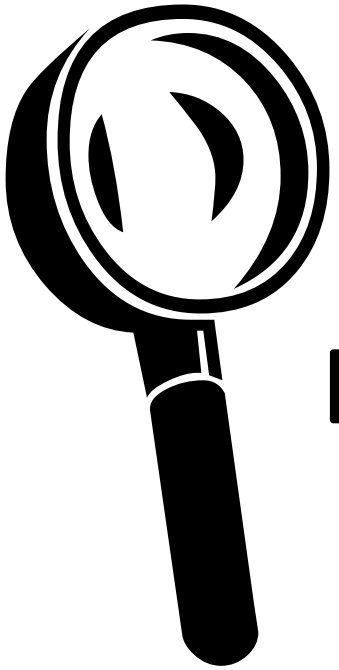
Microbiological institute shows

TRACING THE CULPRIT
Part Four:

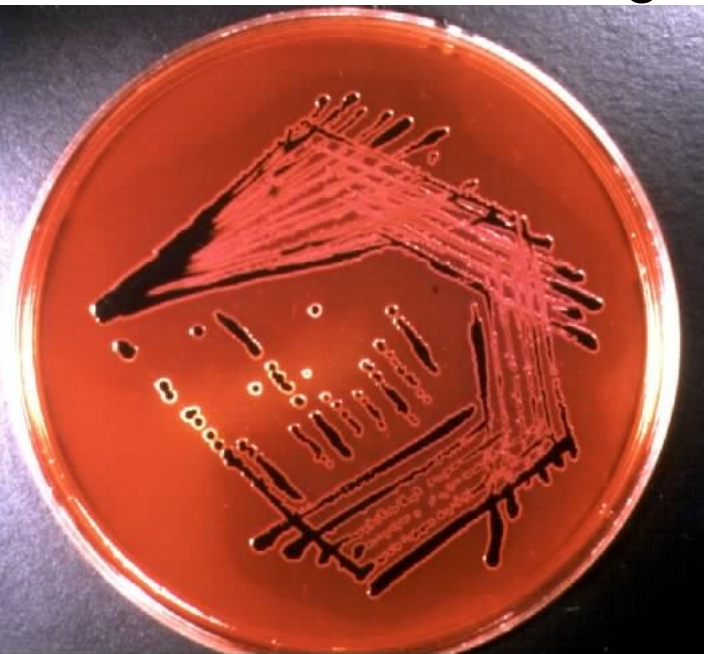


Enterobacteria (& Co.)

A poem for beginning…

http://www.uwec.edu

Salmonella on MAL agar



We can check a healthy hen.
Everything OK. But then
Pigeons start to fly to it
Infect others, that‘s their hit.
Then the egg is used for ice
Looking very, very nice.
But salmonellas can hide
invisible, still inside.
And then youngsters eat the ice…
…and their speed increases twice!

www.medmicro.info, photo author: prof. Boris Skalka

Survey of topics

Clinical description: *Enterobacteriaceae*

Cl. descr.: *Campylobacter, Helicobacter, Vibrionaceae*

Pictures of bacteria

Diagnostics of *Enterobacteriaceae*

Dg. of *Campylobacter, Helicobacter, Vibrionaceae*

Clinical description: *Enterobacteriaceae*

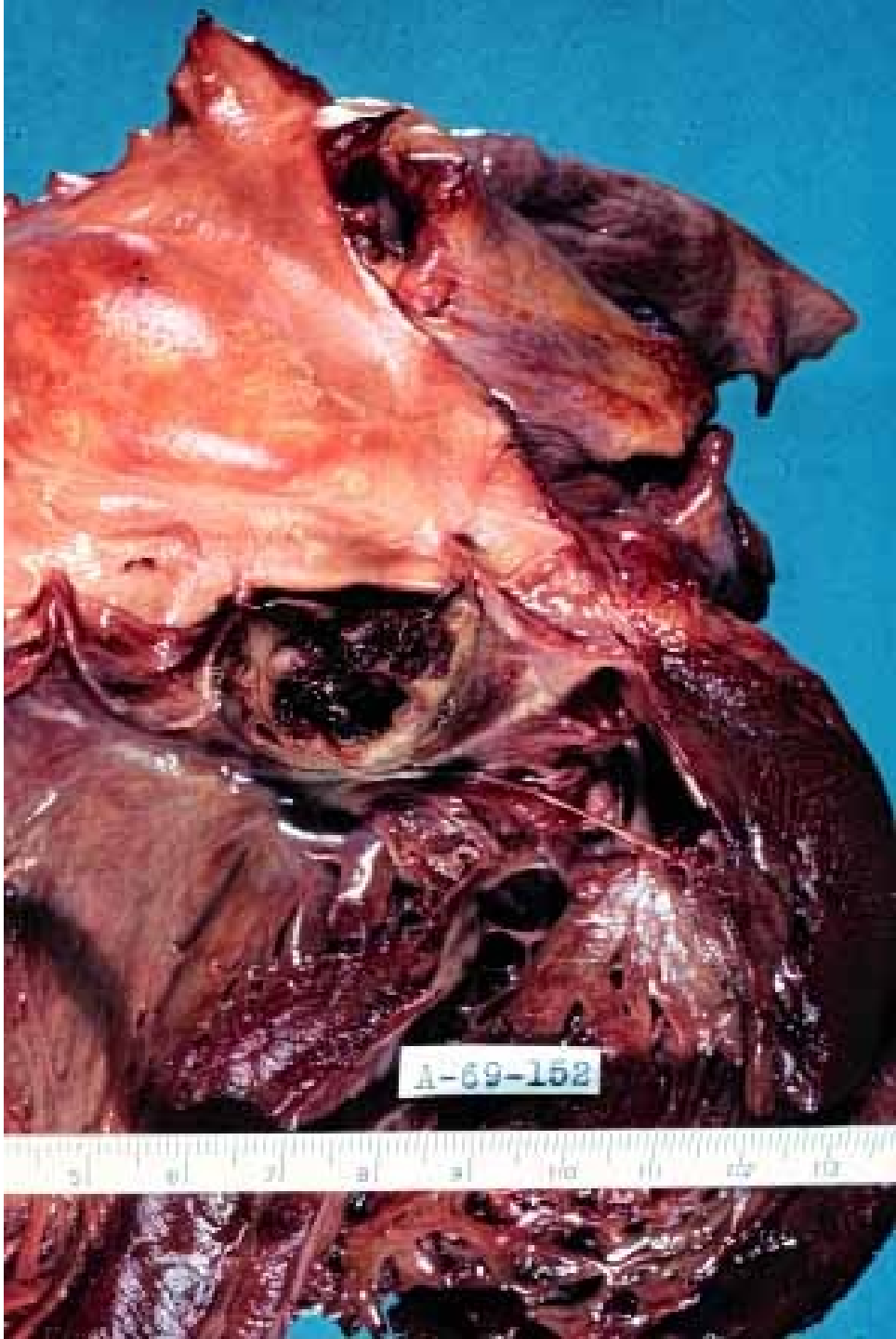
Story one

- