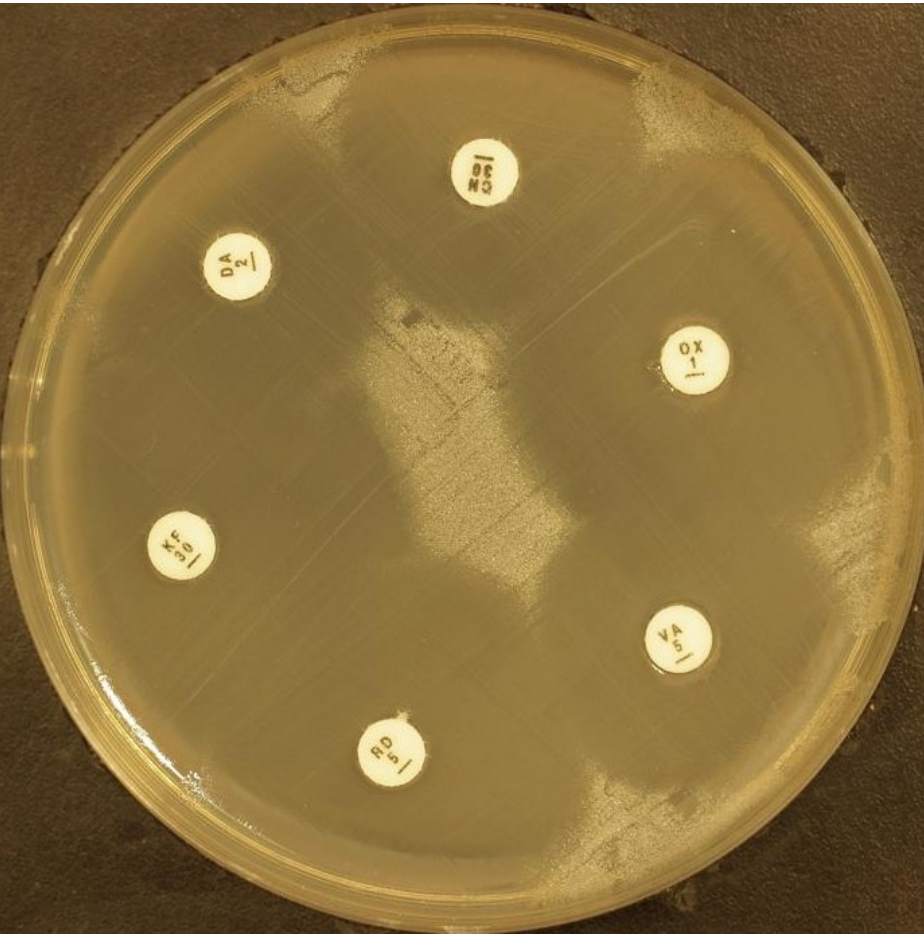
- Drug of choice for *S. aureus* infections is generally **oxacillin**, but there exist exceptions (**lincosamids** for bone infections, **cephalosporins** for UTI)
- **Macrolids** and **tetracyclins** should be used as second choice (for allergic patients). Vancomycin or linezolid rather for MRSA strains or coagulase-negative staphylococci.
- 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 pathogens (= not for staphylococci belonging to common microflora)

Reference zones for the most common antibiotics

Antibiotic	Abbr.	Refer. zone
Oxacilin (protistaf. penic.)	OX	13/18 mm
Vankomycin (glycopept.)	VA	12 mm
Rifampicin (rifamycine)	RD	20 mm
Cefalotin (cephalo. 1. g.)	KF	18 mm
Linkomycin (lincosamid)	MY	21 mm
Gentamicin (aminoglyk.)	CN	15 mm

OX: 13 mm *S. aureus*, 18 mm coagulase negative st.

Susceptibility test in *S. aureus* and koag.-neg. staph



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 MRSA.

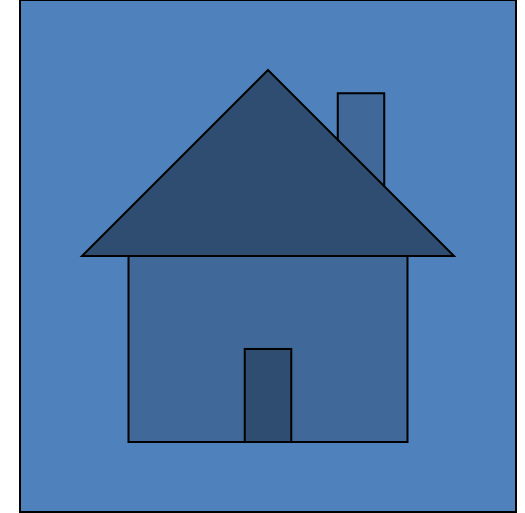
Anti-staphylococcal drugs

- In staphylococci, the drug of choice is **oxacillin**, in UTI **cephalosporins of first generation**. Often used **macrolids** are good in allergic persons only, **lincosamids** are good in locomotor system infections and **aminoglycosides** in combination only. **Glykopeptidic antibiotics (vankomycin and teikoplanin)** are a reserve. They are used in strains resistant to oxacillin, so named **MRSA** and **MRSKN**. In strains resistant even to glycopeptides, or in patients that has contraindications, newer antibiotic **linezolid** can be used.

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)
- Problem is seen by a small zone in oxacillin. But it is not a clear proof.
- The proof is, when the zone is small not only in oxacillin, but also cefoxitin

The End



A victim of a
staphylococcal
infection

