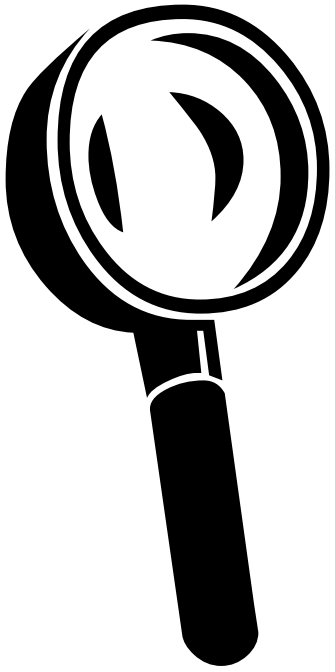
Institute of Microbiology shows:



TRACING THE CULPRIT
Part one: Staphylococcus

Contents of the slideshow

Survey of special bacteriology

Special bacteriology (P01–P09)

- We are going to have nine lessons now concerning bacteria
- The list is not complete, and the bacteria are not classified according to taxonomy, but rather to microscopical morphology *(although e. g. being a coccus does not mean to be related to other cocci)*
- Some bacteria (mycoplasms, chlamydias, rickettsias) are not mentioned in practical sessions; some have been mentioned during virology or methodology classes
- Of course, these bacteria, too, will be supposed to be known during the final examination

Special bacteriology (P01–P09)

- Gram-positive (G+) cocci: P01, P02, P03 (part)
- G+ bacilli: P03 (part)
- G– bacilli: P04, P05, P06 (part)
- G– cocci: P06 (part)
- Strictly anaerobic bacteria: P07
- Acid fast, partially acid fast and branched rods: P08
- Spirochetes: P09

If we say „Gram-positive cocci“, it is not a taxonomy group. Some G+ cocci (e. g. *Staphylococcus*) are phylogenetically more closely related to some G+ rods (e. g. *Bacillus*) than to some other cocci (*Streptococcus*, or even *Micrococcus*)

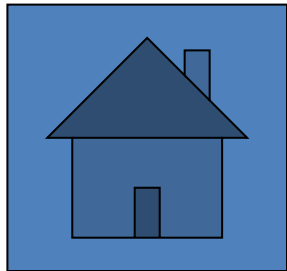
Even Gram staining is not sure. Mycoplasms are phylogenetically related to G+ bacteria, but they have no cell wall, so they stain weakly pink (like Gram-negative organisms).

Medicaly important G+ bacteria (+ acid-fast bacteria and mycoplasms) in modern taxonomy

- **G+ bacteria, phyllum Firmicutes, class Bacilli**
  - Order Bacillales: families *Bacillaceae* (P03), *Listeriaceae* (P03), *Staphylococcaceae* (P01)
  - Order Lactobacillales: families *Lactobacillaceae* (P03), *Streptococcaceae* (P02), *Enterococcaceae* (P03)
  - Order Erysipelotrychiales: family *Erysipelotrichiaciae* (P03)
  - ?Order Mollicutes: family *Mycoplasmataceae* *(but sometimes in a special phyllum!)*
- **G+ bacteria, phyllum Firmicutes, class Clostridia**
  - Order Clostridiales (P07): families *Clostridiaceae*, *Peptococcaceae*, *Peptostreptococcaceae*
- **G+ bacteria, phyllum Actinobacteria, class Actinobacteria**
  - Order Actinomycetales: families *Actinomycetaceae*, *Nocardiaceae, Corynebacteriaceae*, *Mycobacteriaceae* (P03 and P08)
  - Order Bifidobacteriales: family *Bifidobacteriaceae* (e. g. intestinal microbiome microorganism *Bifidobacterium* and vaginal bacterium *Gardnerella*)
  - Order Coriobacteriales: family *Coriobacteriaceae*, e. g. vaginal genus *Atopobium*

Medicaly important G– bacteria (+ spirochetes) in modern taxonomy

- **G– bacteria, phyllum Proteobacteria, class Alphaproteobacteria**
  - Order Rickettsiales: family *Rickettsiaceae* (not included in practical sessions)
- **G– bacteria, phyllum Proteobacteria, class Betaproteobacteria**
  - Order Burkholderiales: family *Burkholderiaceae* (P05)
  - Order Neisseriales: family *Neisseriaceae* (P06)
- **G– bacteria, phyllum Proteobacteria, class Gammaproteobacteria**
  - Order Aeromonadales: family *Aeromonadaceae* (P04)
  - Order Enterobacteriales: family *Enterobacteriaceae* (P04; *new families suggested*)
  - Order Pasteurellales: family *Pasteurellaceae* (P05)
  - Order Pseudomonadales: families *Pseudomonadaceae* (P05), *Moraxellaceae* (P06)
  - Order Xanthomonadales: family *Xanthomonadaceae* (P05, including *Stenotrophomonas*)
  - Order Vibrionales: family *Vibrionaceae* (P04)
- **G– bacteria, phyllum Proteobacteria, class Epsilonproteobacteria**
  - Order Campylobacteriales: families *Campylobacteriaceae* and *Helicobacteriaceae* (P04)
- **G– bacteria (?), phyllum Planctobacteria, class Chlamydiae**
  - Order Chlamydiales: family *Chlamydiaceae* (not in practicals)
- **G– bacteria (?), phyllum Spirochaetes, class Spichochaetia**
  - Order Spirochaetales: families *Leptospiraceae* and *Spirochetaceae* (P09)



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 ill 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 cases pyogene 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:
  - **Enterotoxicosis** = 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 students is hospitalized on emergency unit of infectology clinic…

Who is guilty?

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

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*…

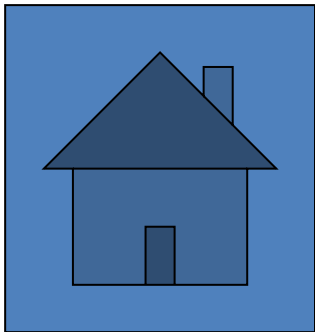
- …but the ratio of strains producing them is different:
  - nearly 100 % for coagulase, hyaluronidase, clumping factor (that is why they are used as diagnostic factors)
  - 20–80 % for haemolysins (alpha, beta, delta)
  - 0,1 % and less for rare, but very serious factors – exfoliatins A and B, enterotoxins A and B, toxic shock syndrome toxin (TSST-1), PVL (Panton-Valentine leucocidin) and others

Abscesses

- Unlike streptococci, producing in tissues mostly uncoated **phlegmonas,** staphylococci form mostly coated abscesses.

Skin infections

- Typical staphylococcal infections are skin infections, like
  - impetigo
  - boil (furuncle)
  - carbuncle
  - folliculitis



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

Story Three

- **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

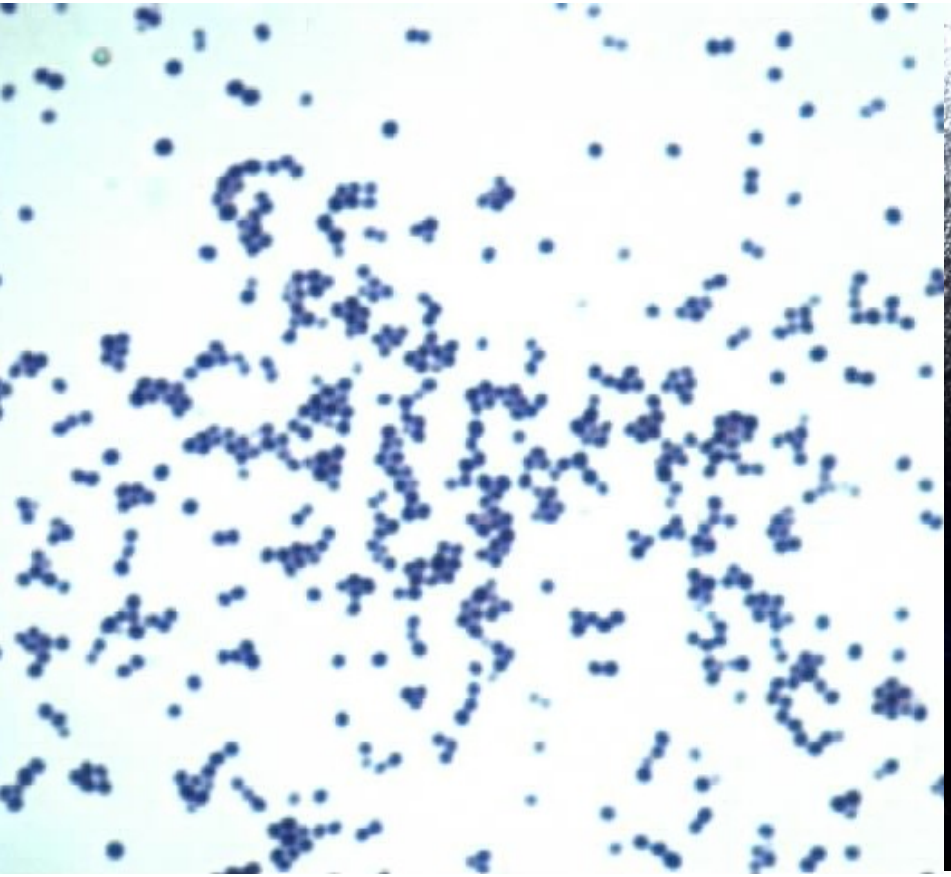
And who is guilty now?

- The guilty is ***Staphylococcus epidermidis***, the most common among **coagulase negative staphylococci**
- Coagulase negative staphylococci belong to **the same genus as „golden staphylococcus“**
- The 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

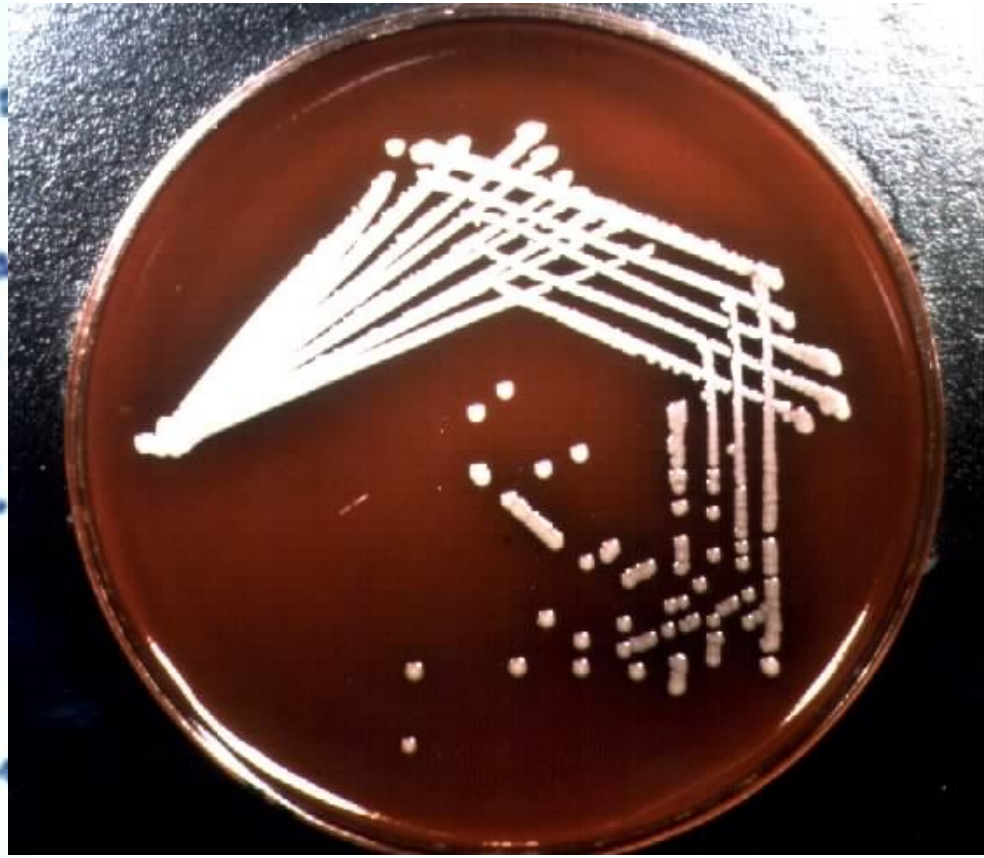
Why „coagulase negative staphylococci“? See later…

Staphylococcus epidermidis

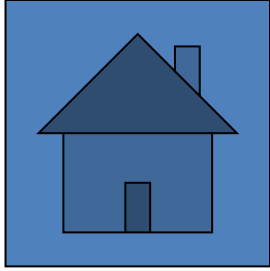
Author: Prof. MVDr. Boris Skalka, DrSc.



