

Institute of Microbiology shows:



TRACING THE CRIMINAL

Part one: Straphylococcus

Overview

Clinical characteristics: *Staphylococcus aureus*

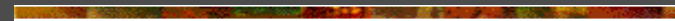
Clinical characteristics: CONS (coagulase-negative staphylococci)

Diagnostics of staphylococci

Differential diagnostics of staphylococci I

Differential diagnostics of staphylococci II

Antibiotic susceptibility testing and antibiotic treatment



Clinical

characteristics:

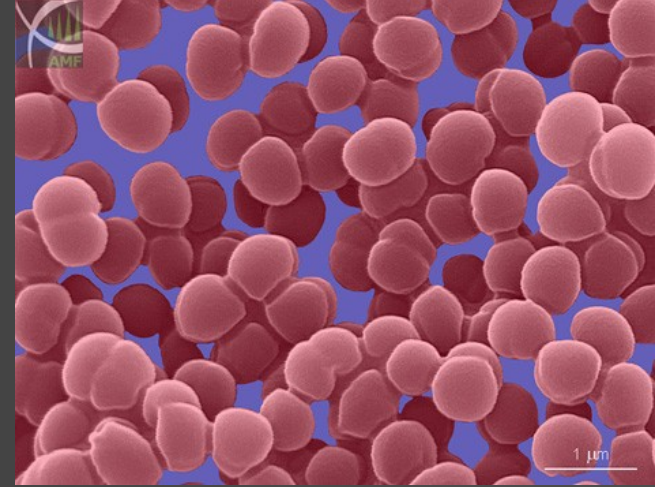
Staphylococcus

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 will be only slightly heated).
- 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...

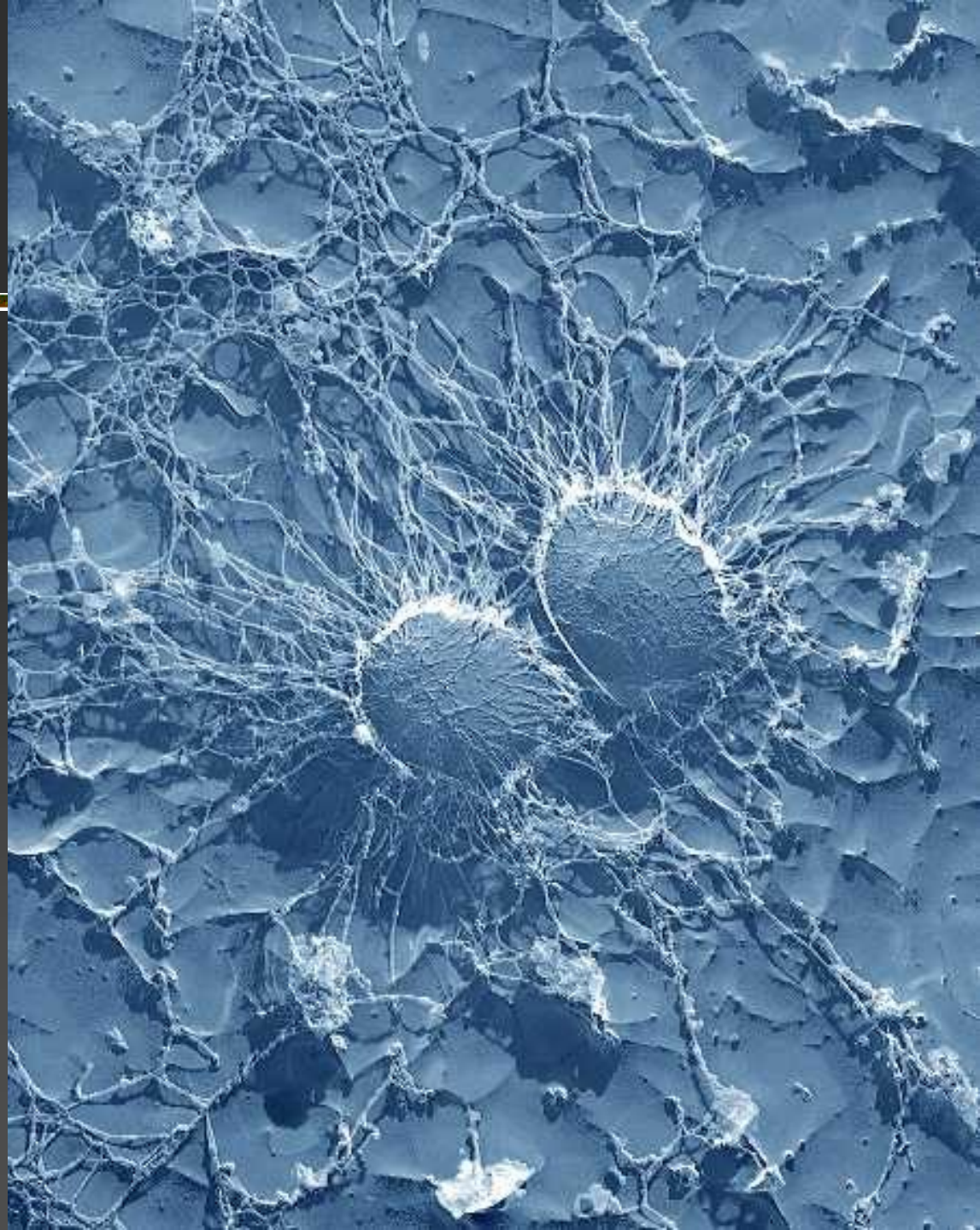


Who is guilty?

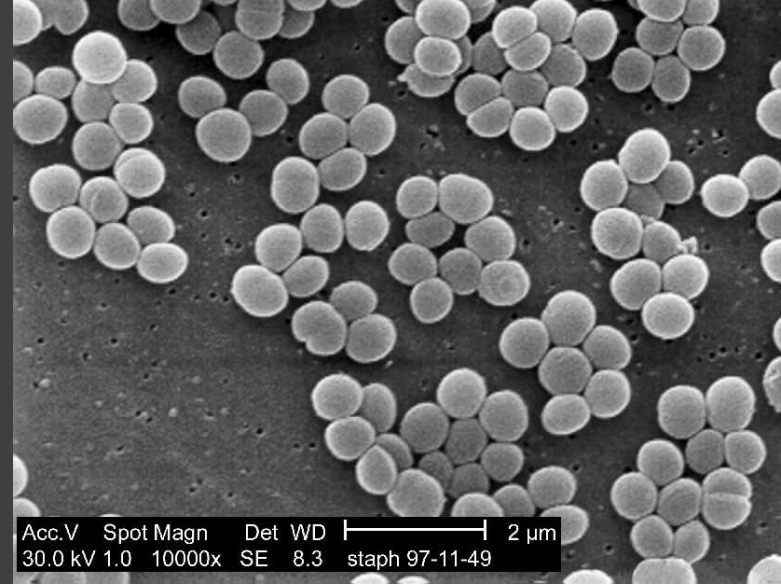
- It is *Staphylococcus aureus*
name from greek staphylé = grape
- This „golden staphylococcus“ often causes pyogenic infections of skin, hairs, nails etc.
- Some strains produce **enterotoxins**, that act as so named **superantigens**
- Intoxication by a bacterial toxin usually starts quickly and finishes quickly, unlike a bacterial infection (e. g. salmonellosis)

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

- Golden staphylococcus, 10 000 × magnified in an electron microscope



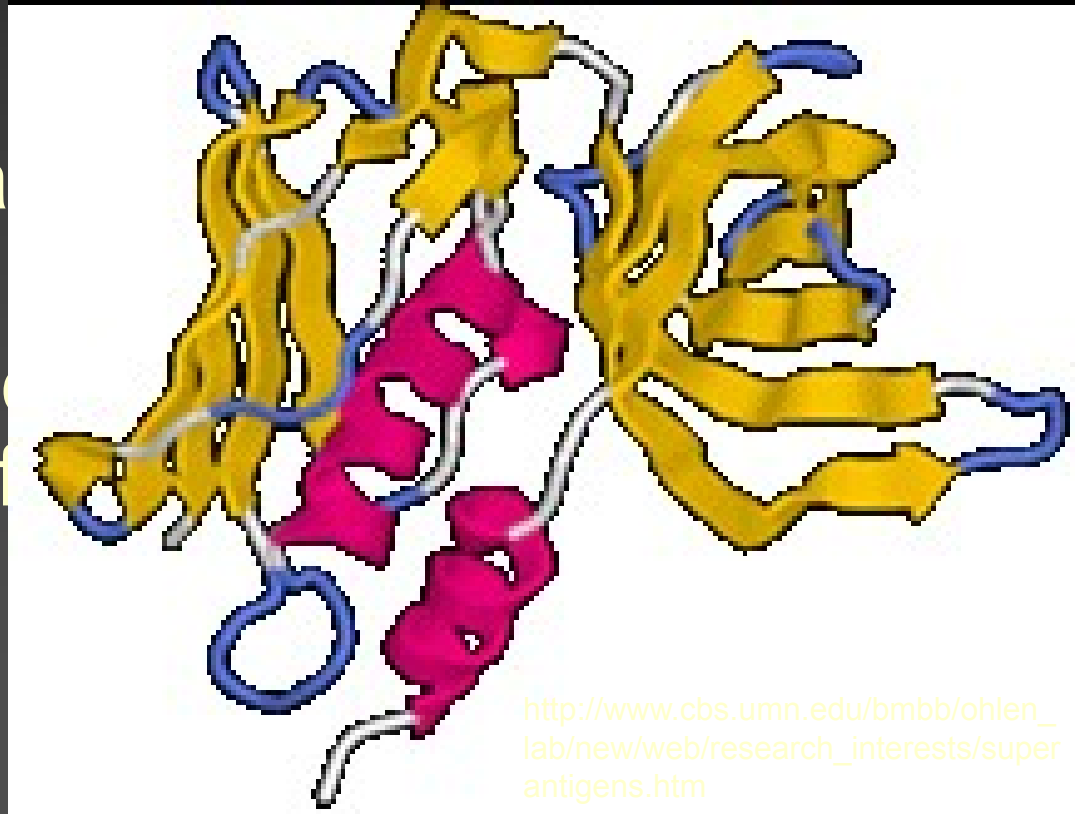
Story Two



- **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 now?

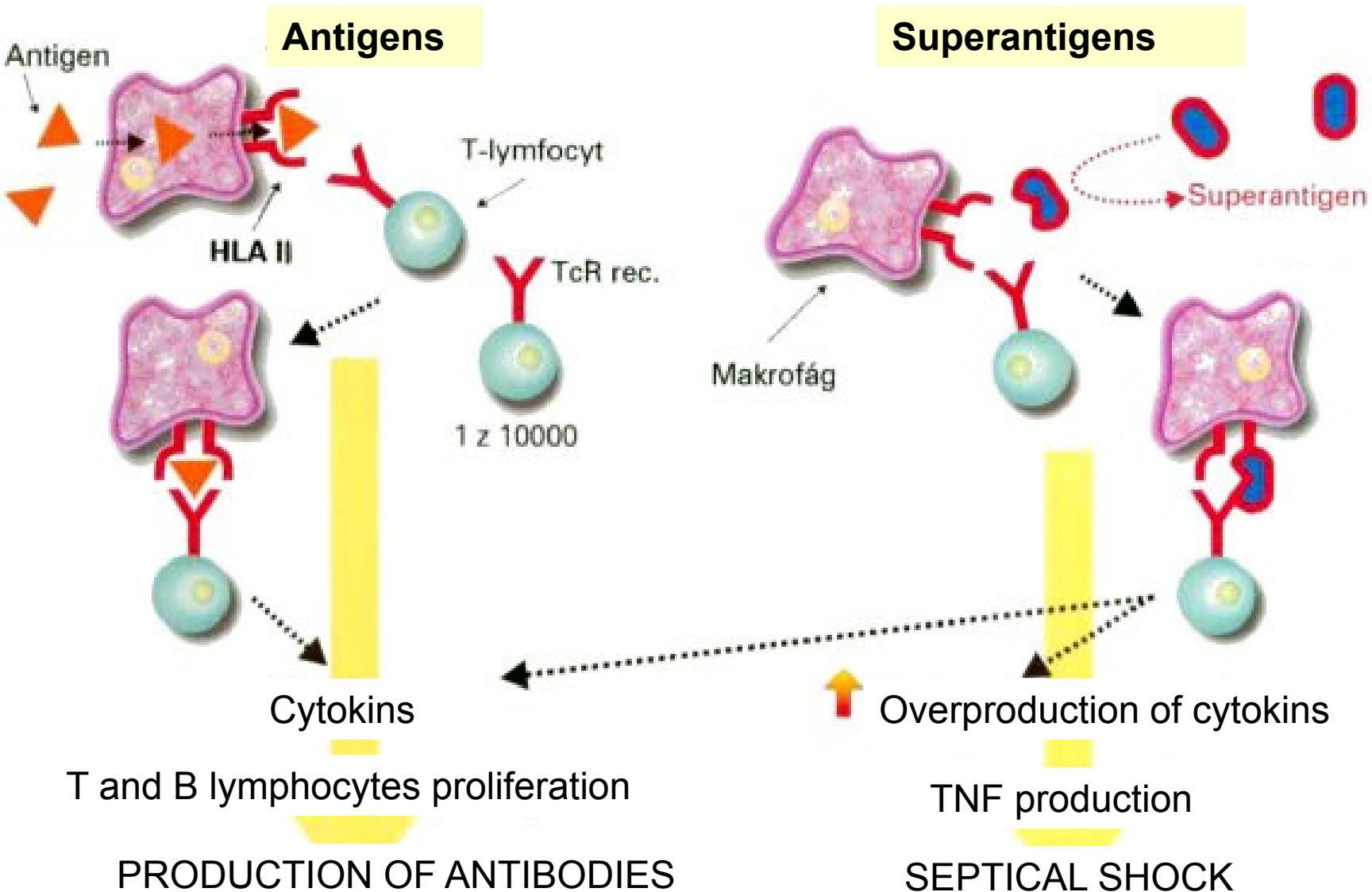
- Again, it is *Staphylococcus aureus*, now a strain called **TSST-1** (toxic shock syndrome toxin)
- This toxin, too, is a **superantigen**
- It causes toxic shock typically in users of **menstruation tampones**



http://www.cbs.umn.edu/bmbb/ohlen_lab/new/web/research_interests/superantigens.htm

