Institute for microbiology shows:

TRACING THE CRIMINAL

Part Five:

Gram-Negative Criminals II

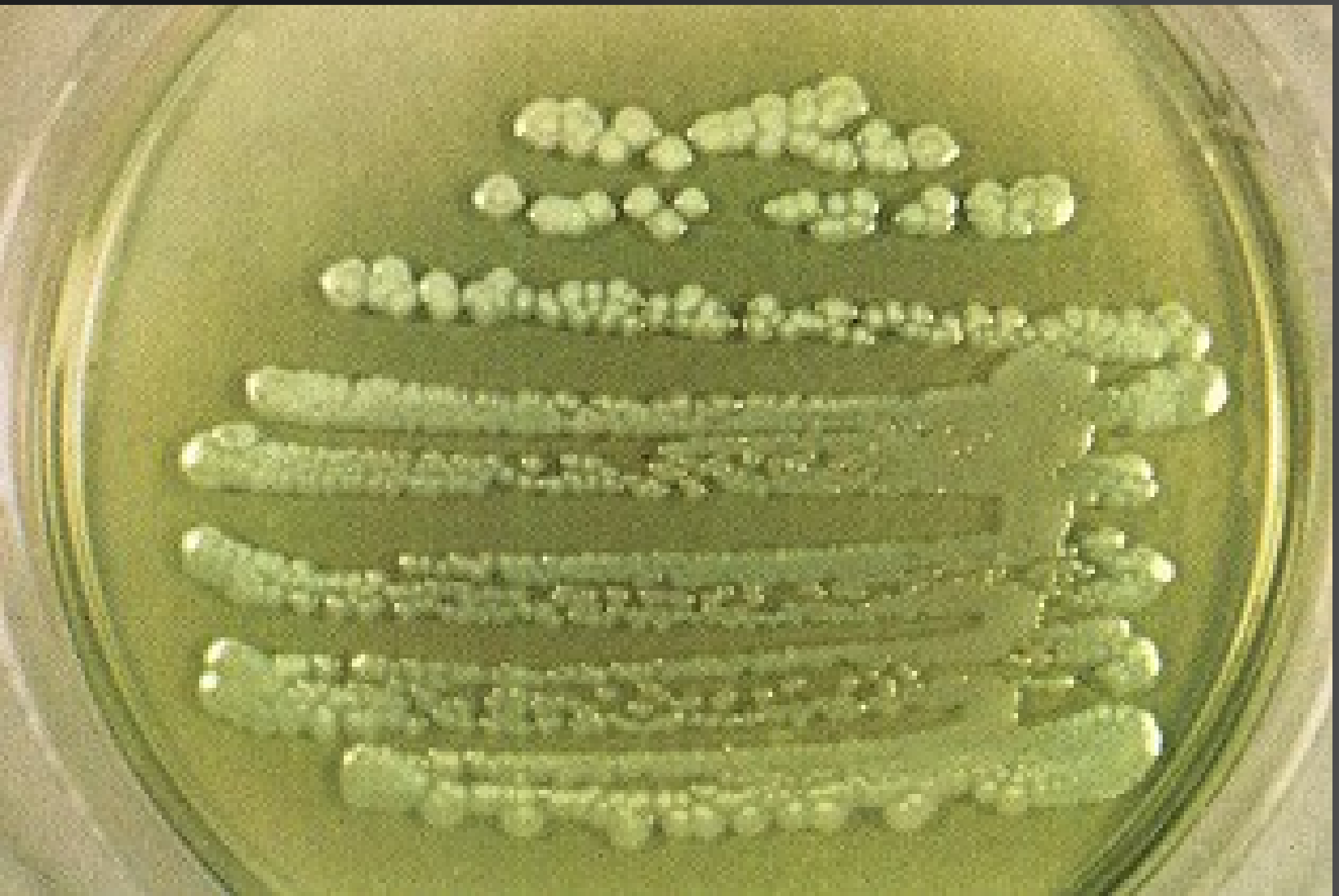
http://medinfo.ufl.edu

Survey of medically important G– rods

Story	Endo	Group
P04	grows	*Enterobacteriaceae* (GLC +, OXI –)
P04	grows	*Vibrionaceae* (GLC +, OXI +)
P04	does not	*Campylobacter* and *Helicobacter*
3. + 4.	grows	G- non-fermenters (GLC –)
1. + 2.	does not	*Pasteurellaceae*
P06	does not	*Legionella, Bordetella, Brucella* etc.

Pseudomonas aeruginosa – a microbiological everGREEN ☺

textbookofbacteriology.net



Survey of topics

Clinical characteristics – *Pasteurellaceae*

Clinical characteristics – G– glucose non-fermenters

Diagnostics of *Pasteurellaceae*

Diagnostics of G– glucose non-fermenters

Clinical characteristictics – *Pasteurellaceae*

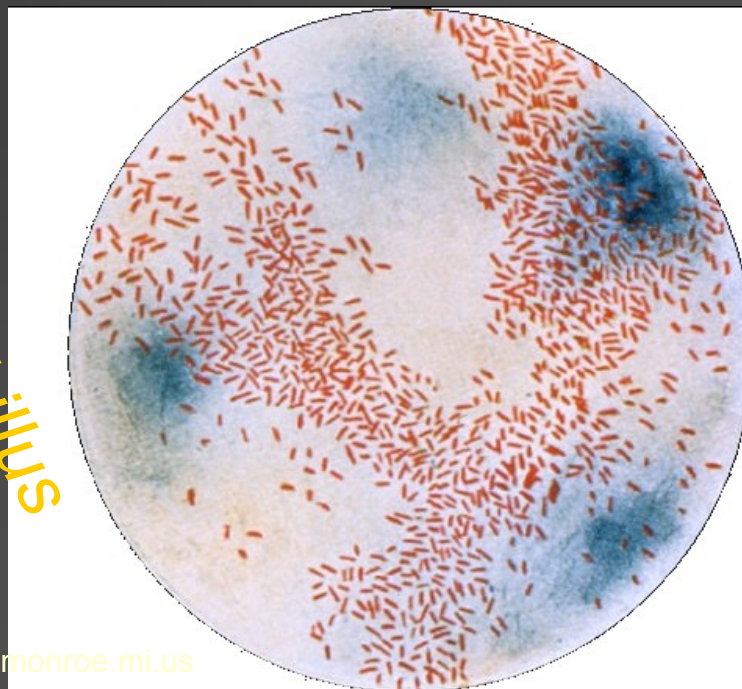
http://www.kinderaerzte-im-netz.de

Story One

- Four-years old Jimmy is a fine boy, but his parents are members of a strange religious society and so they do not wish let him get vaccinated. They would like to keep him at home, but as they have to be at work, they sent him to a nursery.
- After a month Jimmy started to have a cold, difficult breathing, gasping for air, and it become so hard that emergency had to be called. Emergency even thougt about coniotomia, but finally it was not necessary. It was epiglottitis – a disease not too common today…

Who did this to Jimmy?

- Criminal: ***Haemophilus influenzae* ser. b (Hib)**
- Haemophili are **short Gram negative rods**.
- Haemophili belong to the family ***Pasteurellaceae***, together with *Pasteurella* (see later)



Classification of haemophili

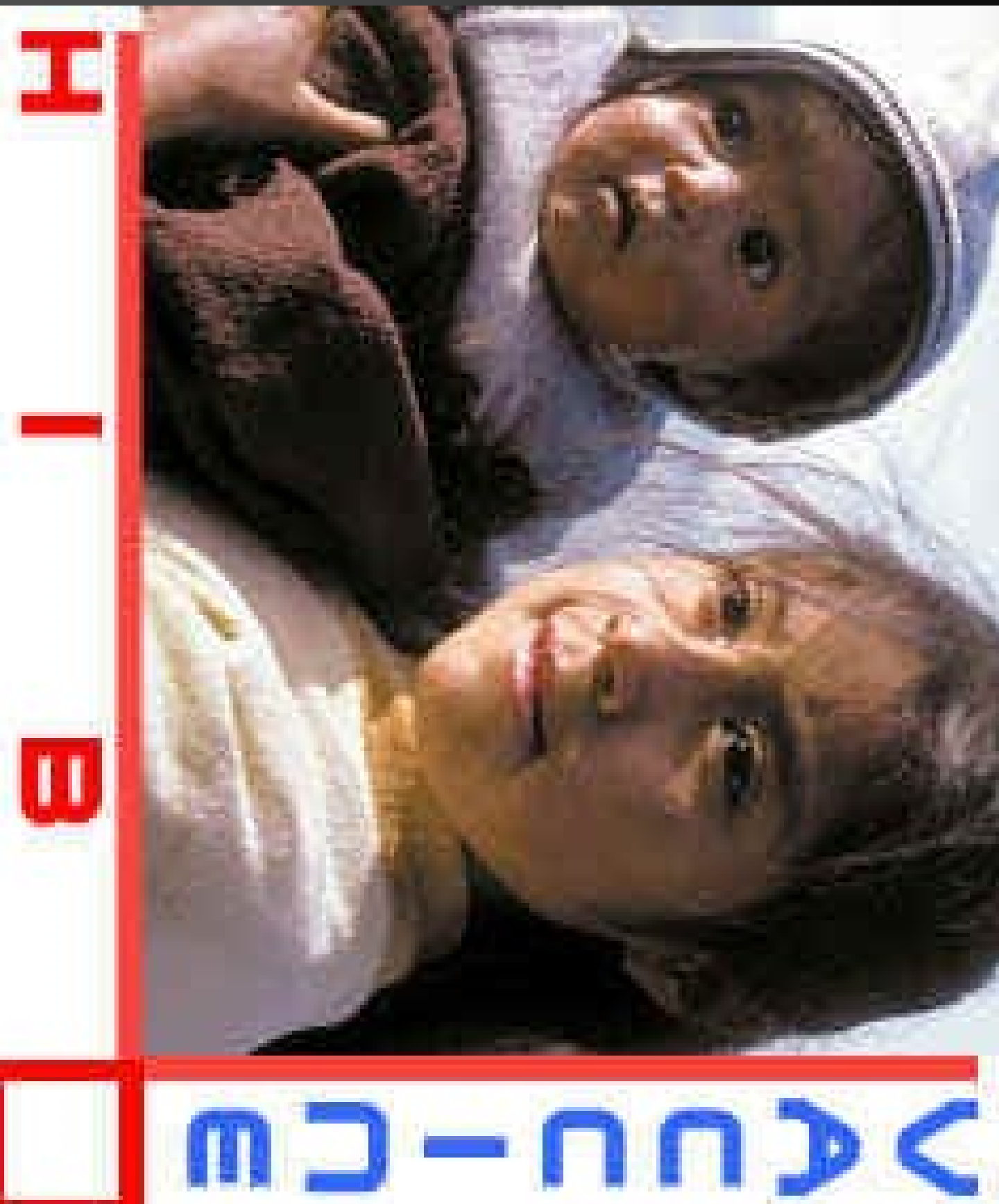
- ***Haemophilus influenzae***
  - **capsullar type b (Hib)** – preventable (vaccine)
  - caspullar types a, c, d, e, f
  - non-encapsulated strains
- ***Haemophilus parainfluenzae*** (much more common, much less pathogenous)
- ***Haemophilus aphrophilus*** and many other species
- ***Haemophilus ducreyi***, causative agent of a sexually transmitted disease **ulcus molle**

Pathogenicity of haemophili

- The most severe diseases caused by haemophili are **epiglottitis, meningitis and sepsis**. This is mostly typical for *Haemophilus influenzae*, serotype b.
- Other common diseases are **otitis media and sinutisitis** (after *Streptococcus pneumoniae* and together with *Moraxella catarrhalis*)
- Their **presence in throat is very common** and their pathogenic role is very query. Especially in case of *Haemophilus parainfluenzae*, we usually do not suppose them to be pathogens.