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.*

Diagnostics 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
- **Identification tests:** catalase positive, oxidase negative, it is possible to differentiate individual species biochemically or using MALDI-TOF
- **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)

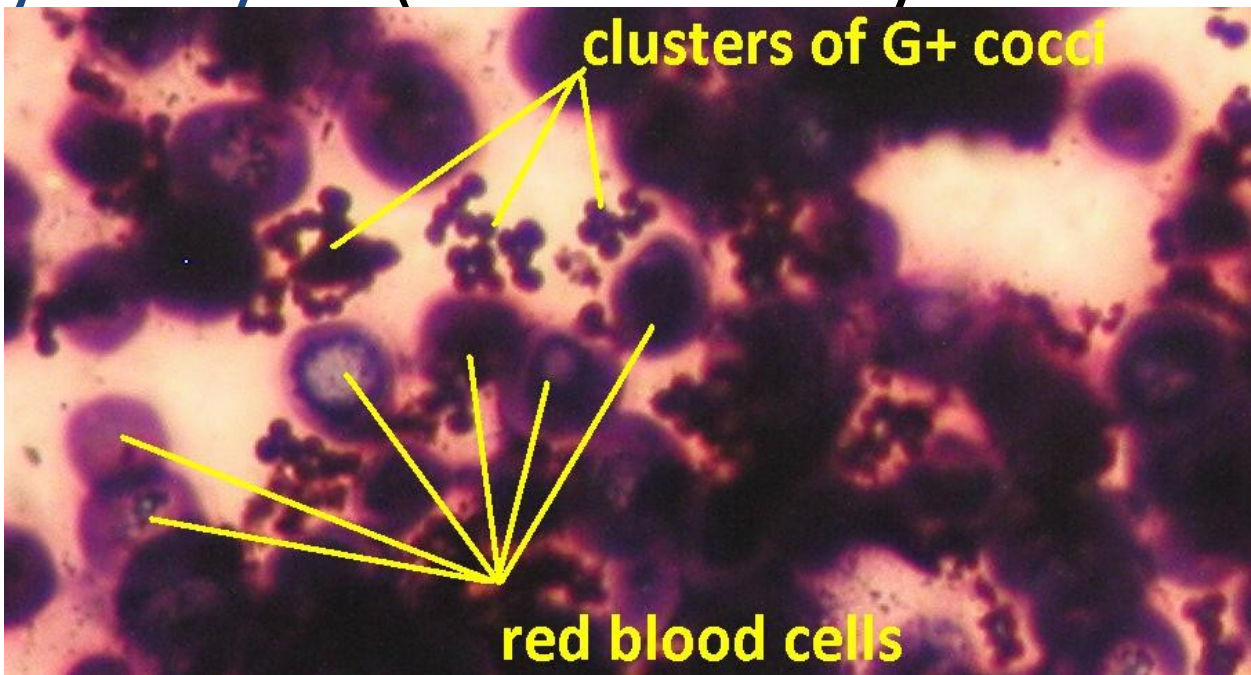
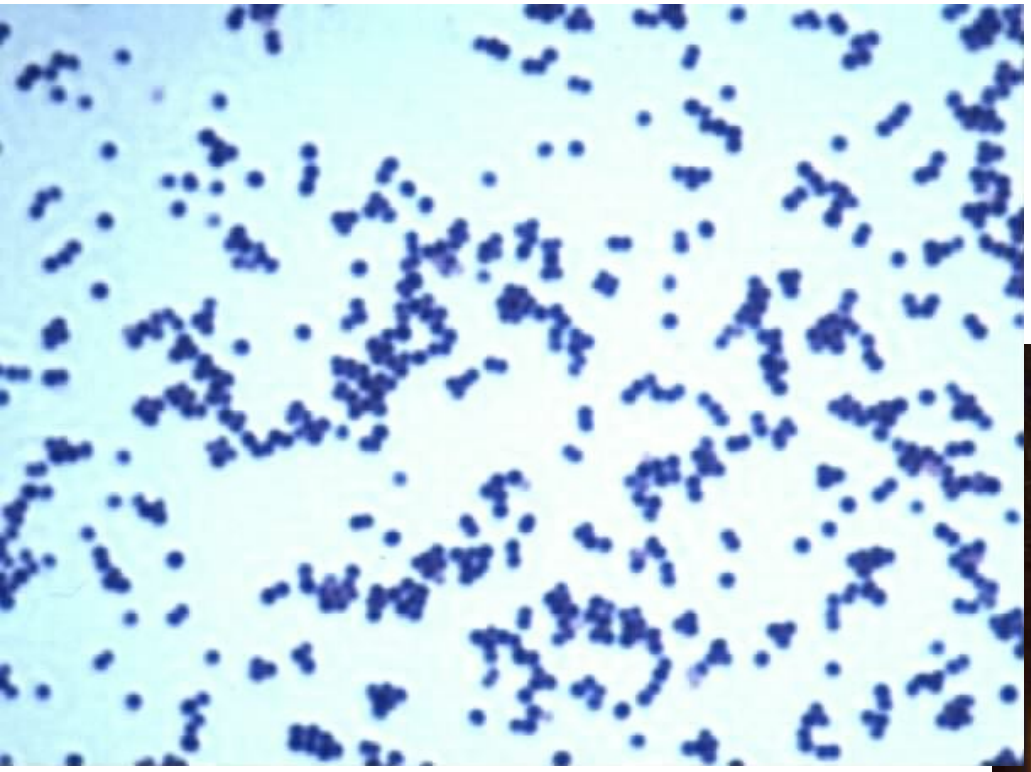
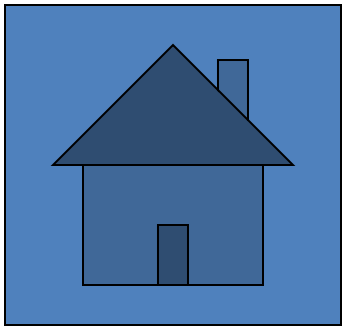


Photo: O. Z.

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)

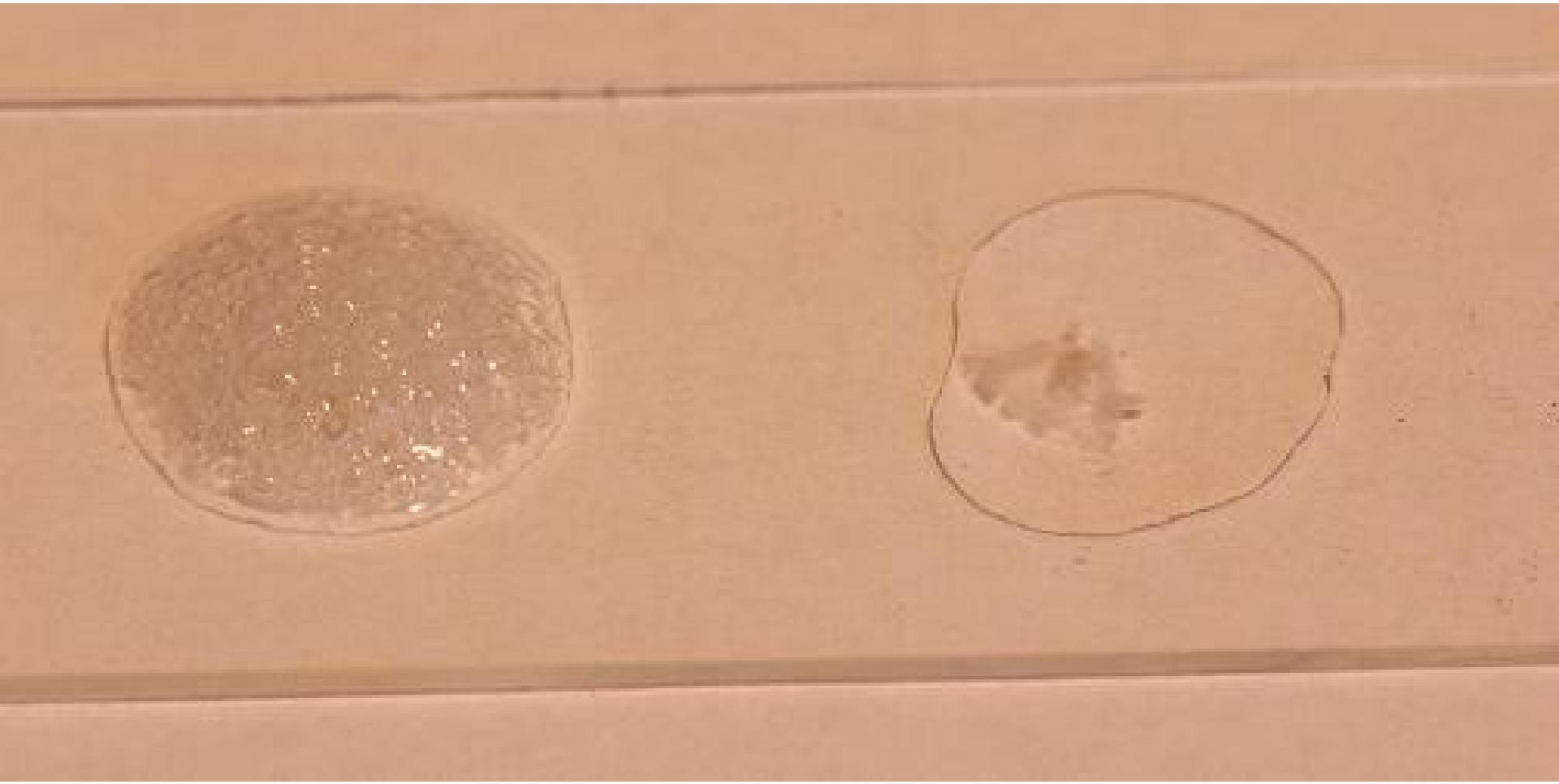


Photo: Veronika Holá

Catalase test + and –

http://www.telmeds.org

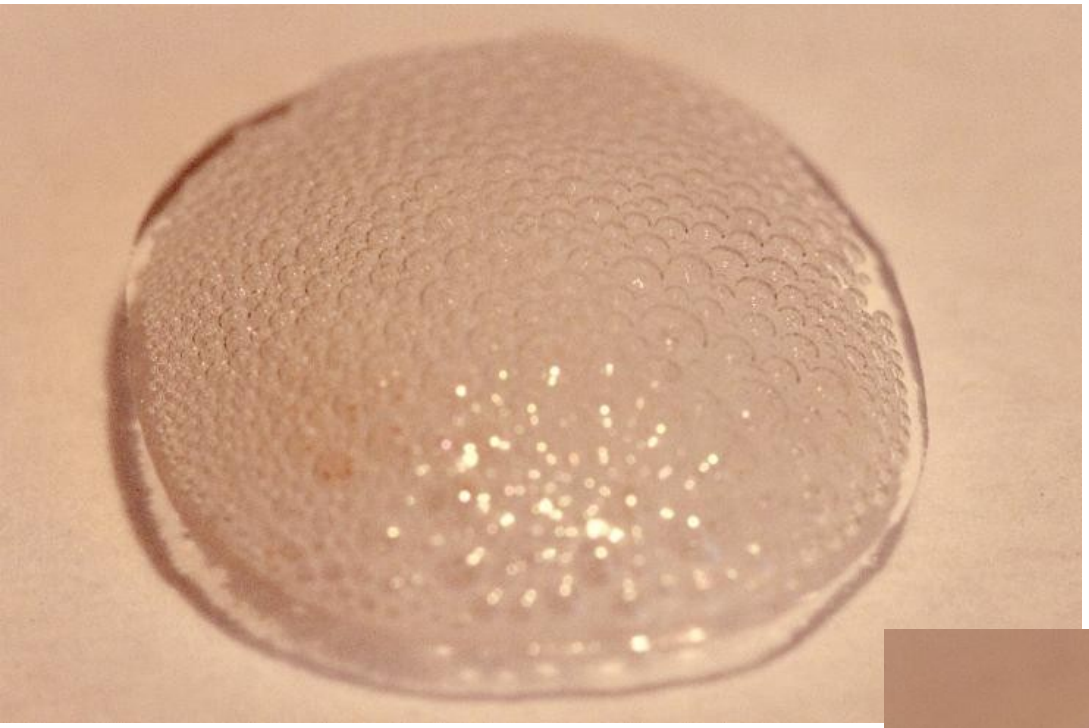
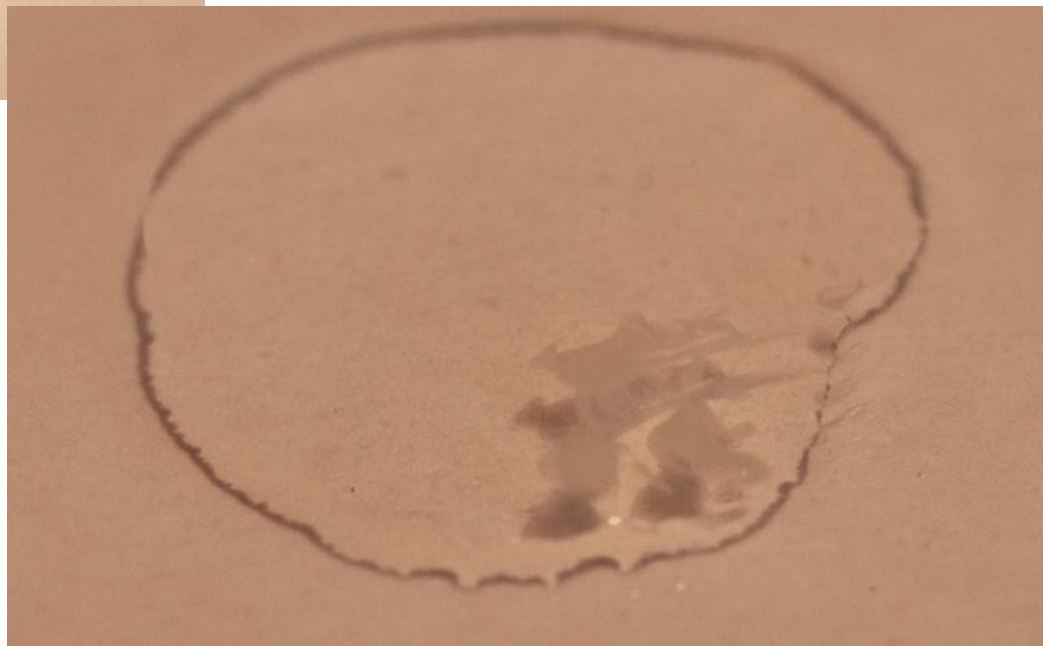


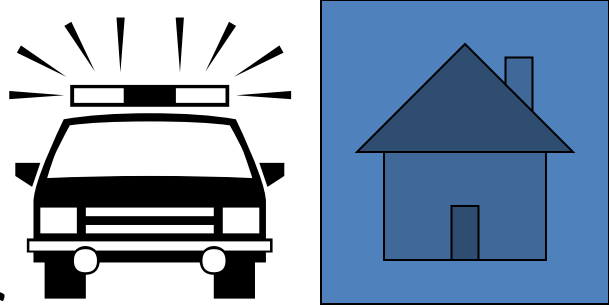
Foto: Veronika Holá



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

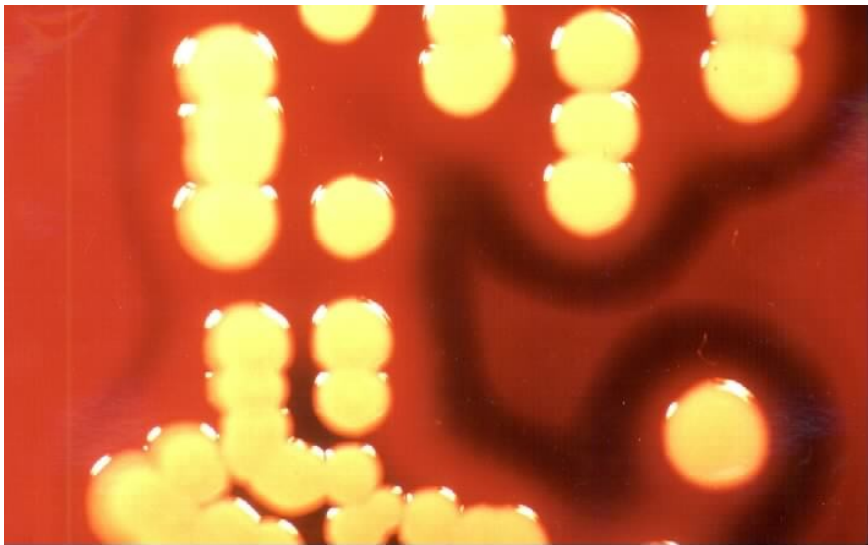
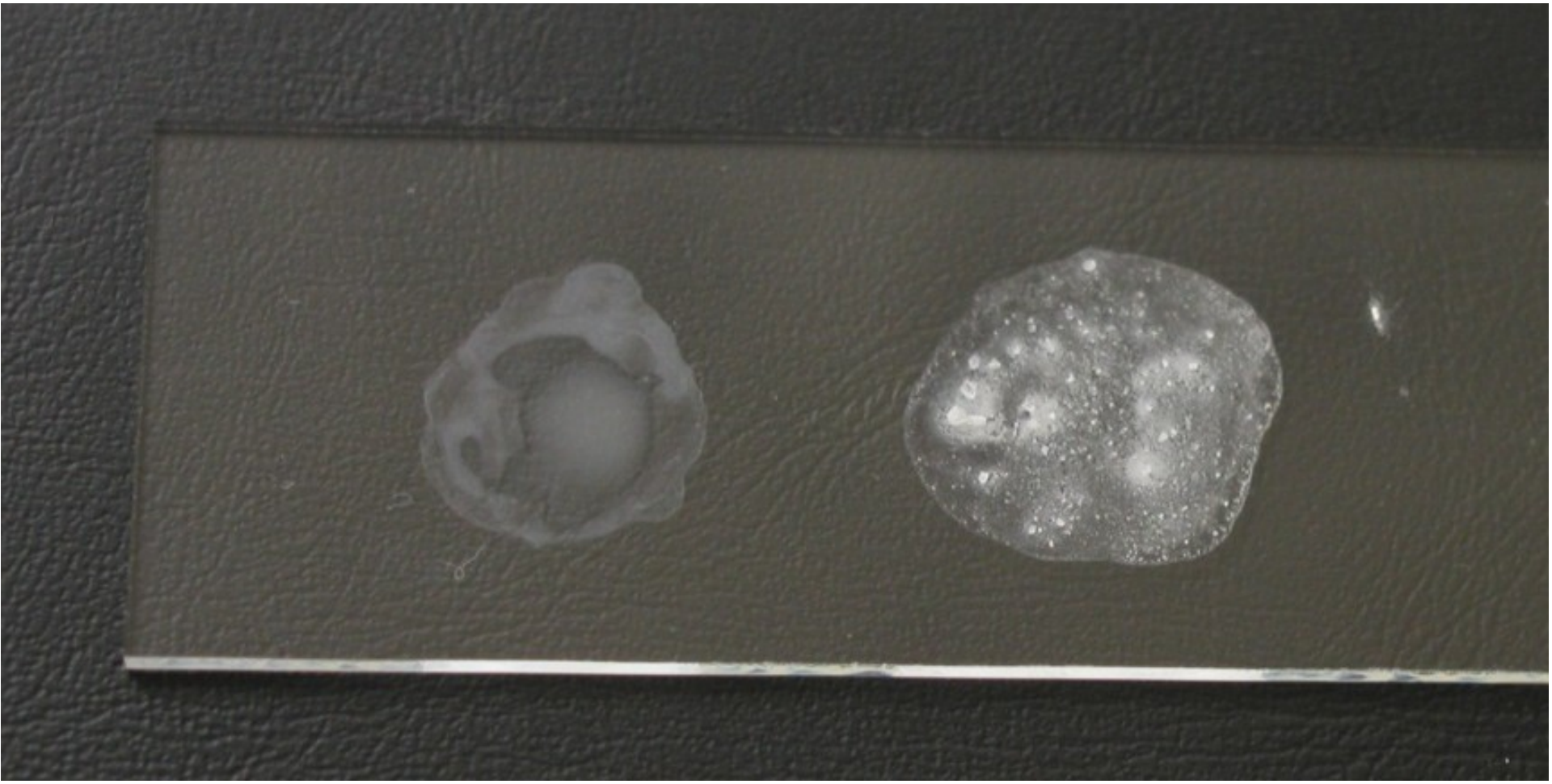


Photo: Archive of Institute of Microbiology

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

Photo: Microbiology Institute



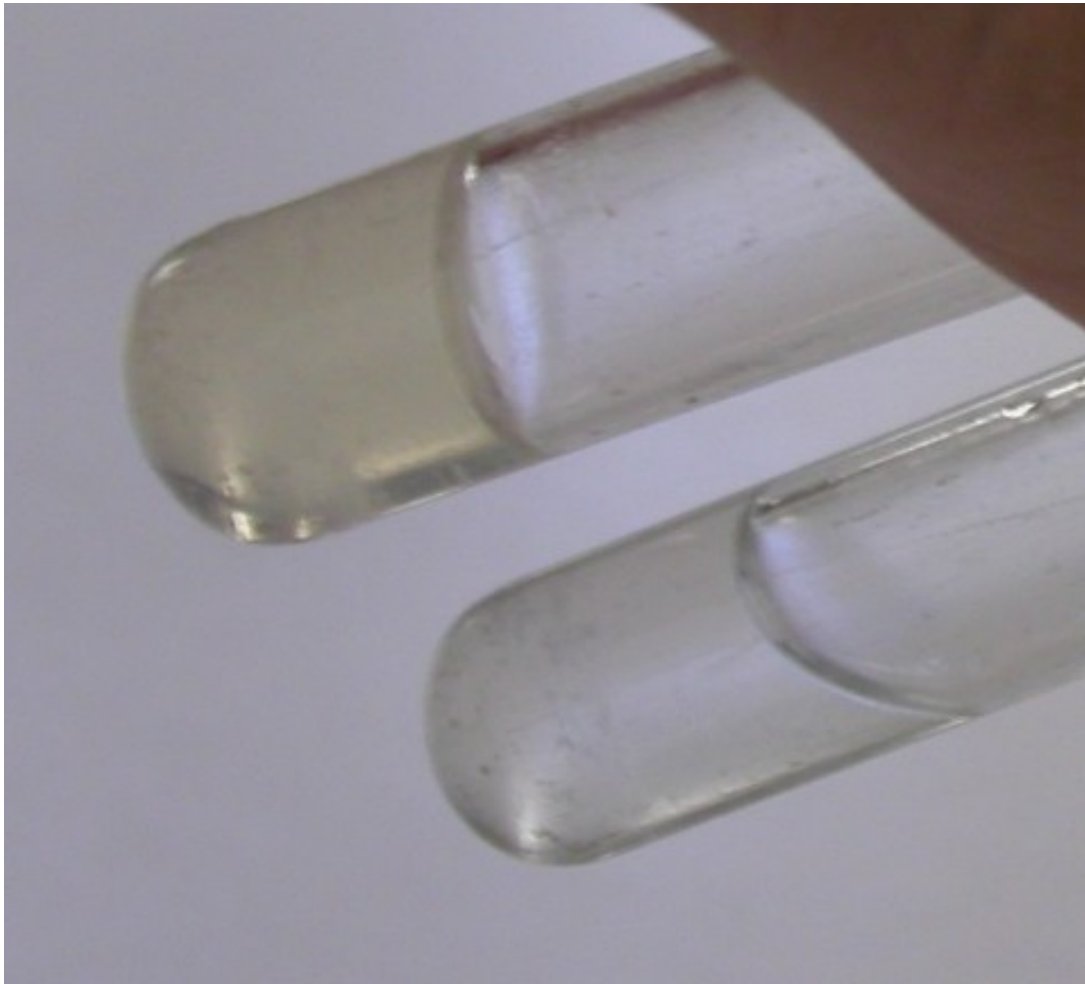
Free coagulase – classic

- 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)

Photo: Microbiology Institute



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**
- **One example is** Staphaurex

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“)

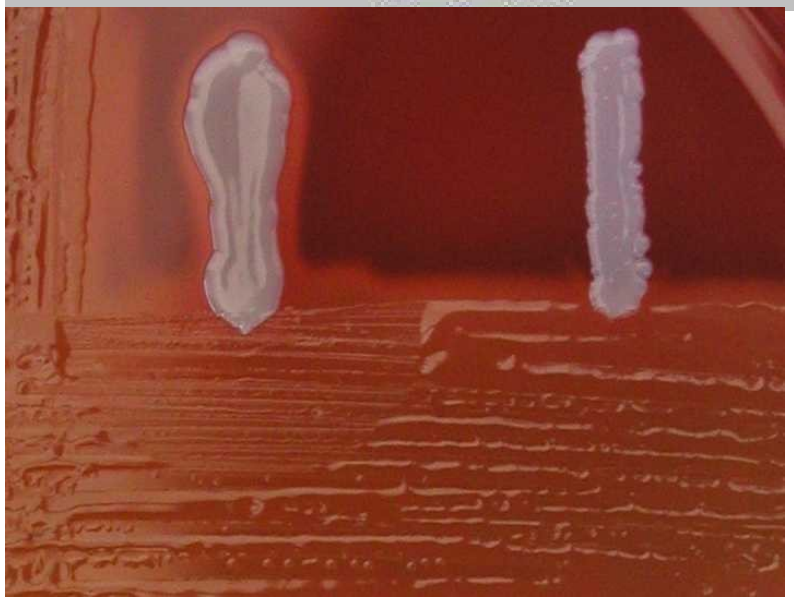
Yellow – *Streptococcus equi* (mucous)

White – tested staphylococci

Results for this example:

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

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



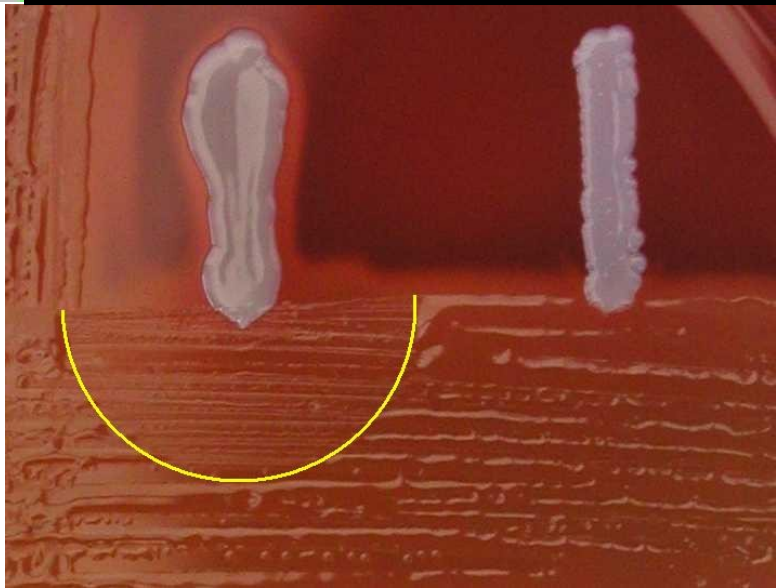


Foto: O. Z.

Foto: O. Z.

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 by several ways
- MALDI-TOF is recently most used way of species identificacion. Nevertheless, we should have alternatives for any case
- STAPHYtest 16 is the most typical Czech variant of a biochemical testing system of staphylococci. It should be done according to guidelines.
- Both MALDI-TOF and STAPHYtest 16 are mostly used for **diagnostics of coagulase negative staphylococci;** nevertheless, both of them can be eventually used for „confirmation“ of diagnostics of *Staphylococcus aureus*, although basis of its diagnostics is still in hyaluronidase or plasmacoagulase tests

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 Tube	2 H	3 G	4 F	5 E	6 D	7 C	8 B	9 A	10 H	11 G	12 F	13 E	14 D	15 C	16 B	17 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 Tube	2 H	3 G	4 F	5 E	6 D	7 C	8 B	9 A	10 H	11 G	12 F	13 E	14 D	15 C	16 B	17 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

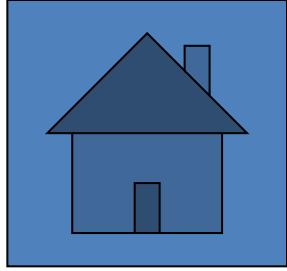
- Not regarding the producer, the principle is the same – **combination of many enzymatic reactions**, that can be seen as **colour change**

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

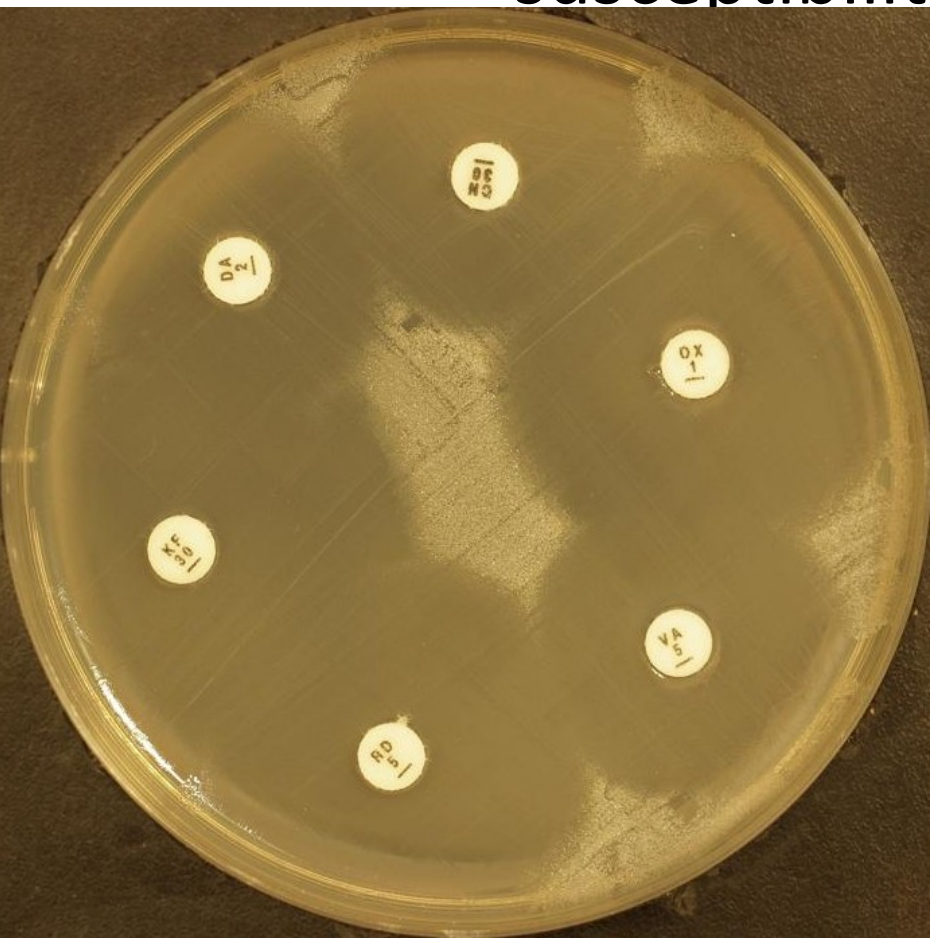
- Drug of choice for *S. aureus* infections is generally **oxacillin**, but there exist exceptions
- **Macrolids** and **tetracyclins** should be used as second choice (for allergic patients).
- **Cephalosporins** of 1st or 2nd generation recomended for UTI rather than oxacillin (that has poor access to the urinary systém)
- **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 ot 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
Chloramphenicole	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)



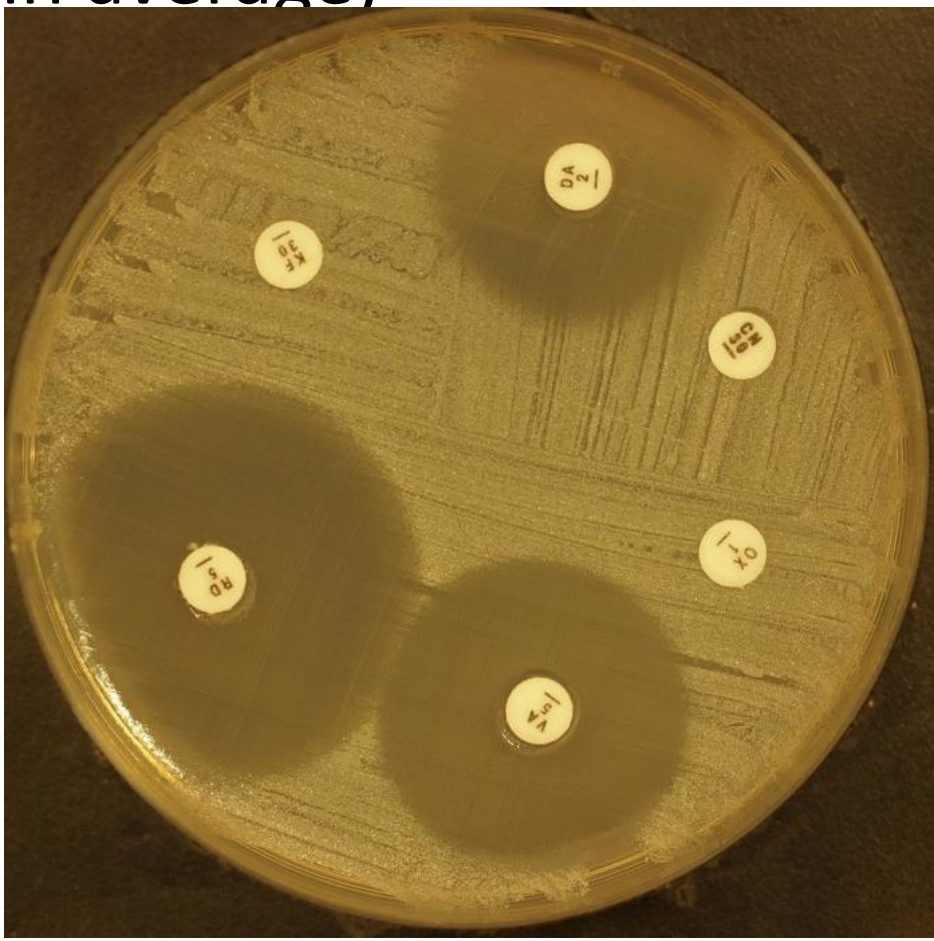


Photo: V. Holá

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

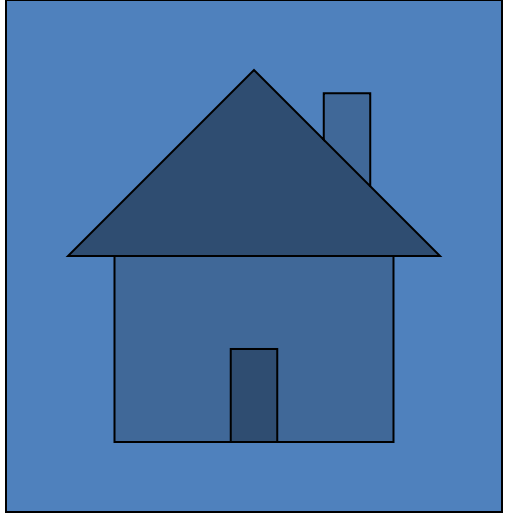


Photo: O. Z.