Superantigens

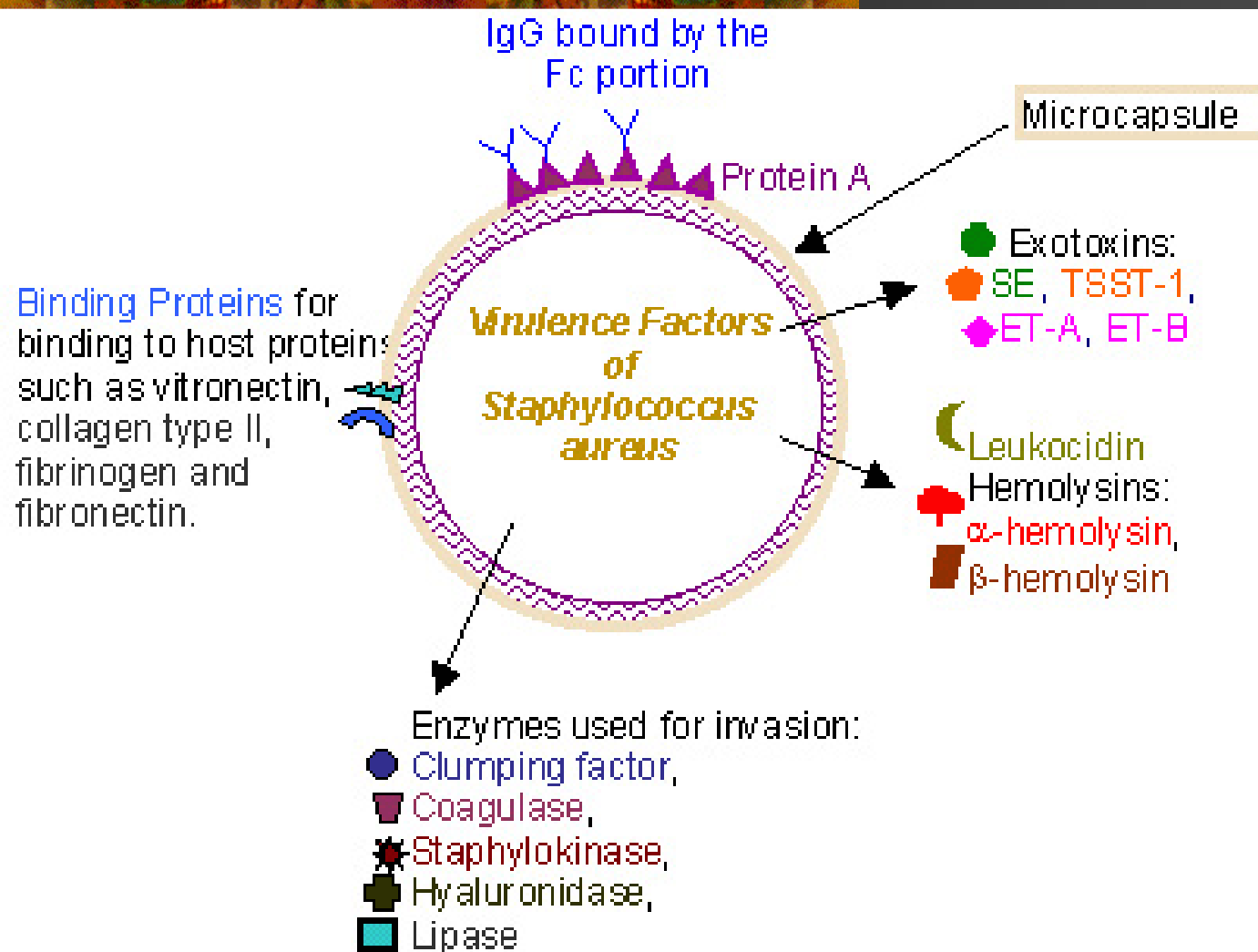
Immunity response



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 abscessi 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

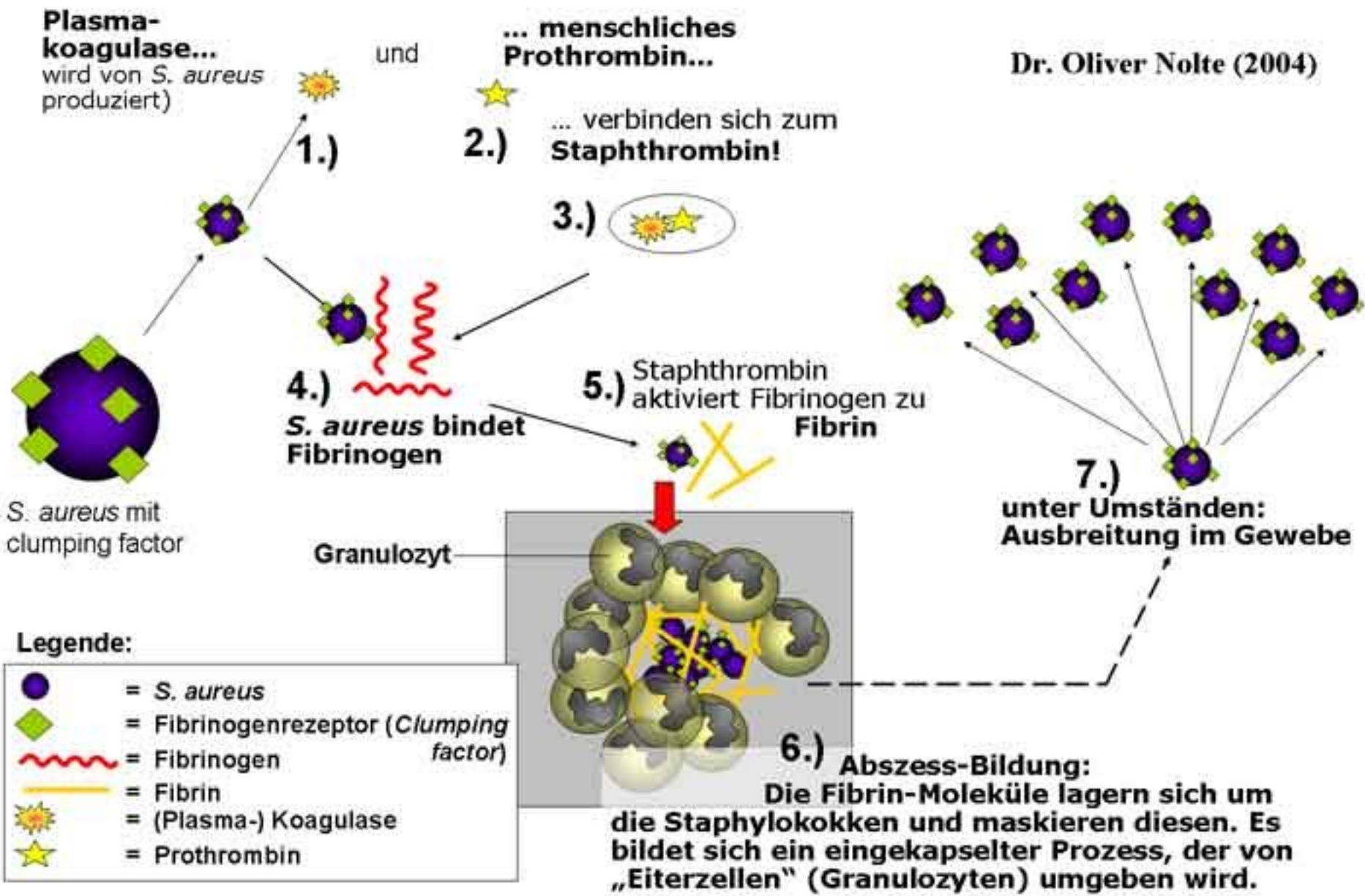
There exist plenty of virulence factors found in *S. aureus*...



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

Abscessi

- Unlike streptococci, producing in tissues mostly uncoated **phlegmonae**, staphylococci form mostly coated **abscessi**. Formation of an abscessus (using so named clumping factor and plasmacoagulase – see further!) is in the next scheme, from a german website
-



Examples of infections caused by staphylococci: Impetigo...



...bullous impetigo...



...otitis externa
with a
furuncle...



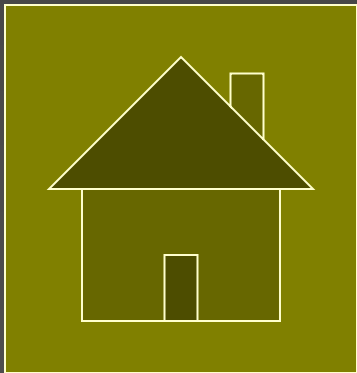
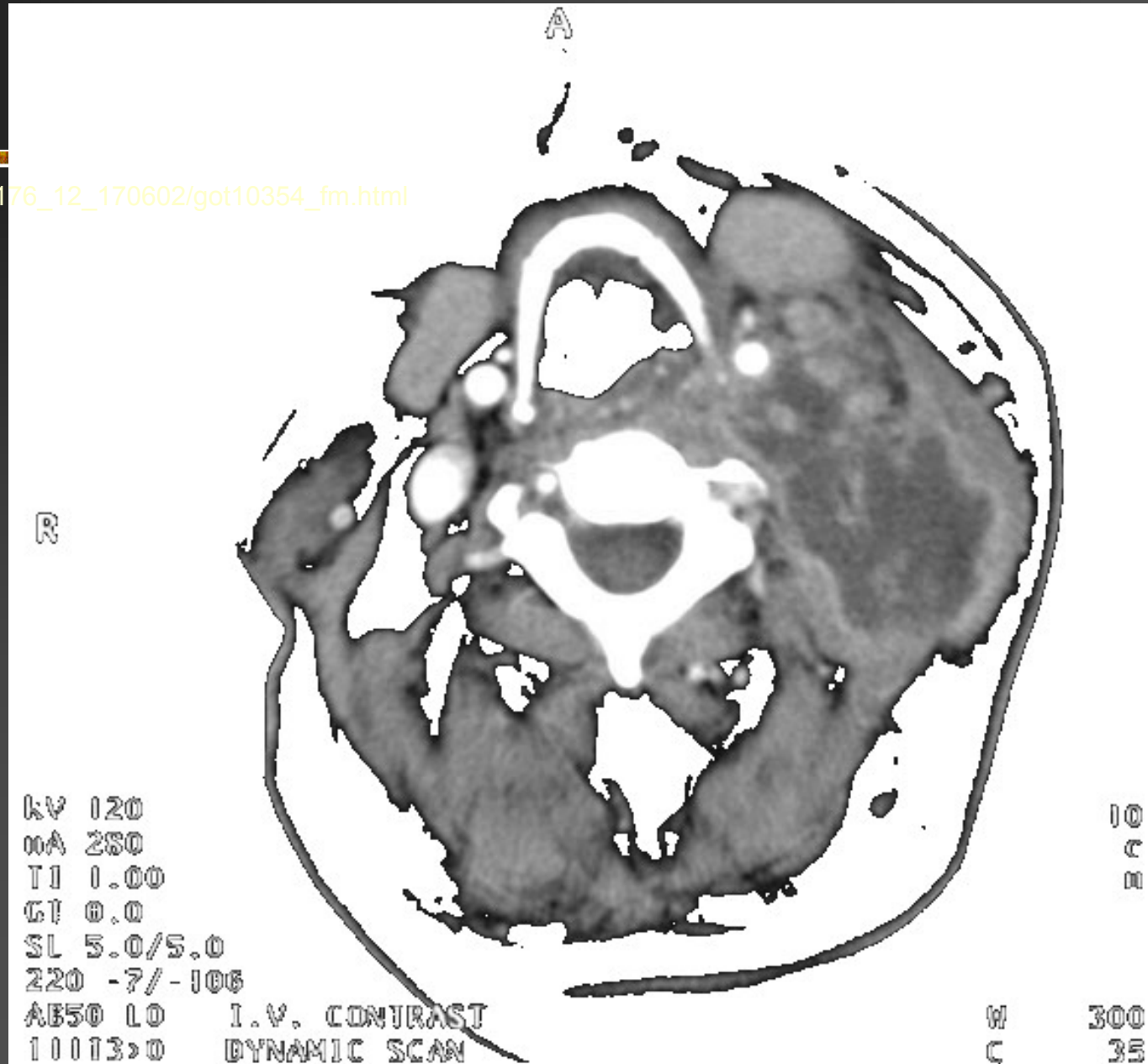
...or skin infection with crusts...

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



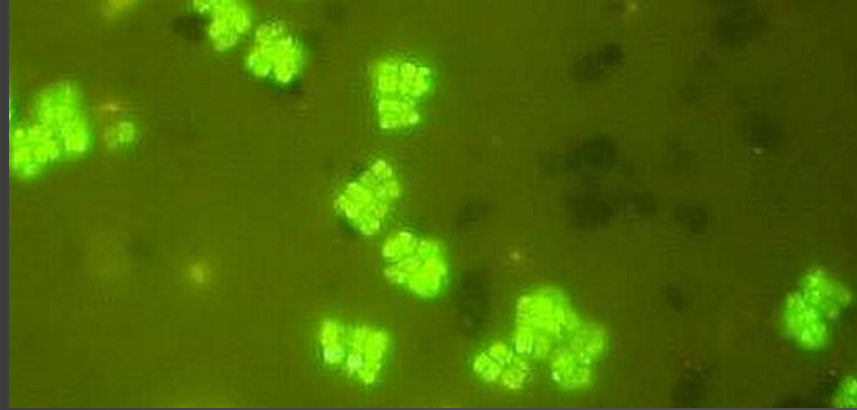
Clinical

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 acutely**, 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?

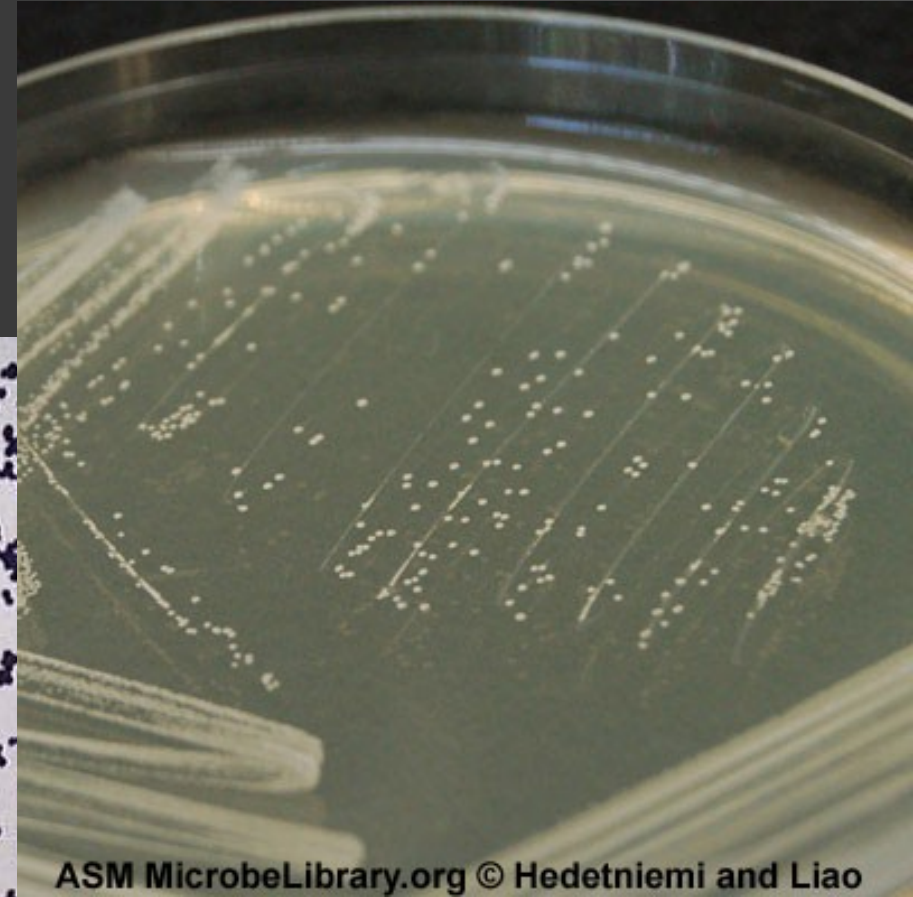
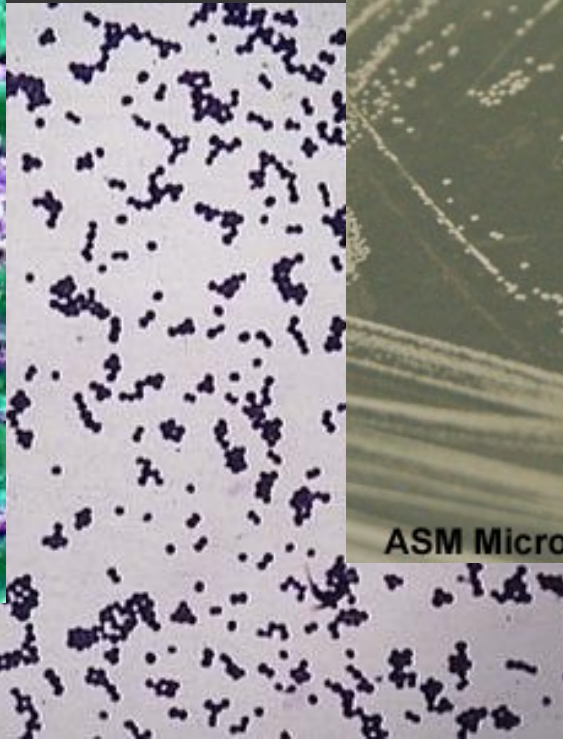
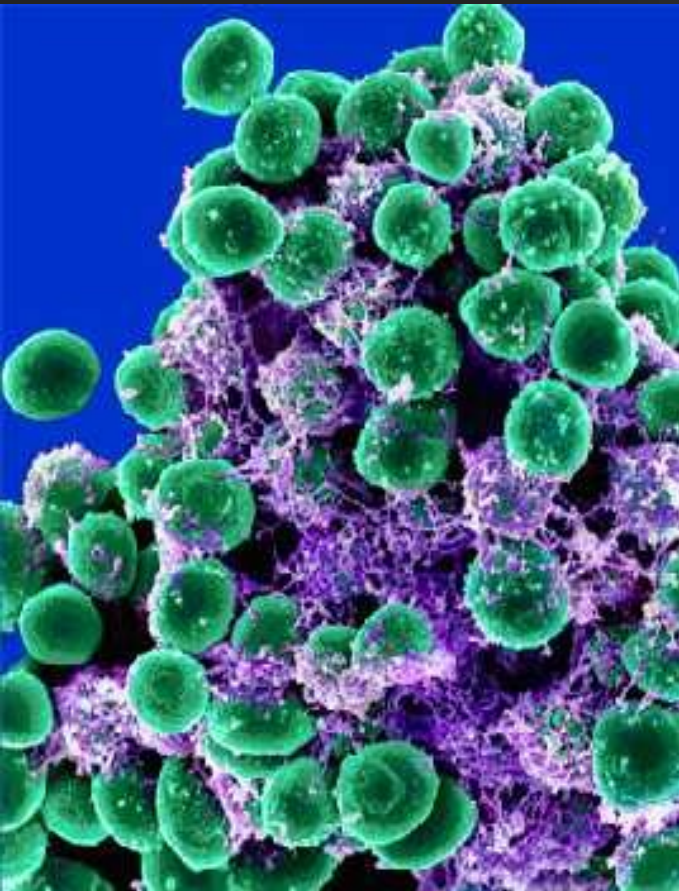
- The guilty is *Staphylococcus epidermidis*, the most common among coagulase negative staphylococci
- Coagulase negative staphylococci belong to the same genus as „golden staphylococcus“
- They are much less pathogenic
- In last decades, they started to be very important causative agents of infections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

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

Staphylococcus epidermidis

<http://www.microbelibrary.org>

<http://www3.niaid.nih.gov>



ASM MicrobeLibrary.org © Hedetniemi and Liao

<http://www.difossombrone.it>

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, wound infections, catheter sepsis 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
staphylo-
cocci today

S. simiae (AY727531)

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

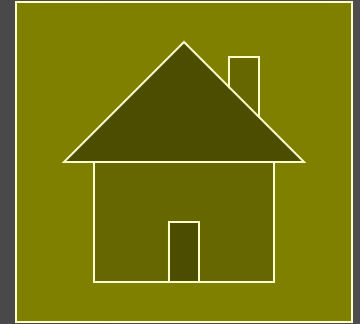


Diagnositics of staphylococci

Description of criminals (diagnostics) 1

- **Microscopy:** grampositive 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



www.medmicro.info (our webpage)



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

Survey of step-by-step diagnostics of a staphylococcal infection

■ ~~Microscopy of SPECIMEN (e. g. sputum)~~

■ Microscopy of an isolated STRAIN

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

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, there do not have clusters*
-

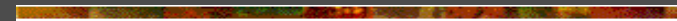
Gram stain (repeating)

- **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, Lugol 30 s, alcohol 15 s, water, safranin 60 s, water, dry, immersion obj. 100× magnifying)
 - **Now, we can exclude** all object that are gram negative and/or rods, eg. that do not belong into group of „G+ cocci“
-

Catalase test (for remembering)



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

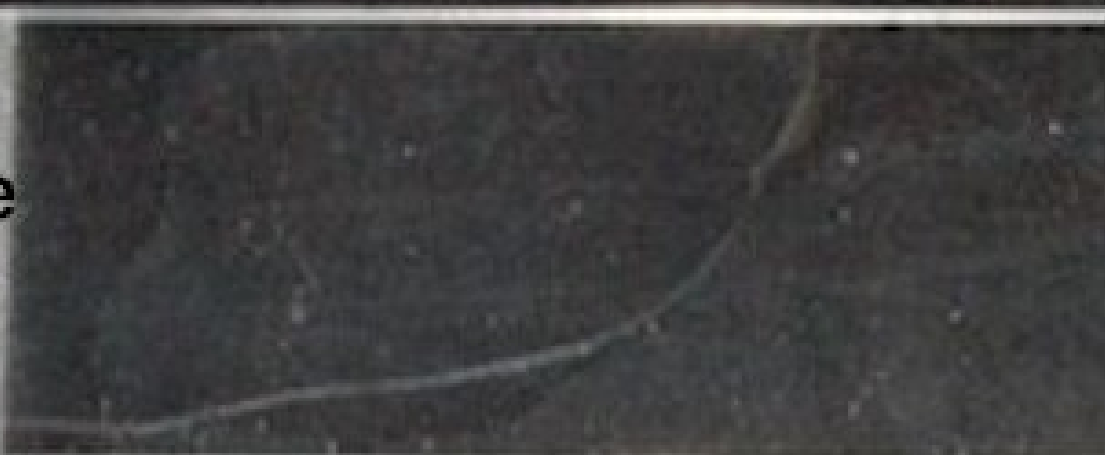


Catalase test

CATALASE TEST

<http://www.telmeds.org>

Negative



Positive



Survey of diagnostics (simplified)



(or other tests)



Enterococcus or

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 peroxyde, bubbles = positivity).

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!

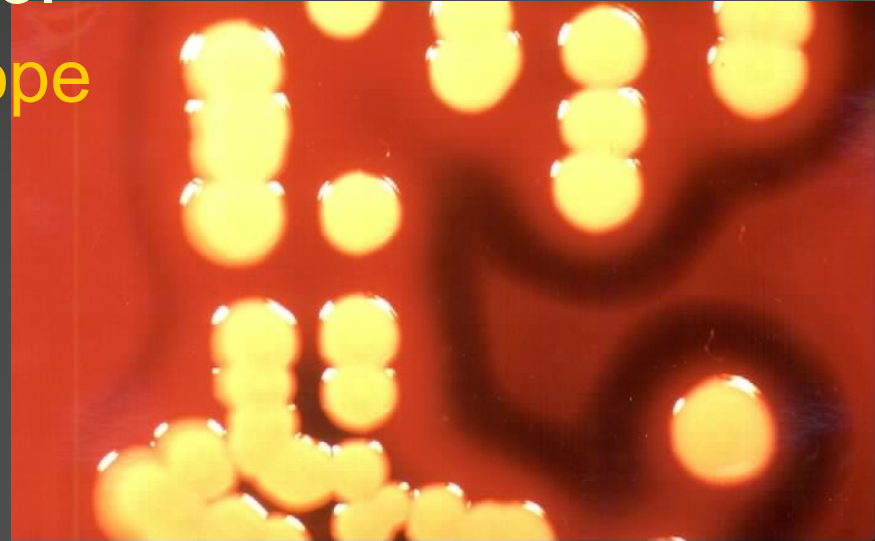
Differential
diagnostics of
staphylococci II:
steps inside genus
Staphylococcus

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!

- **Hemolysis:** Coagulase negative staphylococci may produce delta haemolysin, „Golden“ staphylococci may produce alfa, 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



Clumping factor or bound plasmacoagulase – quick

- Colonies are mixed with a drop of rabbit plasma on a slide
 - Positivita is 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



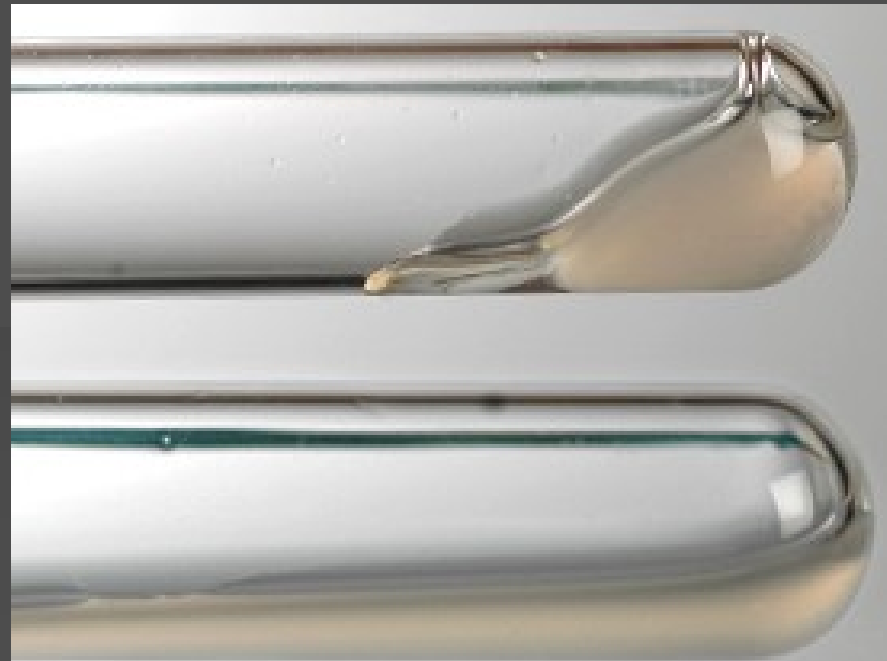
<http://memiserf.medmikro.ruhr-uni-bochum.de>

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



COAGULASE
TEST



POSITIVE

<http://www.telmeds.org>



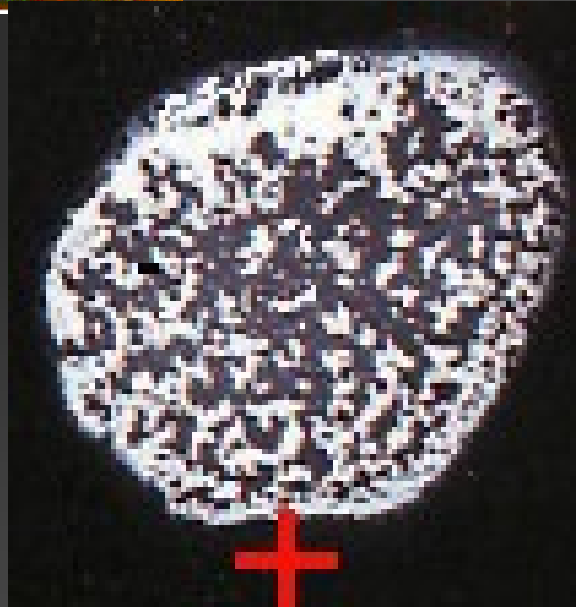
NEGATIVE

More pictures of plasmacoagulase



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 quite expensive

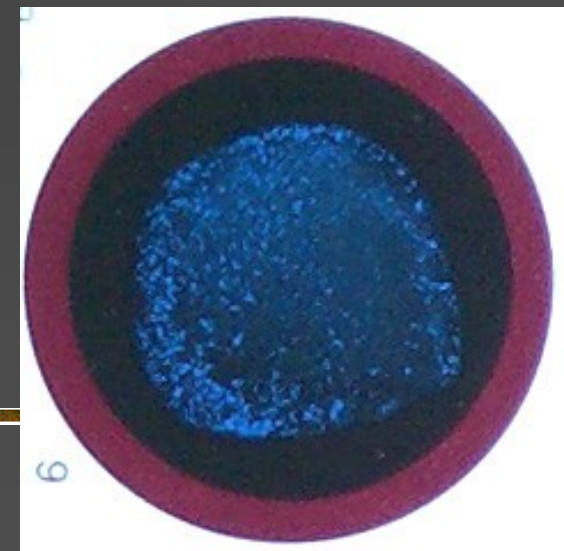
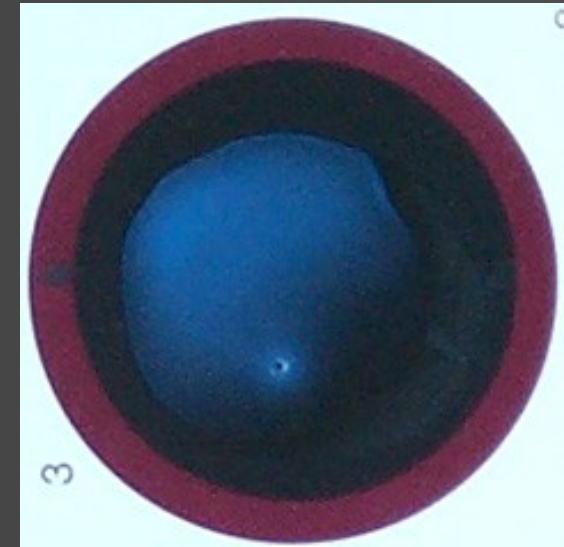


www.microbes-edu.org

Staphaurex kit and results

<http://www.pathologyinpractice.com>

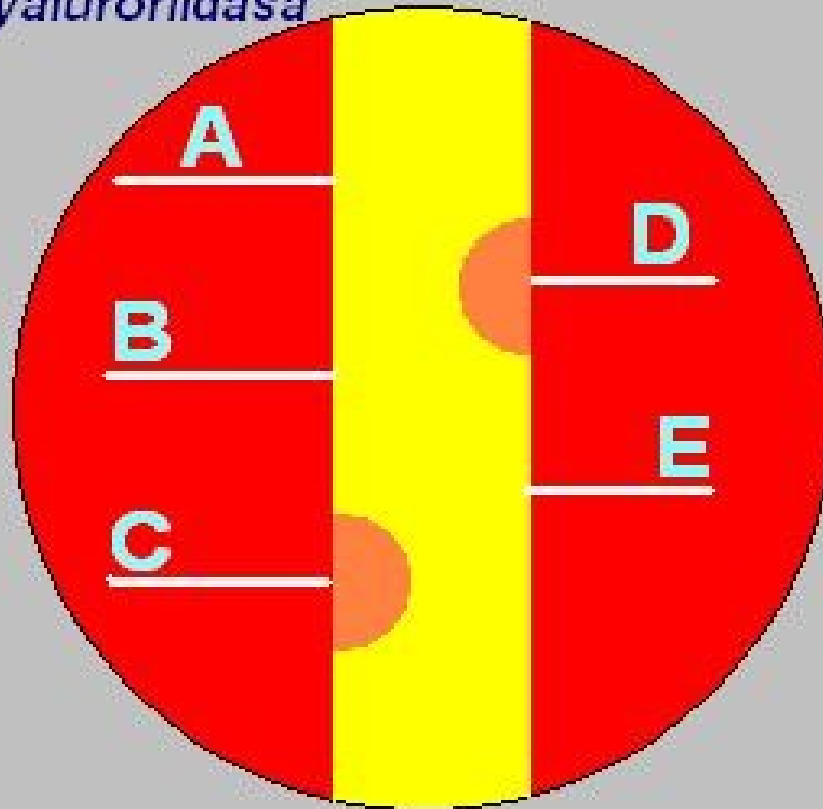
www.microbes-edu.org



Hyaluronidase (decapsulation)

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

Hyaluronidasa



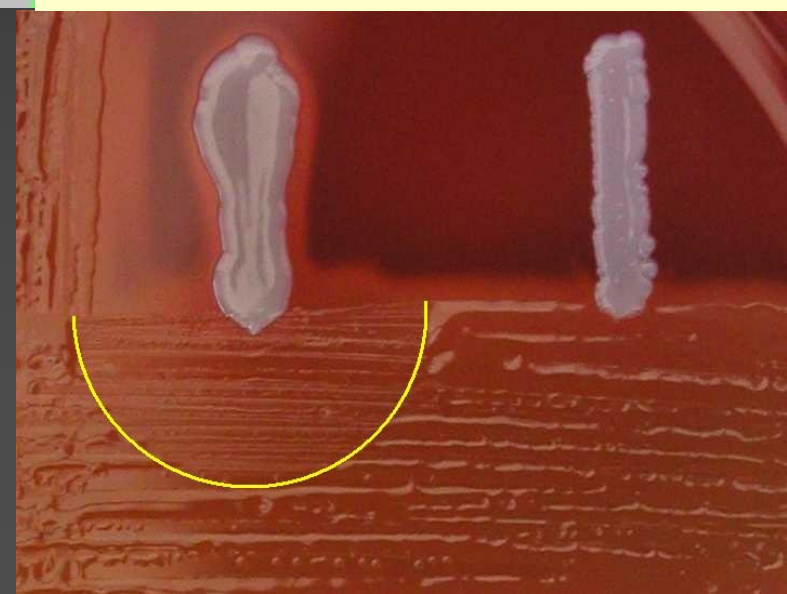
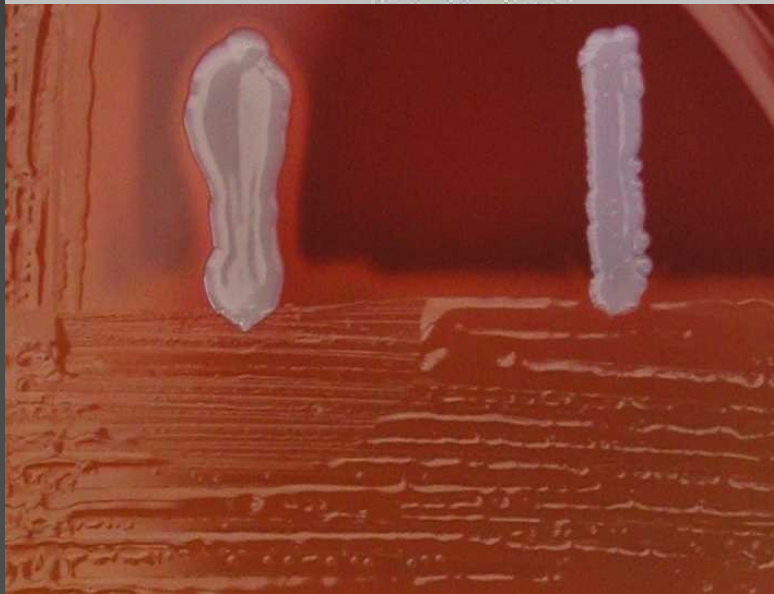
Yellow – *Streptococcus equi*
(mucous)

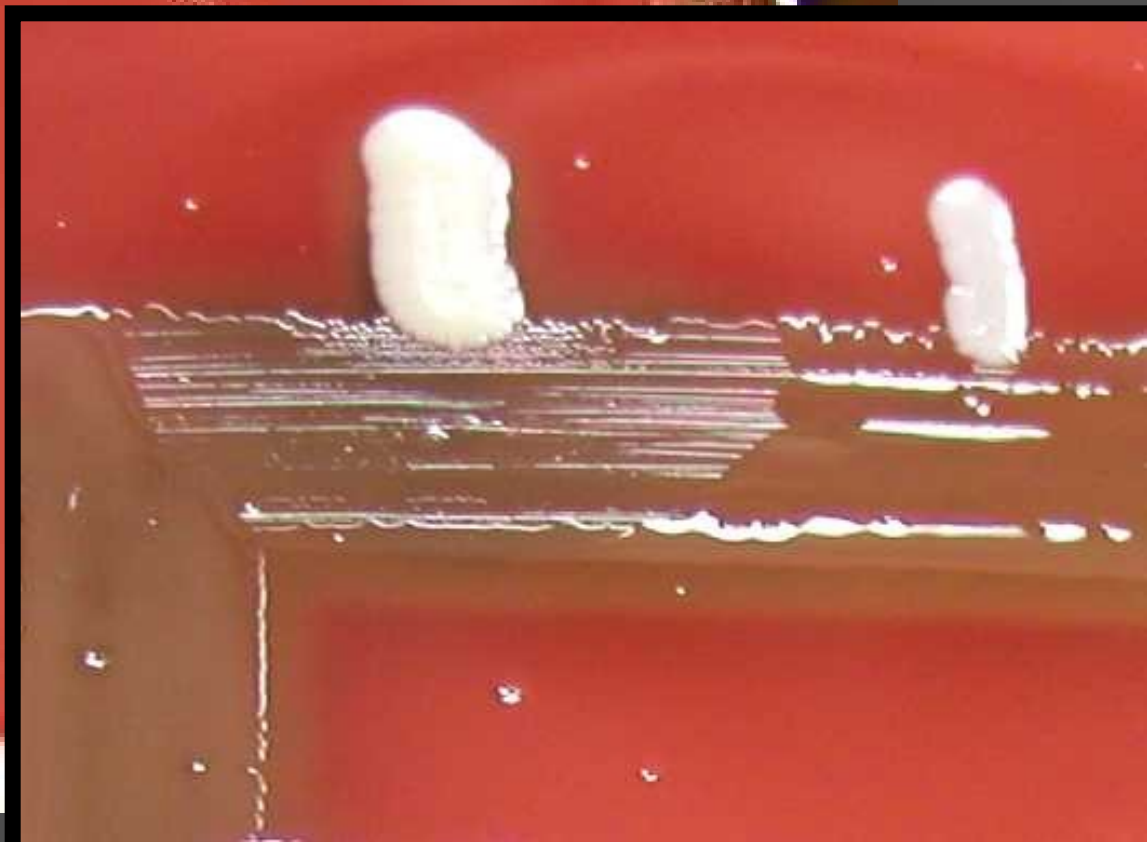
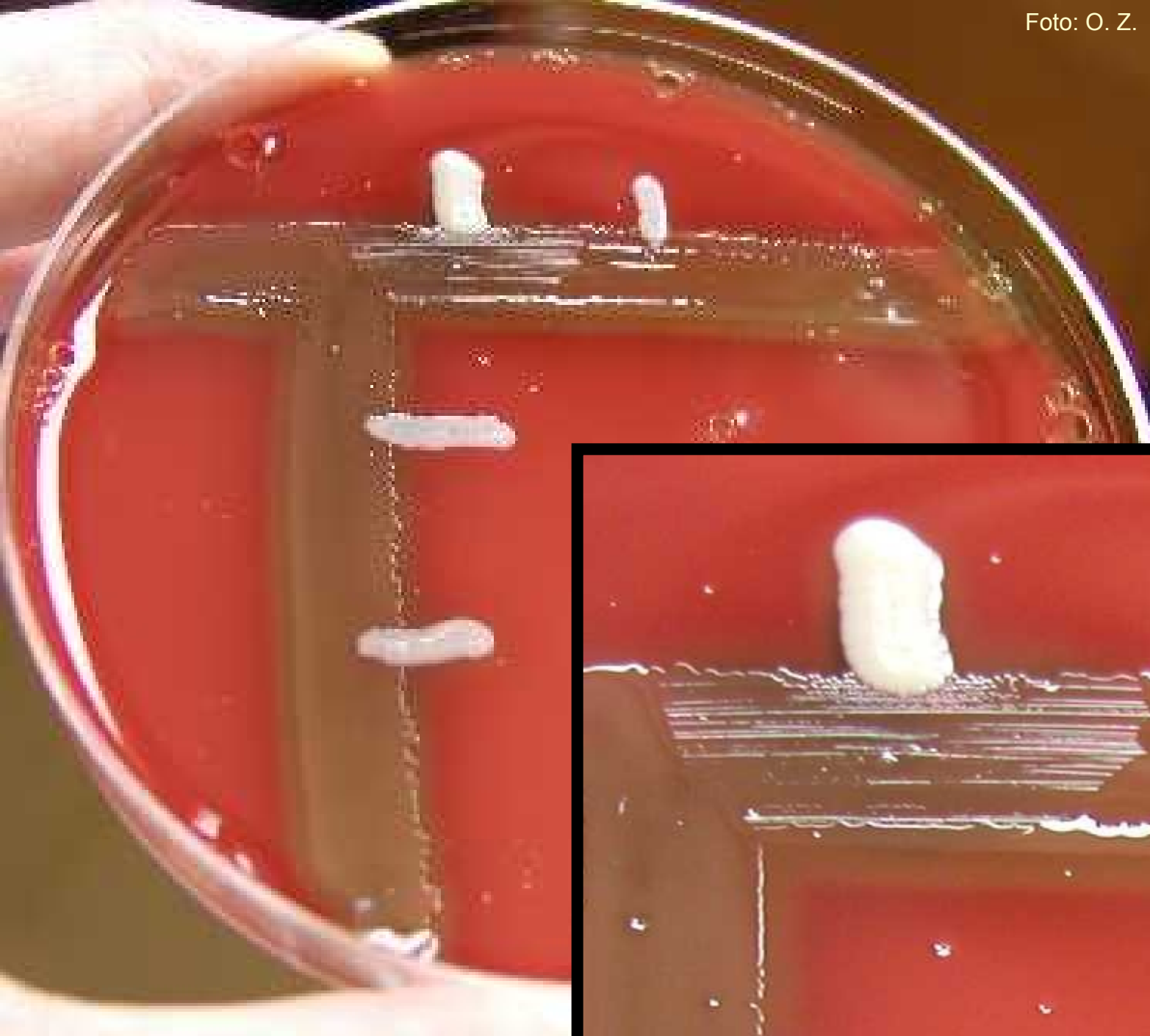
White – tested staphylococci

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 (Pliva-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, colorless fluid = negative
- First row of the STAPHYtest = 2. – 9. react.
- Second row of the STAPHYtest = 10. – 17. 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 two remaining test (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	
		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

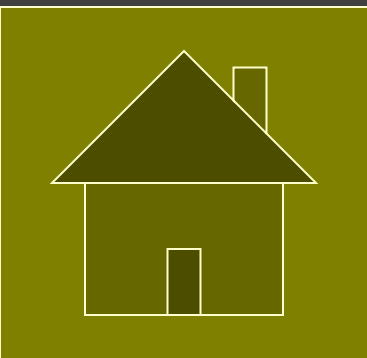
- Not regarding the producer, the principle is the same – combination of many enzymatic reactions, that can be seen as colour change

<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



Antibiotic

susceptibility testing

and antibiotic

treatment of

staphylococcal

infection

Susceptibility testing

- To check secondary resistances, we mostly use a **diffusion disk test** – we measure the inhibition zones and compare with reference zones
- Among used antibiotics we use commonly e. g. OX = oxacilin, VA = vankomycin, RD = rifampicin, KF = cefalotin, MY – linkomycin CN = gentamicin
- 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.

Illustration photo



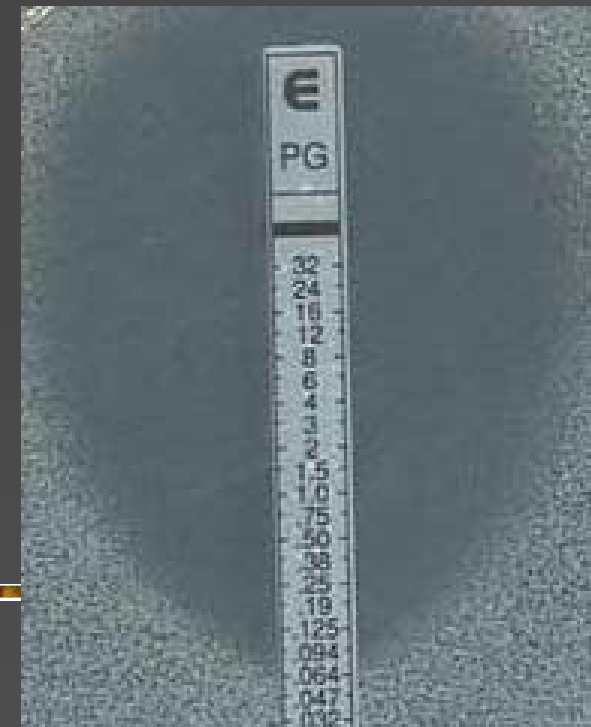
Quantitative and qualitative tests

- 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 →



Usual law: 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.

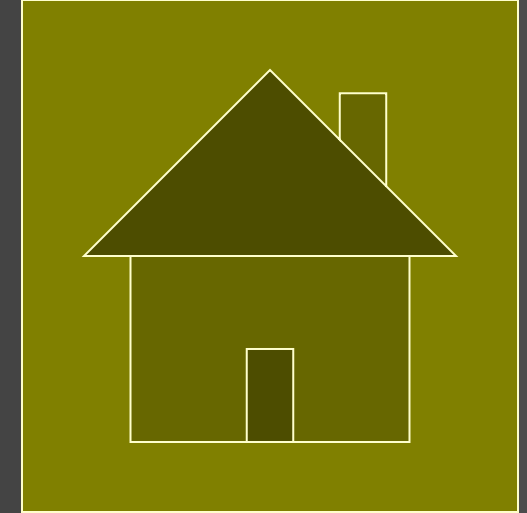
Anti-styphylococcal drugs

- In staphylococci, the drug of choice is **oxacilin**, in UTI **cefalosporins 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 oxacilin, so named **MRSA** and **MRSKN**. In strains resistant even to glycopeptices, or in patients that has contraindications, newer antibiotic **linezolid** can be used.

MRSA and their detection

- Methicilin 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 oxacilin. But it is not a clear proof.
 - The proof is, when the zone is small not only in oxacilin, but also cefoxitin
-

The End



A victim of a
staphylococcal
infection