A *Haemophilus* disease

http://www.immune.org.nz



Ulcus molle

- It is a sexually transmitted disease found mostly in sub-tropical and tropical countries

Ulcus molle – chancroid – caused by *Haemophilus ducreyi*

Ulcus durum – chancre – one of symptoms of syfilis, caused by *Treponema pallidum*

Story Two

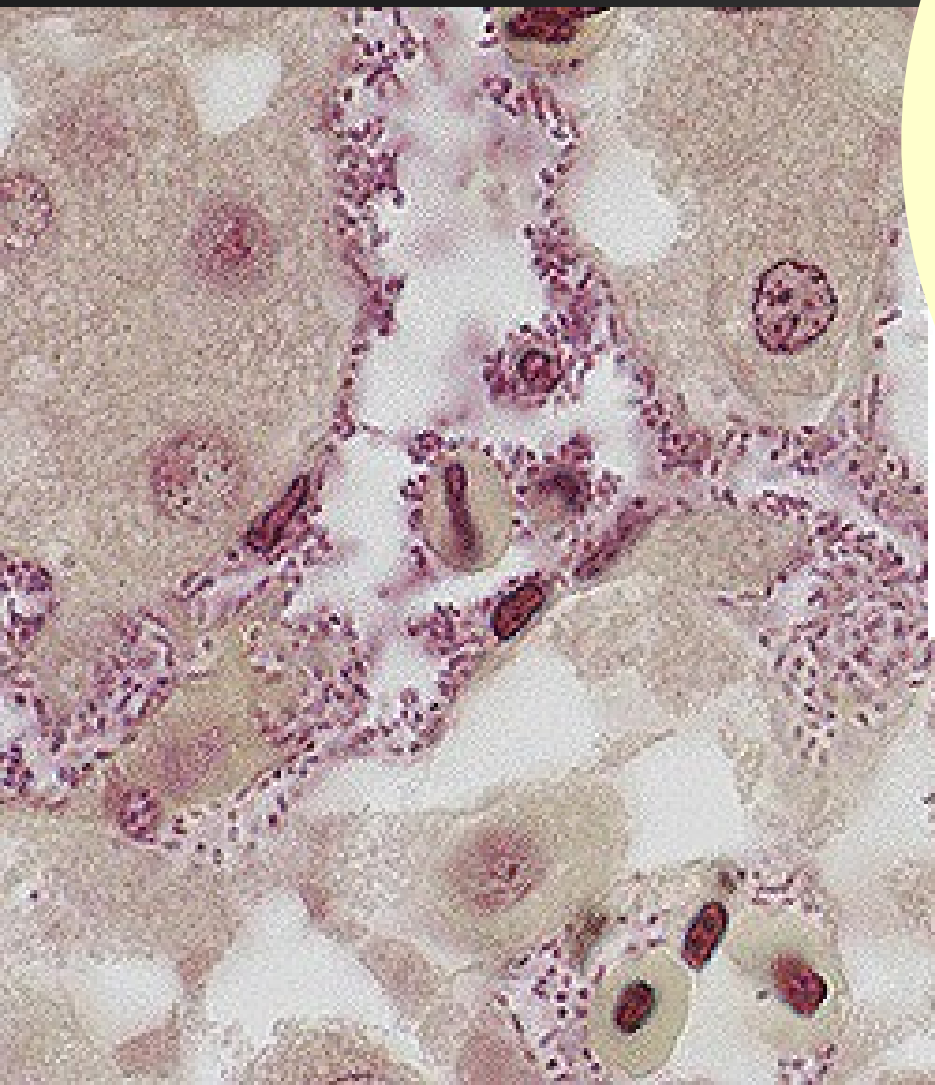
- Joana did walking in gardens as usually. Unfortunatelly, one garden fence was too old and rotten and the dog inside too strong. The dog run out and just met Joana. So, Joana was bitten into her leg.
- The owners of the dog had proven that the dog was vaccinated against rabies. Nevertheless, some pus was found soon in the wound. So the pus was sent to the laboratory. And the criminal was…

Pasteurella multocida

- *Pasteurella multocida* is common flora in dog chaps.
- In humans, it causes mainly pyogene wound inflamations after being bitten by a dog or another animal.
- It smells simillarly as *Haemophillus* (some people say „like old rag“, but unlike *Haemophilus*, it grows on blood agar (but not Endo agar).
- The morphology is something between *Streptococcus* and *Enterococcus*, but it is Vancomycin resistant and this gives a suspicion to the microbiologist (especially at parallel susceptibility to penicillin)

Pasteurella multocida

http://library.thinkquest.org



Clinical characteristictics – Gram– glucose non-fermenters

http://faculty.plattsburgh.edu

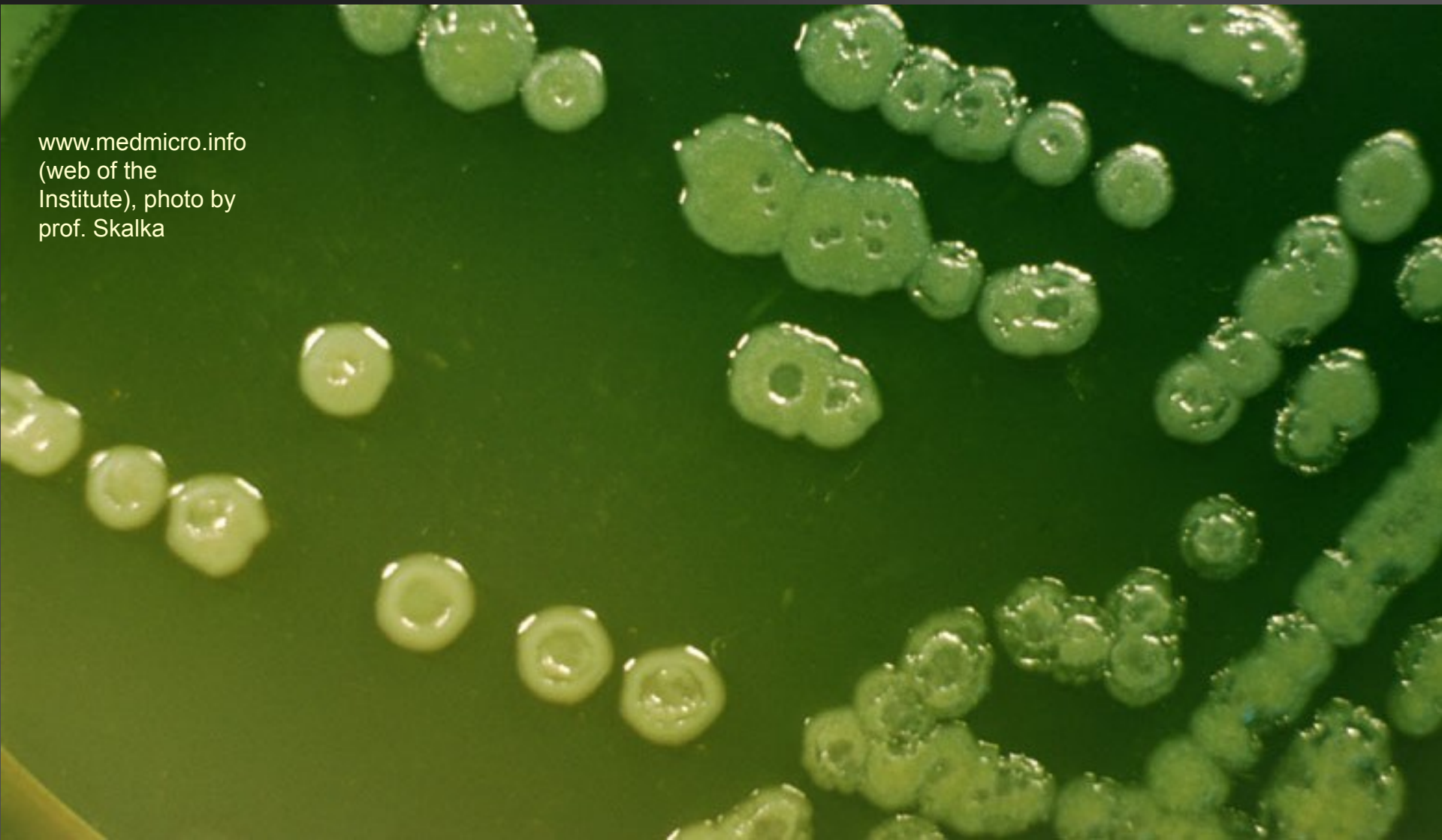
Story Three

- Mr. Phosphoros is a pyroman. Several days ago, he burned himself. Now, his burn is inflamated. He is hospitalised on a specialized centre and feels very badly. Doctors knew that it has no sense to try antibiotics accidentaly, so they performed a swab. Thanks to this, a target therapy was found, and Mr. Phosphoros healed. Of course, only temporarily: sooner or later, he will probably play with his matches again *(like some students of the practical)*.

Who is guilty this time?

- It is *Pseudomonas aeruginosa*, the most common so named „Gram– non-fermenters“ (G– NF)
- On the other hand, the guilty one could be any of that group, e. g. *Acinetobacter*, *Burkholderia cepacia* or *Stenotrophomonas maltophilia*
- Those bacteria are mostly strict aerobes, instead of fermentation sugars, they breakdown them by aerobic respiration, and their adaptation to outer environment is clear also of other properties – they use to have low temperature optimum and they are often pigmented, so they fight with sun in outer environment

Green pigmented strain of *Pseudomonas aeruginosa* on MH

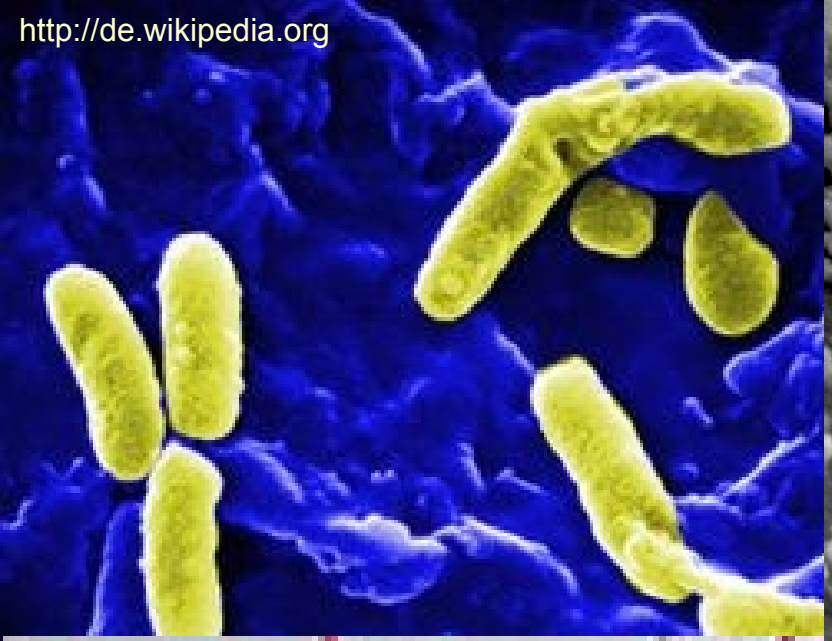


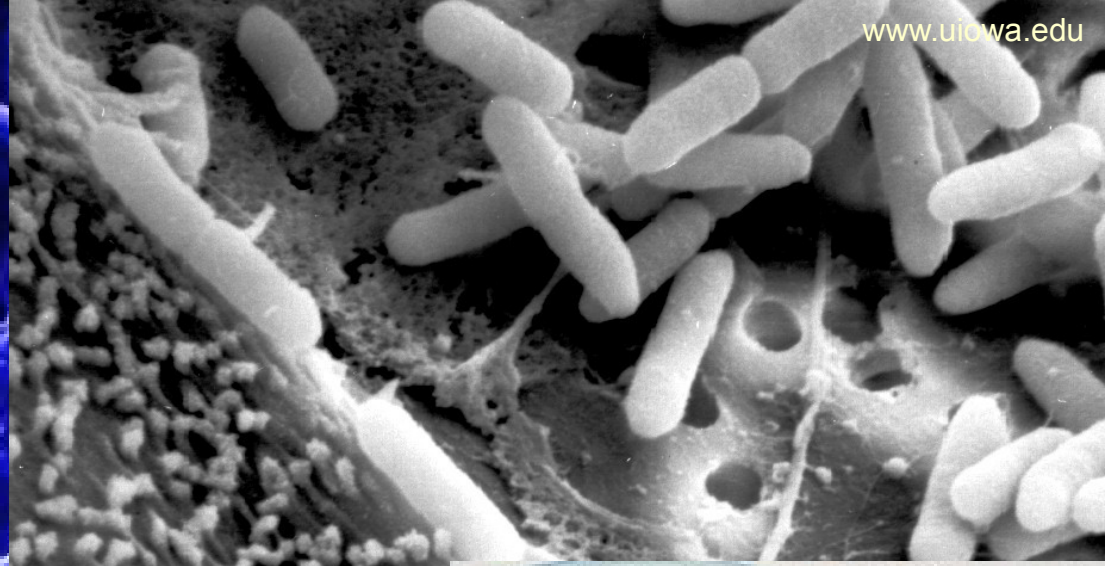
www.medmicro.info (web of the Institute), photo by prof. Skalka

Another picture of *Pseudomonas aeruginosa*

www.medmicro.info (web of the Institute)

photo by prof. Skalka


http://de.wikipedia.org


www.uiowa.edu


textbookofbacteriology.net


textbookofbacteriology.net
Exceptional
Pseudomonas
strain with blue
pigmentation

Pathogenicity of G– NF

- Commonly: they are bacteria from outer environment, often plant pathogens, „not-brave-bacteria“, which are not able to infect a healthy person. Their target are patients with burns, clients of emergency units, transplant centers, e. t. c.
- In hospitalized persons they often cause not only wound infections, but we find them also in respiratory ways, and even in the bloodstream.
- So they are important agents of nosocomial infections
- Sometimes it is difficult to differenciate between an infection and a colonisation – especially in superficial wounds it is often useless to use other than topical antibiotics at finding of some of those bacteria

In disabled persons, they can cause even such problems as a nail inflamation

www.kvarts.is

Dr. Zahradníček‘s autocauistics

- Friday, 13th January 2006: fall into a not-covered canalisation hole in the city of Padang, West Sumatera, Indonesia. Quite large wound, reaching tibial periost
- Some three weeks later, the wound started to smell like *Pseudomonas*, and really, this bacterium was sucsesfully cultured from it.
- Dr. Zahradníček decided for local treatment (gentamicin + polymyxine) – in such wound infections topic terapy uses to be more important than systemic treatmend (also because of likely presence of wound biofilm)
- The therapy was successfull

Padang

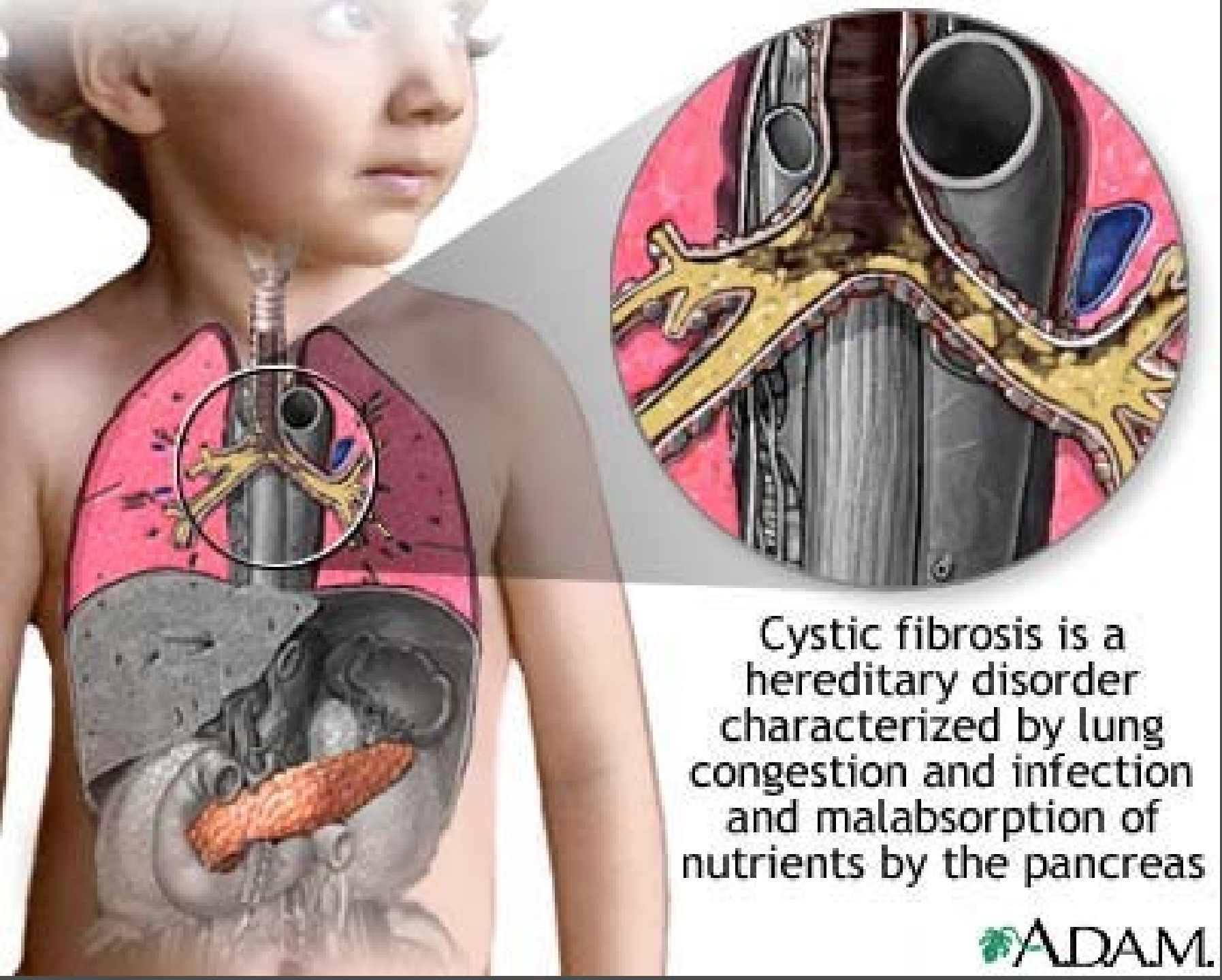
Photo O. Z.

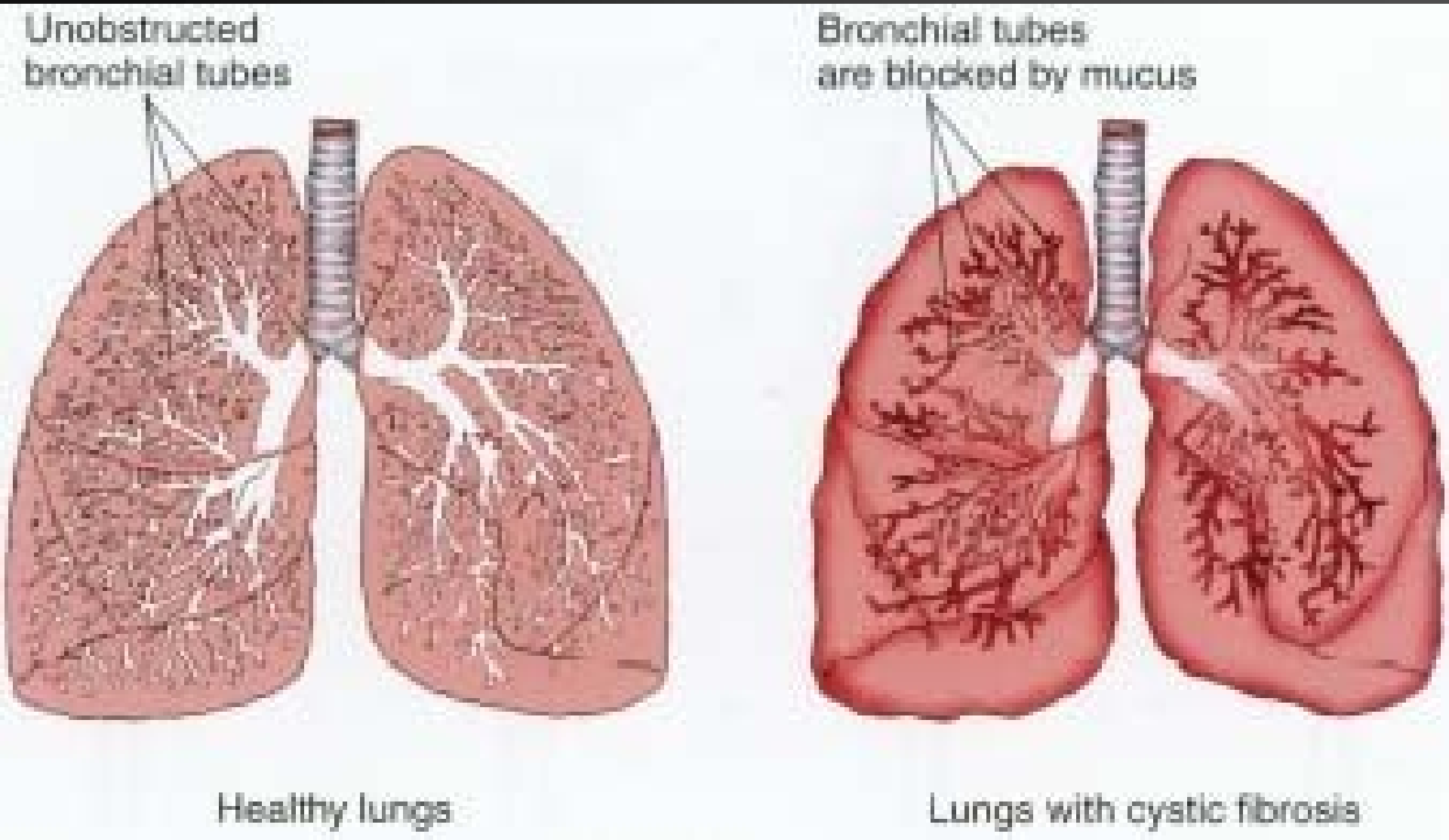
Story Four

- Linda was a poor girl: she suffered because of **an inborne disease, cystic fibrosis.**
- Her **lung surfactant was different from surfactant of healthy people**. So, he was often infected.
- Last time it was *Staphylococcus aureus*. This time it was different: **the causative agent was *Burkholderia cepacia***, one of G– non-fermenters.

Non-fermenters and Cystic fibrosis

- Cystic fibrosis is a severe, **inborn lung disease**, with failure of production of normal lung surfactant. This leads to changed characteristics of lungs, including many times increased risk of infection
- **Most common causative agents** are *Pseudomonas aeruginosa, Burkolderia cepacia* and *Staphylococcus aureus*. Strains often become **polyresistant** and many children with cytic fibrosis die very young.





http://www.humanillnesses.com

More Gram non-fermenters: *Pseudomonas fluorescens*

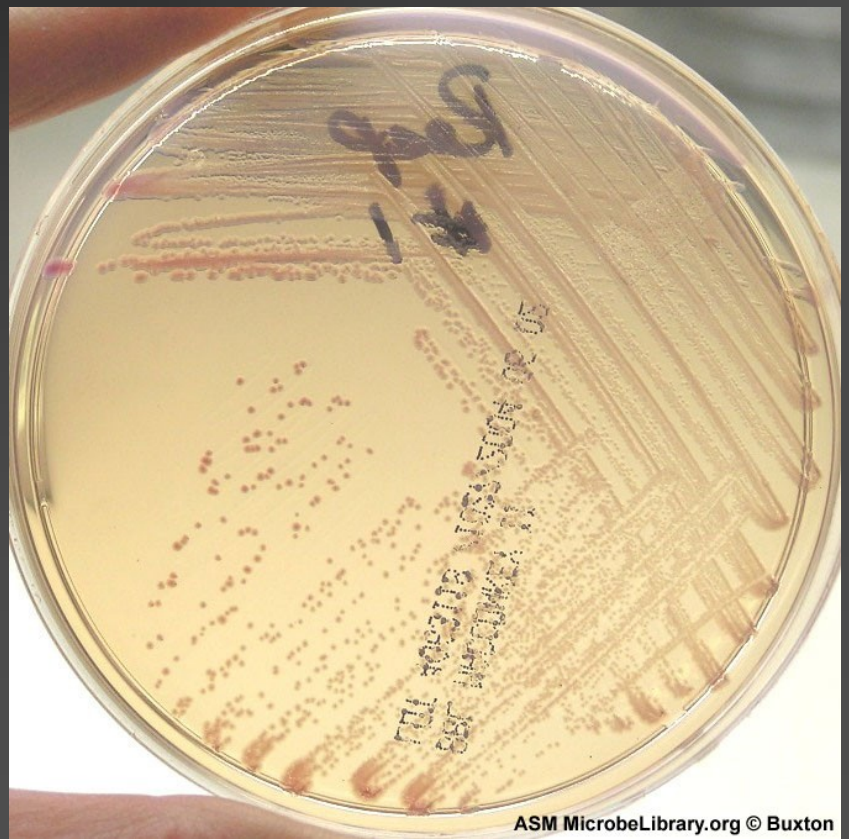
P. fluorescens is very simillar to *P. aeruginosa,* but under UV-lamp, fluorescence occurs

http://www.bact.wisc.edu

http://www.apsnet.org

Burkholderia cepacia

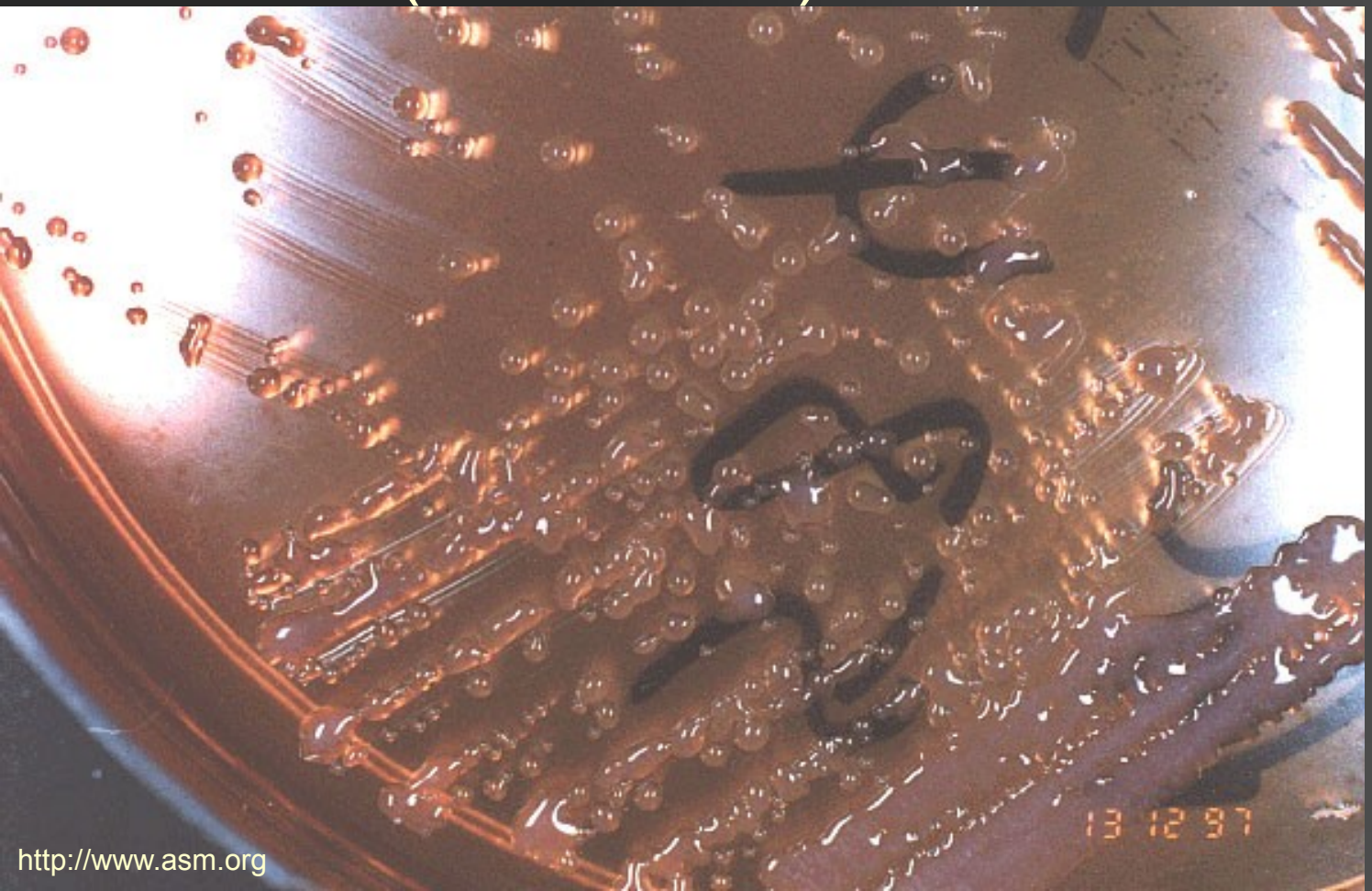
Burkholderia cepacia is responsible for rotten onions (*Allium cepa*), so it is really a typical plant pathogen



http://www.microbelibrary.org

Burkholderia pseudomallei

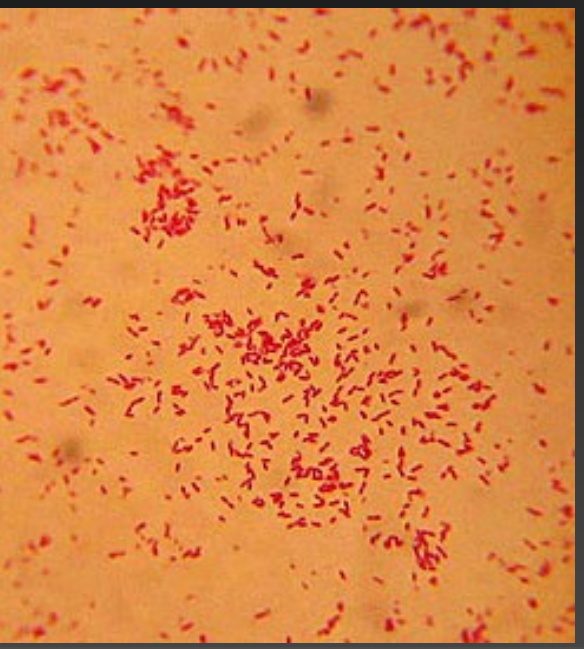
Burkholderia pseudomallei is causative agent of mellioidosis. Related B. *mallei* is causative agent of malleus (a zoonosis)



http://www.asm.org

Stenotrophomonas maltophilia

http://www.scielo.cl

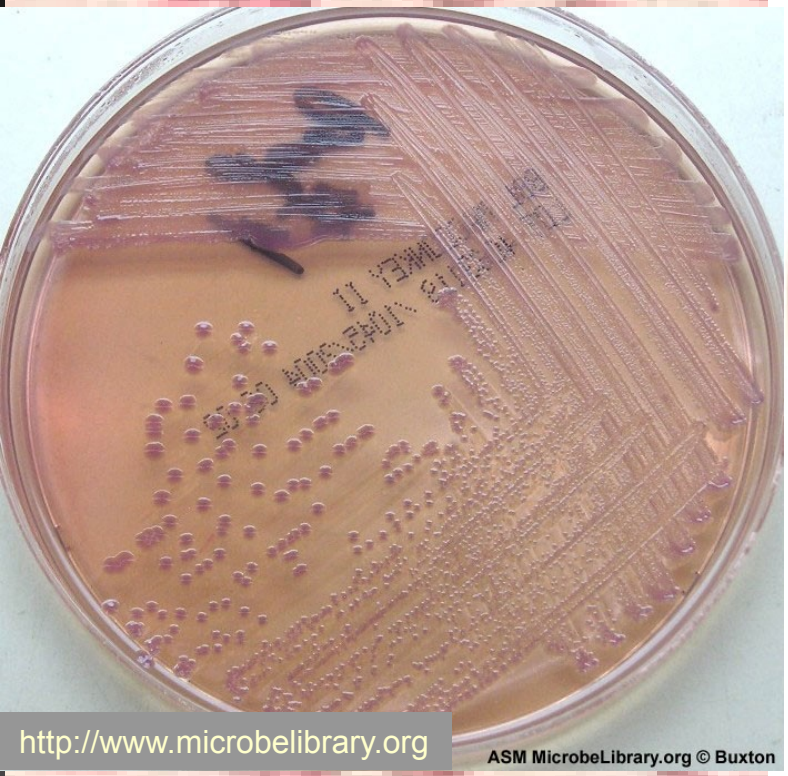


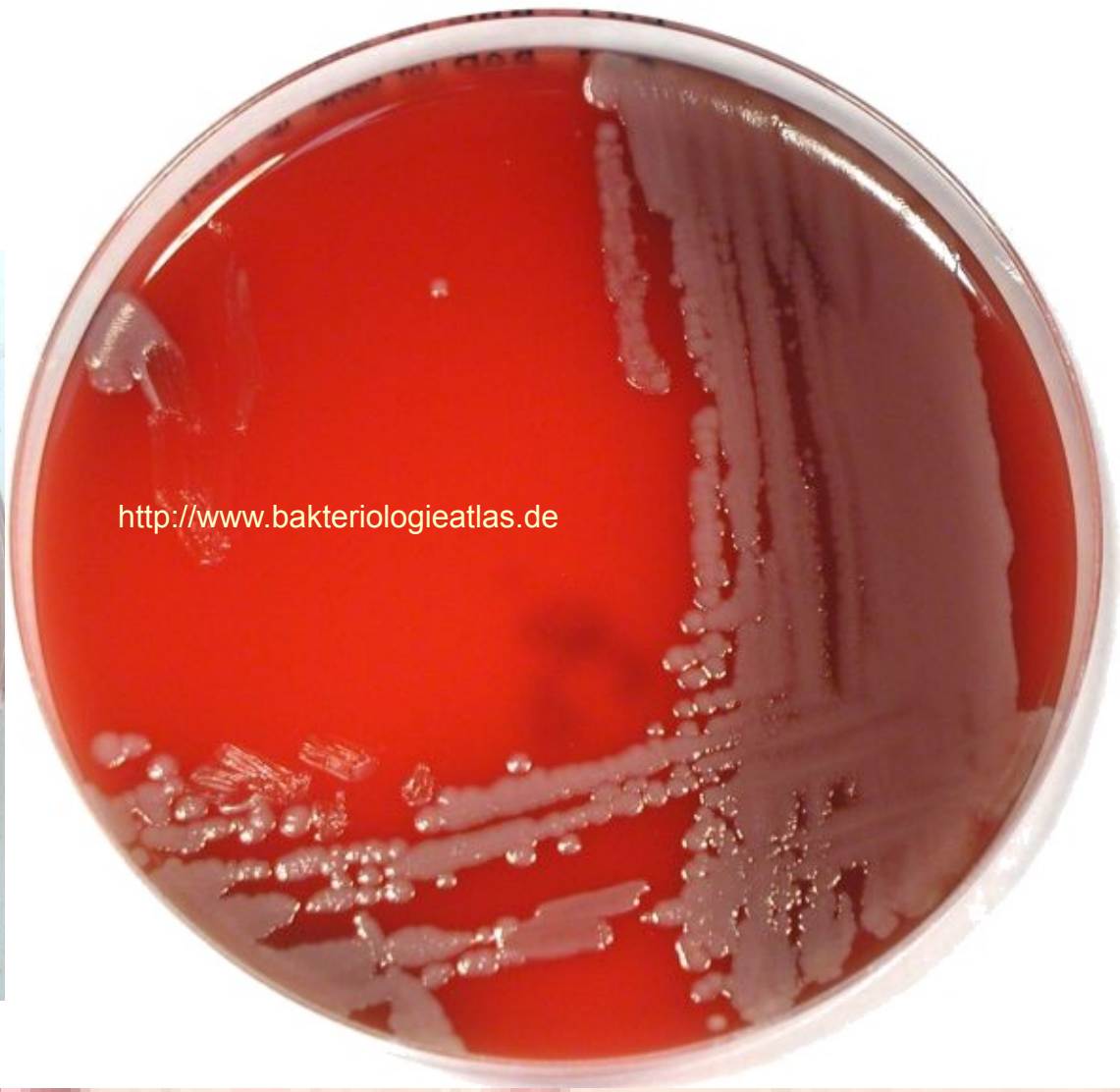
http://clinicalmicrobiology.stanford.edu

http://www.microbelibrary.org

Stenotrophomonas maltophilia is a long name, but it is possible to learn it easily: it is narrow-nutrition-unit maltose-loving, so it is a „bacterial panda“, chewing maltose instead of bamboo ☺.

Acinetobacter





http://www.buddycom.com

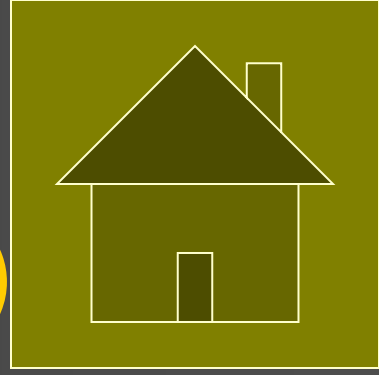
Greek: a-kineto- = „non motile“

Bacterial metabolism and relation of bacteria to oxygen

We know already that G– non-fermenters are bacteria that do not ferment sugars, but performs aerobic respiration. Let‘s compare now two bacteria:

- *Escherichia coli* lives in the intestine. It has enough nutrients, but not enough oxygen (unlike other gases ☺) preferes glucose (and other substrates) fermentation. *Escherichia coli* is a **facultative anaerobe**. Some other intestinal bacteria are **strict anaerobes**.
- On the other hand, *Pseudomonas* has oxygen enough, but nutrients not enough. It uses aerobic respiration: enables better exploitation of nutrients. *Pseudomonas* is a **strict aerobe**.

Pseudomonas as a strict aerobe (unlike other bacteria)



- Unlike strain I (*Escherichia coli*) and strain II (*Bacterioides fragilis*, a strict anaerobe), *Pseudomonas aeruginosa* (strain III) is a strictly aerobic bacterium (*Bacteroides fragilis*, more in P07)

Strain	Broth	VL-broth	Result
III	growth	clear	Strictly aerobic bacterium
II	clear	growth	Strict anaerobe
I	growth	growth	Facultative anaerobe

Diagnostics of *Pasteurellaceae*

Survey of methods in *Pasteurellaceae* diagnostics

- Direct methods
  - Microscopy – short G– rods
  - Culture – *Pasteurellaceae* do not grow on Endo agar, *Haemophilus* even does not grow on Blood agar (except being co-cultivated with another microbe)
  - Biochemical identification – it is possible to use it
  - Antigen analysis – used in haemophili (Hib)
  - Nucleic acid detection – not used routinely

Indirect methods used rarely

Differentiation of *Pasteurellaceae* (differential diagnostics)

- Gram staining: Gram– rods × other bacteria
- Endo medium: as we now, among clinically important bacteria, only *Enterobacteriaceae*, *Vibrionaceae* and Gram– non-fermenters are able to grow. ***Pasteurellaceae* do not grow**.
- *Pasteurellaceae* are detected by typical smell, biochemical properties, growth on individual media, typical antibiotic susceptibility etc.

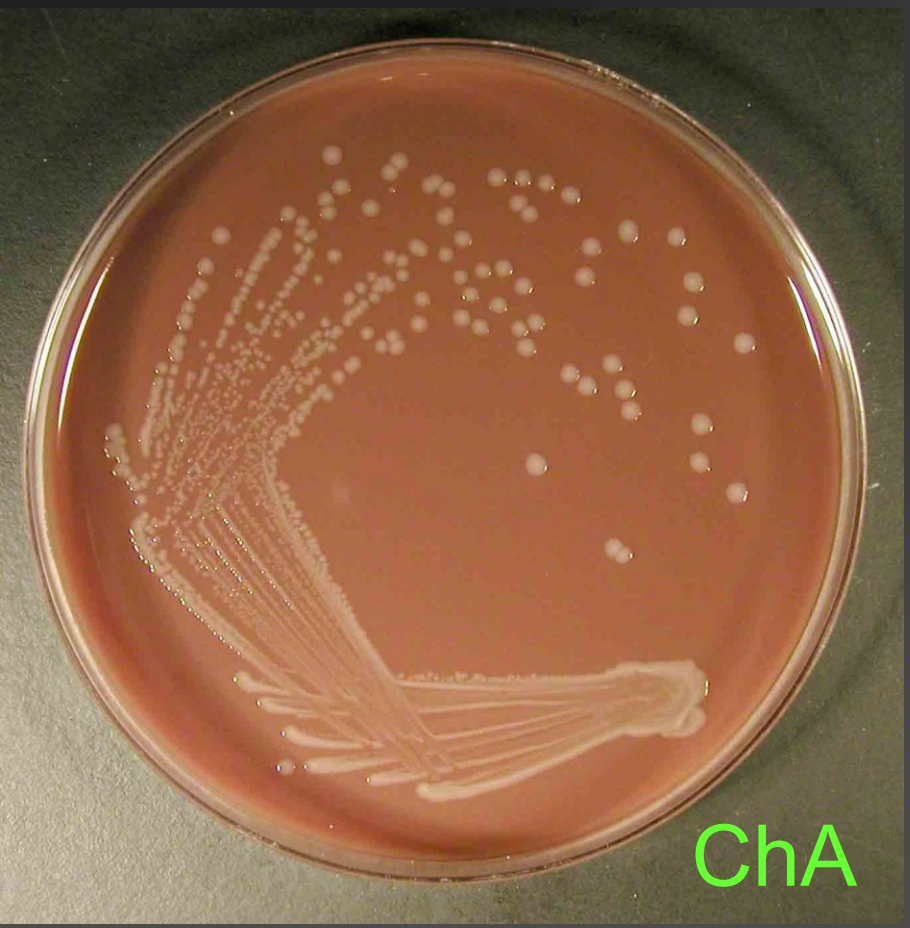
To *Haemophilus* and *Pasteurella* diagnostics

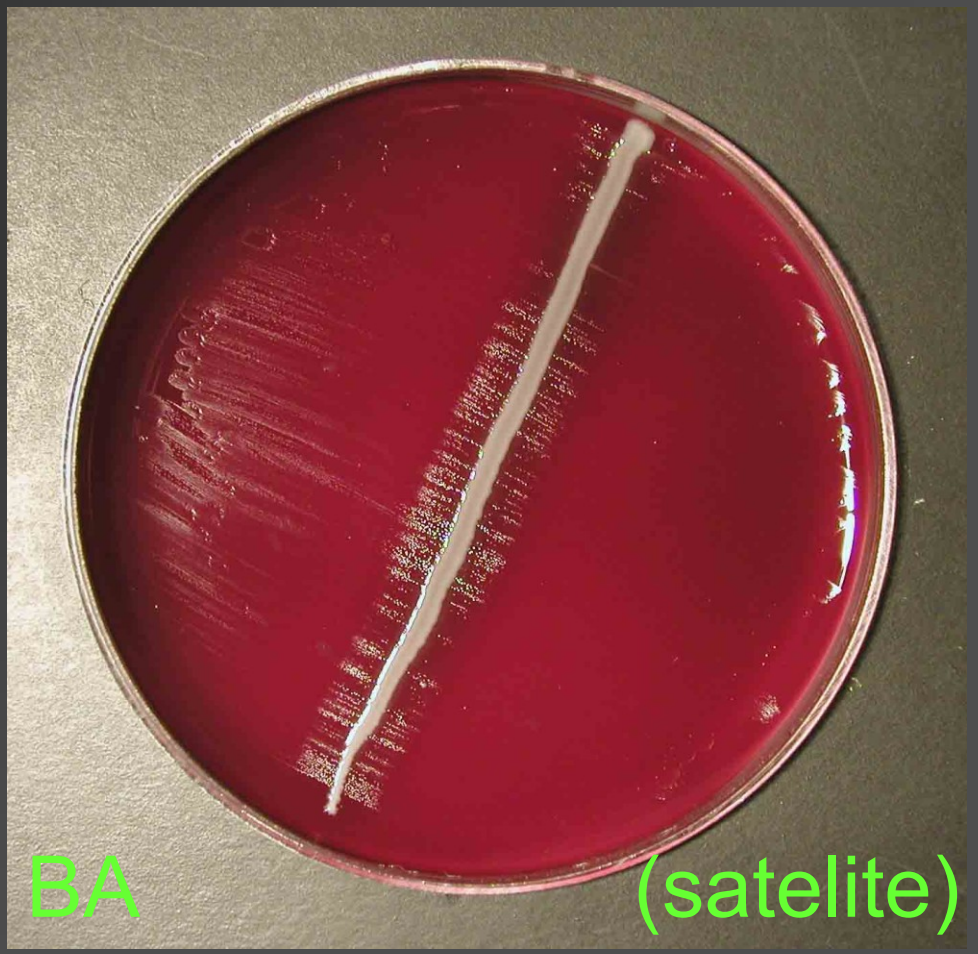
- Pasteurella is able to grow on on blood agar
- Haemophili are not able to grow on blood agar, they are not able to „open the RBC“. So, they grow on chocolate agar or Levinthal agar (filtrated chocolate agar)
- On BA, they are able to grow, if a bacterium that „opens the RBC“ is present (satelite phenomenon). Such bacterium is e. g. golden *Staphylococcus*
- They grow in tiny colonies, so we use a disc to disable growth of other bacteria (bacitracin, but in higher concentration than in bacitracin test)

Satelite phenomenon

- As we know already, haemophilli need factors from RBC, but they are not able to break an RBC themselves. They need the RBCs to be broken
  - by heating – chocolate agar
  - by presence of another microbe
- Satelite phenomenon is an example of the second way how to make haemophilli be able to exploit blood factors. That means the growth of *Haemophilus* around *Staphylococcus* line only.
- Presence of satelite phenomenon is a confirmation, that our bacterium is really a *Haemophilus*

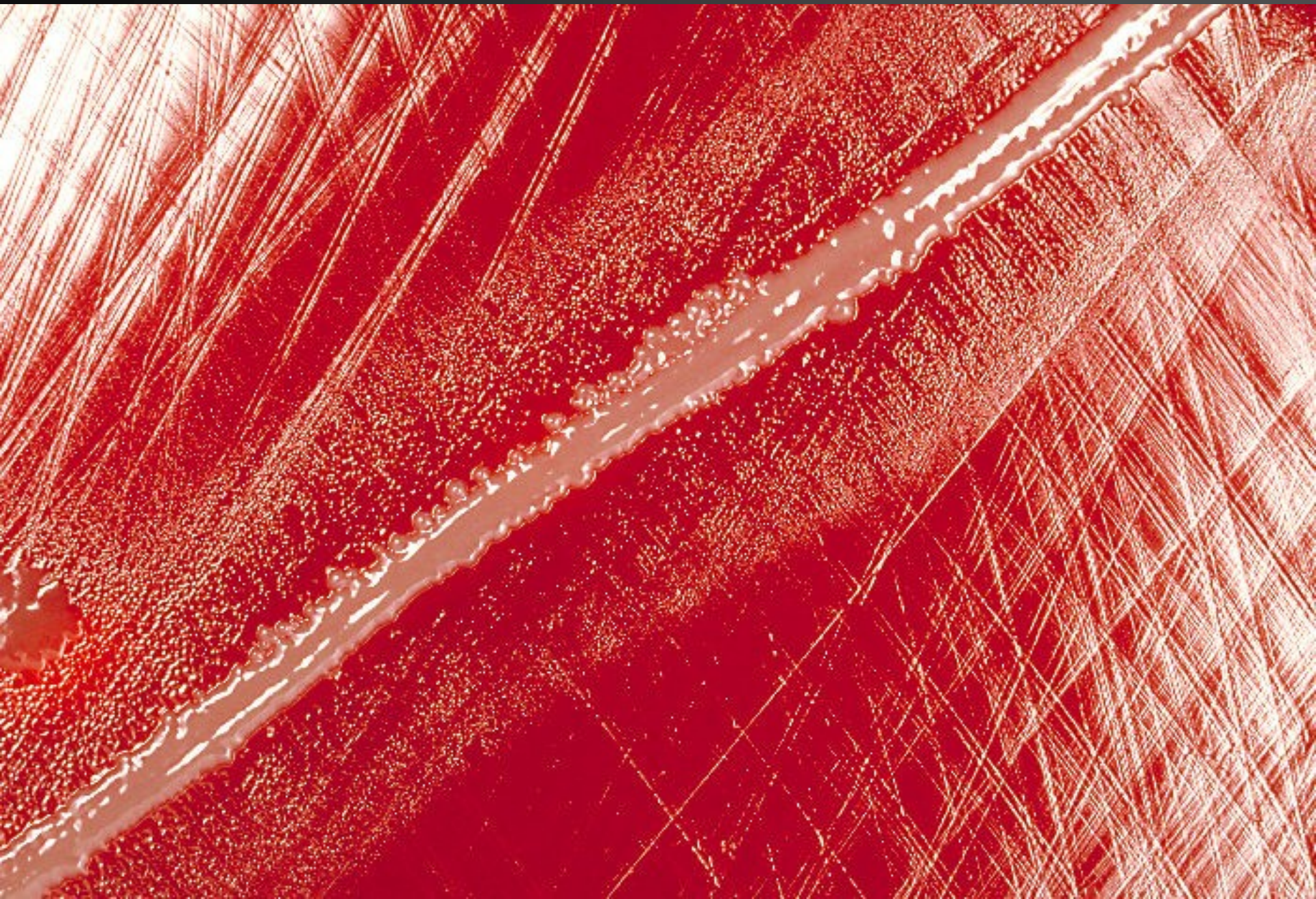
Haemophili on chocolate agar (left) and as a satelite on blood agar





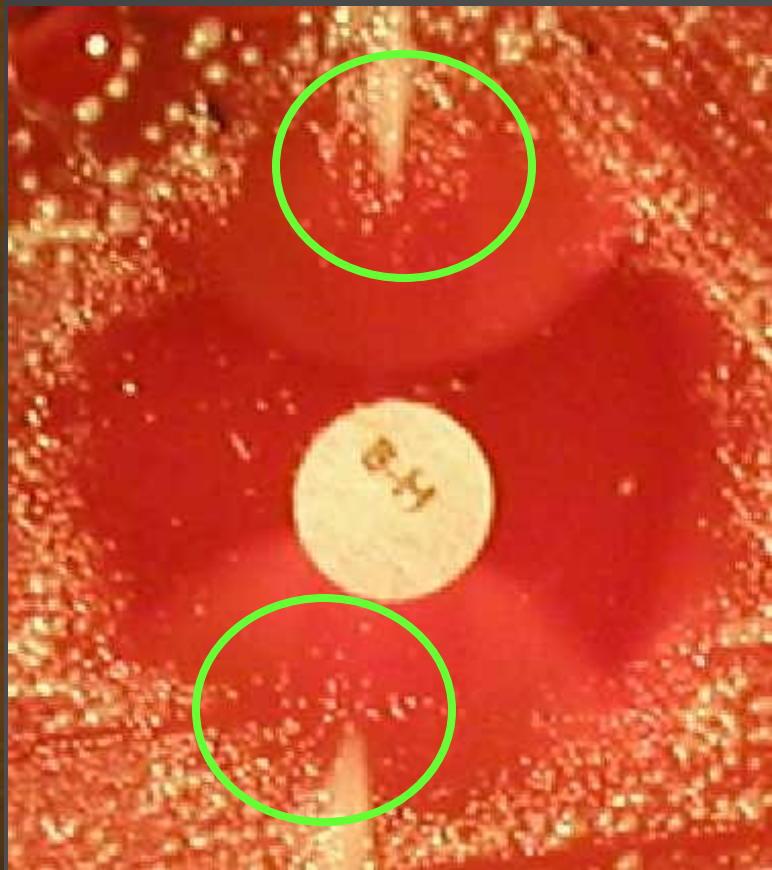
Satelite once more

http://phil.cdc.gov



Detection of haemophili

Haemophili are more resistant than the bacteria of the common flora, so they grow inside the zone, but only around staphylococcus line (satelite phenomenon)



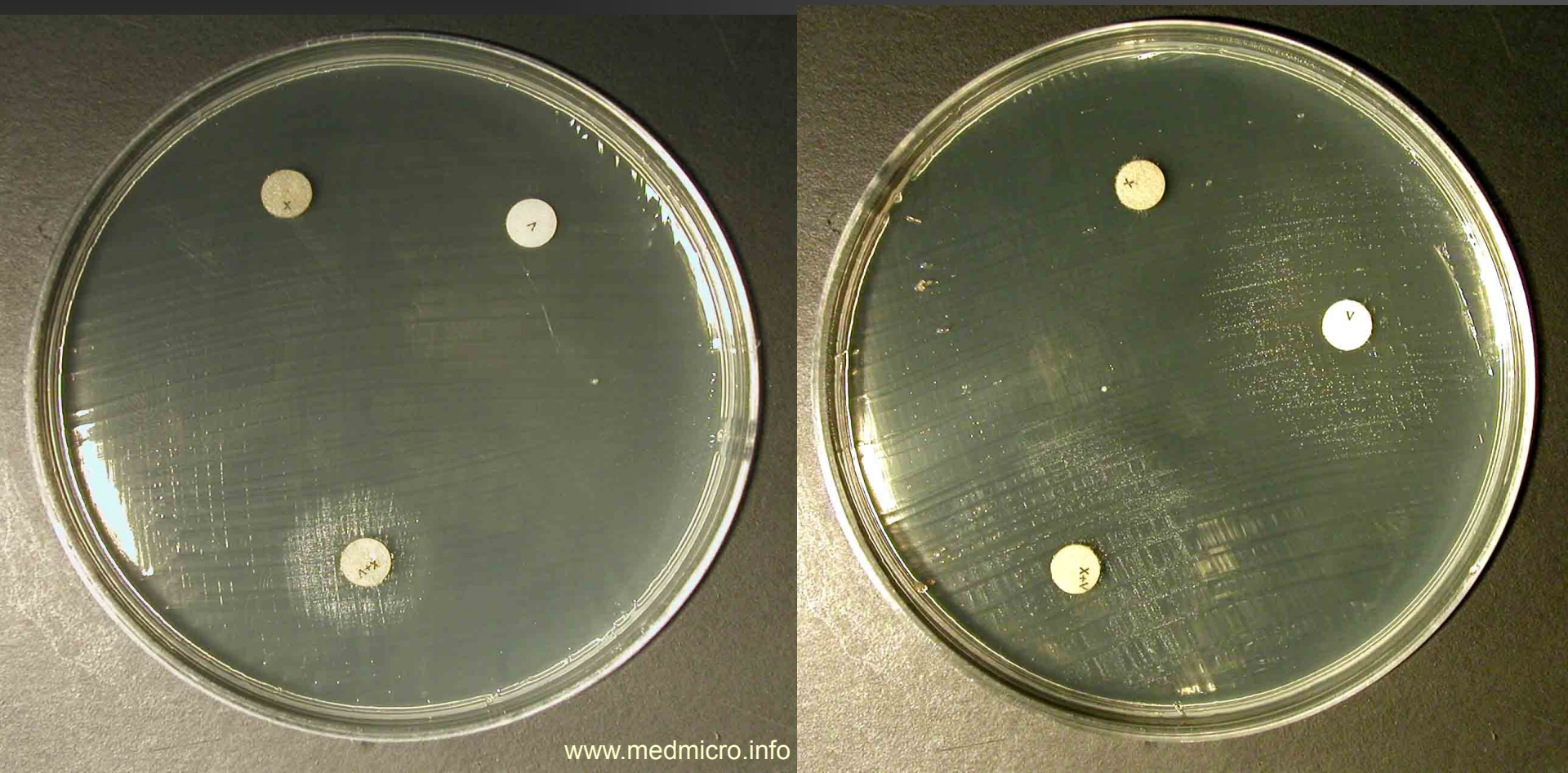
Growth factors of Hemophili (= determination of individual species)

- Haemophilli need factors from blood, but the need of individual factors is species specific.
  - *H. parainfluenzae* needs factor V (= NAD)
  - *H. aphrophilus* needs factor X (= hemin)
  - *H. influenzae* needs both factors.
- We use discs with these factors: one with X, another with V, and the third with a mixture of both of them.

Growth factor test of *Hemophili*

One disk is with factor X, second with factor V, third a mixture

H. influenzae (left), *H. parainfluenzae* (right)

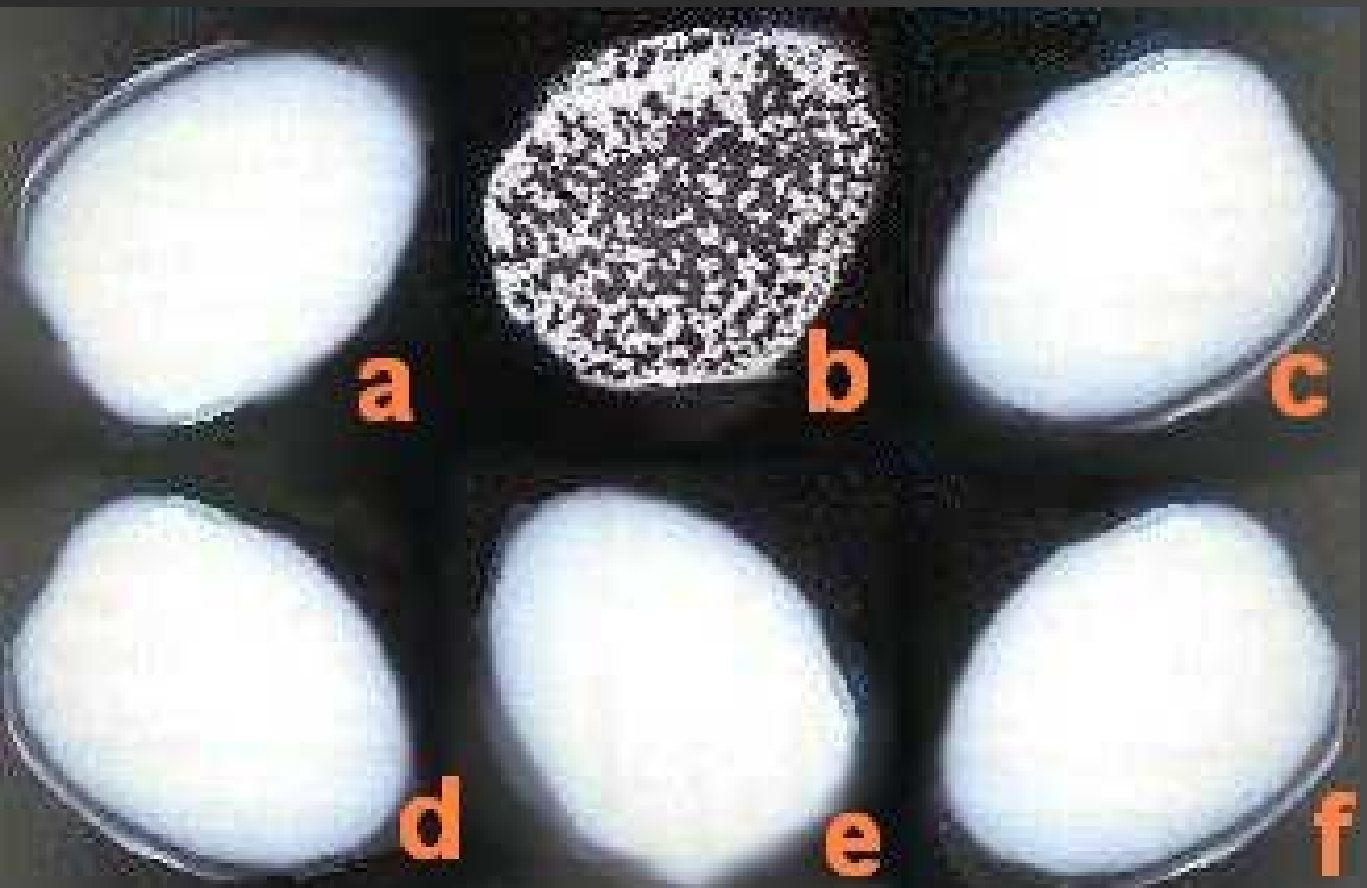


Haemophillus influenzae: antigen analysis (intra-species diagnostics

- Antigen analysis in *Hemophillus influenzae* is performed like in other bacteria. The main goal is diferenciation of Hib. Today, we have comercionally available sets, containing e. g. latex particles. We try to assess the capsular type of H. influenzae (a, b, c, d, e, or f). When the strain does not agglutinate with any sera, it is probably an un-encapsulated strain
- Formerly, so named co-agglutination with *Staphylococcus* strain was used: agglutinate was more dense because of *Staphylococcus* binding the Fc-end of anti-haemophilus antibody

Antigen analysis of *H. influenzae*: an example of the result

The agglutination results for haemophili are observed simillarly as other agglutination reactions



collage with use of:
www.microbes-edu.org

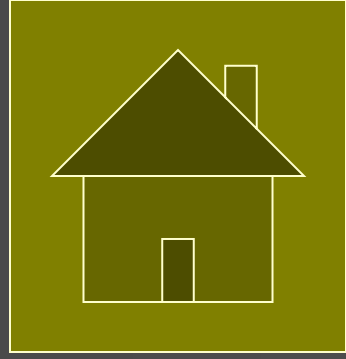
Detection of *Pasteurella* using typical susceptibility pattern

- **No Gram-negative bacterium is susceptible to vancomycin.** Vancomycin can be used for Gram-positive bacteria only, but here it is very strong; all streptococci and majority of staphylococci and enterococci are susceptible
- On the other hand, **very little bacteria are susceptible to penicillin, escepically among G– bacilli.**
- **So, susceptibility to penicillin and resistance to vancomycin is quite typical for *Pasteurella*.**

Tests of atb susceptibility

- Haemophilli do not grow on MH agar
- Usually Levinthal agar (fitrated chocolate agar), is used for diffusion disc test – for this purpose, this agar is better than classical chocolate agar
- Our laboratory uses „Haemophilus agar“, similar to Levinthal agar
- Reading of the zones is the same as for any other bacteria

Antibiotic susceptibility testing: An example of *Pasteurellaceae* antibiotic set



Antibiotikum	Abbrev.	Reference zone
Ampicilin (aminopeniciline)	AMP	22 mm
Co-amoxicilin (am.+inhib.)	AMC	18 mm
Chloramphenicol	C	29 mm
Doxycycline (tetracycline)	DO	29 mm
Co-trimoxazol (mixure)	SXT	16 mm
Azithromycin (macrolid)	AZM	12 mm

Diagnostics of Gram–non-fermenters

Survey of methods for G– non-fermenters

- Direct methods
  - Microscopy – mostly G– rods, but *Acinetobacter* is a G– coccus
  - Culture – non-fermenters grow on majority of media, including Endo agar. As glucose-non fermenters, they are mostly also lactose-non fermenters, but their colonies are sometimes quite dark, because of pigmenation
  - Biochemical identification – possible, but tests cheking aerobic respiration (not fermentation) should be used. We also use mostly decreased temperature and prolonged incubation
  - antigen analysis, nucleic acid detection – not used routinely

Indirect methods used rarely

Differentiation of G– non-fermenters (differential diagnostics)

- Gram staining: **Gram– rods** × other bacteria
- Endo agar: **they grow**. As glucose-non fermenters, they are mostly also lactose-non fermenters, but their colonies are sometimes quite dark, because of pigmenation
- Non-fermenters are differenciated from enterobacteria/vibria by non-fermenting glucose (e. g. **Hajna medium remains completelly red** after culture, no colour change; but **eventual light brown colour does not matter, it is due to presence of pigments**)

Further diagnostics of individual genera and species of G– NFs

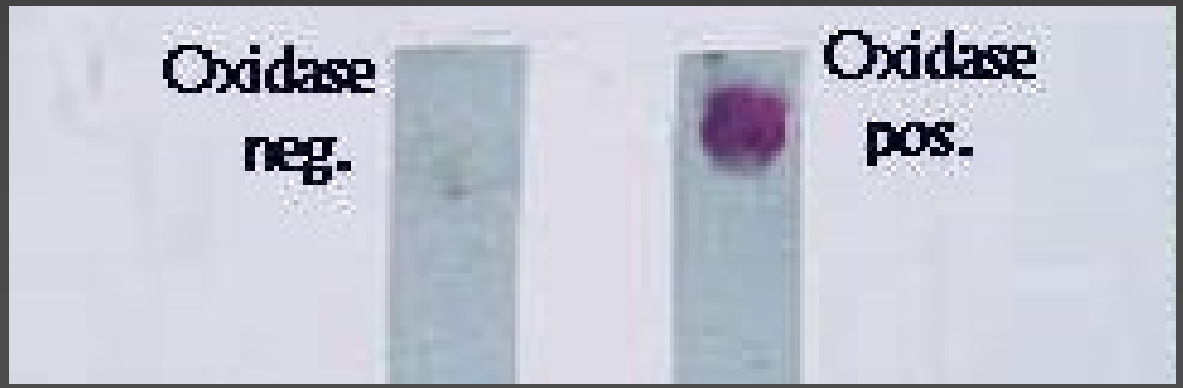
- *Pseudomonas* is usually detected by:
  - Presence of typical odour (young cultures)
  - They form pigments, mostly green, sometiomes blue or maroon. Best visible on MH, worse on BA and Endo agar
  - Positive oxidase
- Other non-fermenters, or not-sure *Pseudomonas*, shlould be differenciated biochemically, e. g. by NEFERMtest 24

Pseudomonas on MH agar and other media

- Remember, that MH agar itself is nearly colourless (or slightly yelowish).
- All green colour you see is product of *Pseudomonas*, or more preciselly, of its pigment pyoverdin
- On BA and Endo, pigment production is not so strong, but partially visible, too. Nevertheless, something more visible on these media is the typical pearl smooth surface of the colonies

Oxidase test in non-fermenters

- Among the most common G– non-fermermenters, ***Pseudomonas*** **is oxidase positive,** ***Burkholderia*** **usually too; on he contrary,** ***Stenotrophomonas*** **is usually negative and** ***Acinetobacter*** **too.**



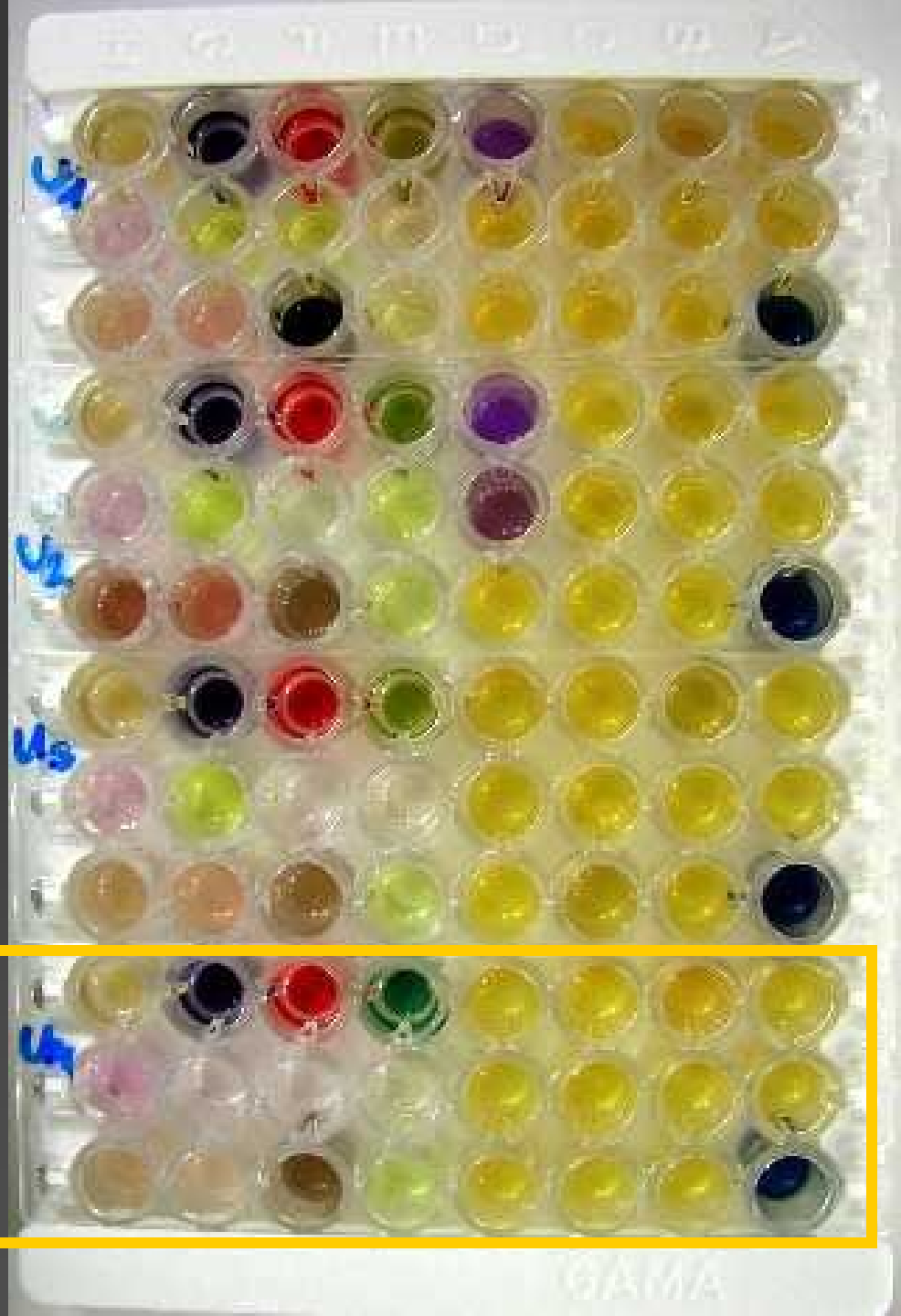
medic.med.uth.tmc.edu/path/oxidase.htm

NEFERMtest 24

- For precise biochemical identification of G– non-fermenters we use mostly NEFERMtest 24 (or a simillar test of other provenience).
- It is a triple-strip (not double as last week)
- There is a different way of code-formation than for (for example) ENTEROtest 16:
  - first number is 0 (oxidase –) or 1 (oxidase +)
  - next 6 numbers come from collumns H to C
  - collumns B and A are not counted (they are eventually used for more detailed determination)

NEFERMtest 24

- One frame is used for four triple-strips (for four strains). Each strain is detected using 24 reactions.
- Requires 30 °C, 48 h



www.medmicro.info (web of the Institute), photo O. Z.

Antibiotics susceptibility of G– NF

- G– non-fermenters may be tested on common media.
- We use strong antibiotics, that should not be used for other infections
- We use here
  - 3rd generation cephalosporins* (but only some of them – „anti-pseudomonad“ ones, like ceftazidime)
  - Anti-pseudomonad penicillins, monobactams and carbapenems* (imipenem, piperacillin/tazobactam)
  - aminoglycosides (gentamicin, amikacin)
  - fluoroquinolones (ciprofloxacin, ofloxacin)
  - polypeptides (colistine)

**or combinations with beta-lactamase inhibitors*

An example of NF atb set

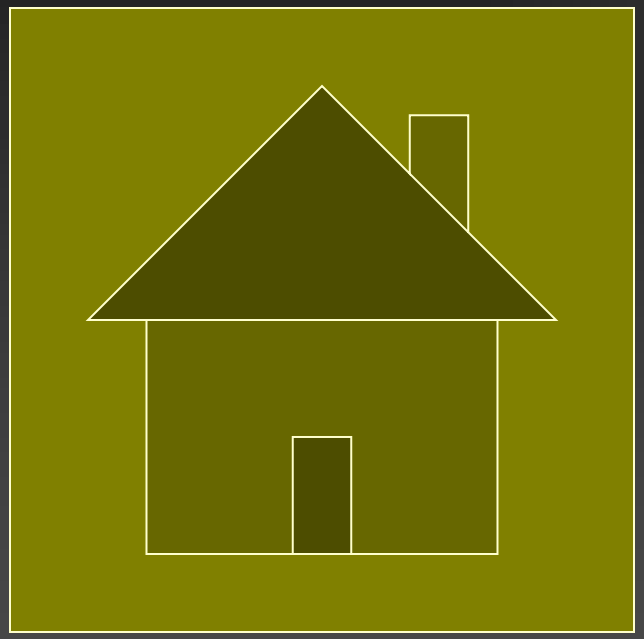
Antibiotic	Abbrev.	Reference zone
Piperacillin + tazobactam*	TZP	22 mm
Gentamicin (aminoglykos.)	CN	18 mm
Imipenem (karbapenem)	IMP/IMI	22 mm
Ciprofloxacin (quin 3 gen)	CIP	29 mm
Ceftazidime (CS 3 gen)	CAZ	16 mm
Colistin (polypeptide)	CT	12 mm
*antipseudomon. peniciline + β-lactamase inhibitor		

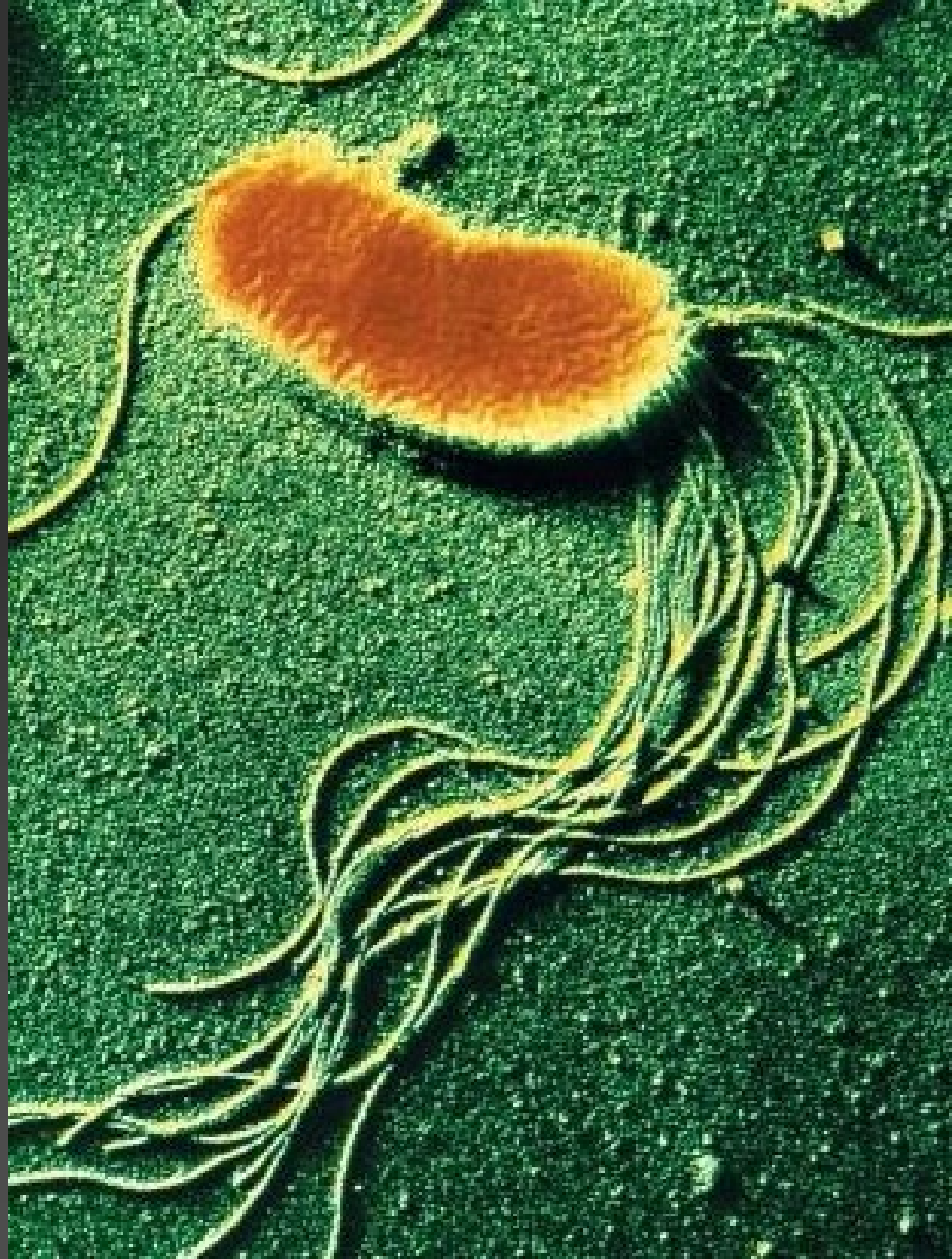
On this picture, *Pseudomonas aeruginosa* is probably susceptible to all tested antibiotics, but it is possible only set only contained discs with special anti-pseudomonad drugs. Even so there exist poly-resitant strains that have secondary resistances even to such antibiotics.

Especially producers of so called metalo-betalactamases (MBL) use to be only susceptible to amikacin and colistin.

It would be also possible to use E-test (here) or microdilution test

The End





http://www.scienceclarified.com

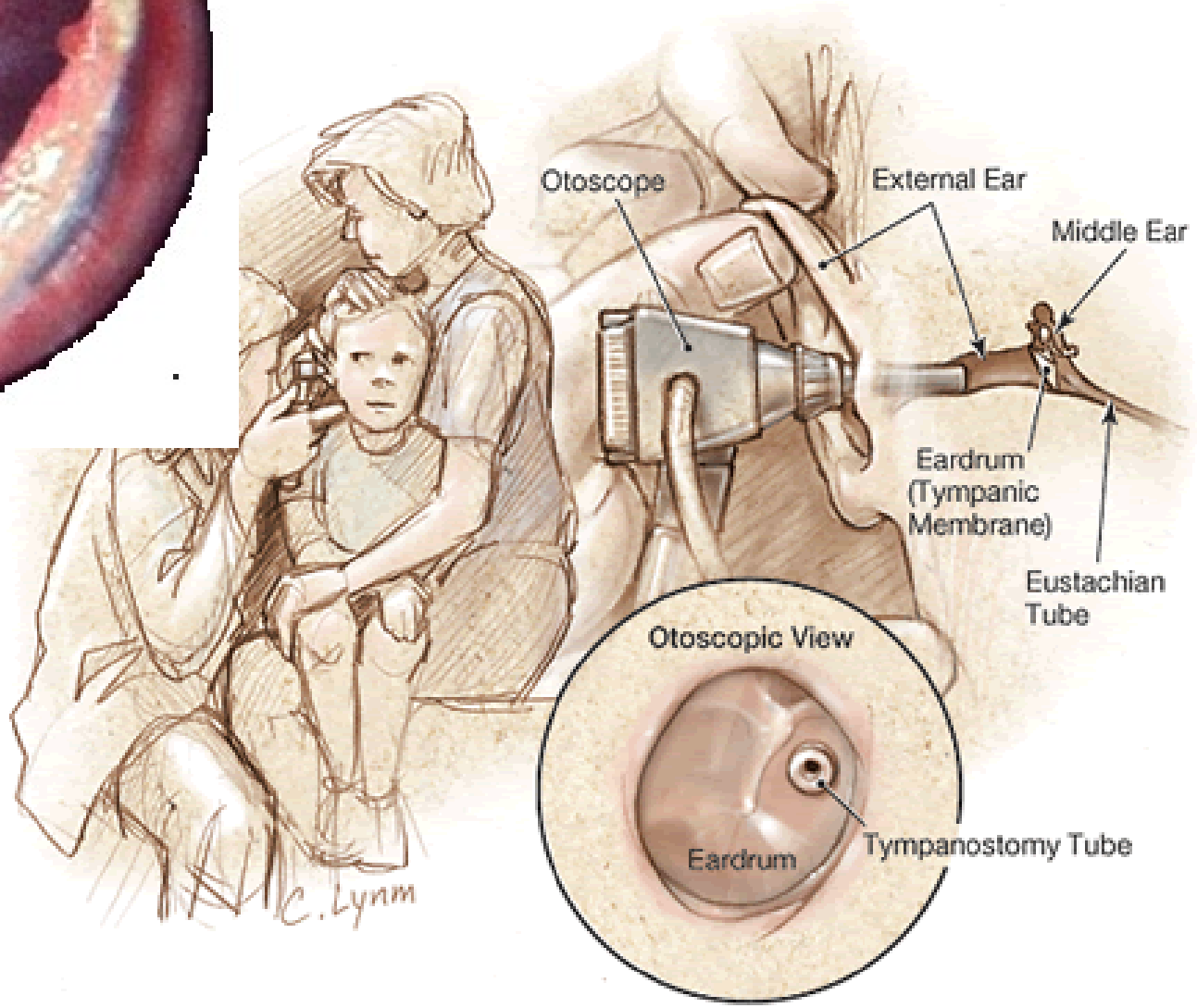
Inflamation of external ear – otitis media (bonus)

- **Common in children** (short horizontal Eustach tube)
- **Caused by:** *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis*
- **In chronical cases** also some G– rods

It is necessary to differenciate otitis externa: here Staphylococcus aureus is the main pathogen (as in other skin inflamations), local therapy, e. g. Framycoin drops

Otitis media

http://www.otol.uic.edu/research/microto/Microtoscopy/acute1.htm



http://www.medem.com/MedLB/article_detaillb.cfm?article_ID=ZZZPMV6D1AC&sub_cat=544

Examination and treatment of otitis media

- **Therapy** is indicated in case of a real inflamation (pain, redness, fever) and it does not react to anti-inflamatory treatment
- **Drug of choice** is amoxicilin (e. g. AMOCLEN), an alternative is co-trimoxazol
- **Ear swab** examination is meaningfull only after paracentesis
- Otherwise it is also possible to examinate **pyogene liquid** taken during paracenthesis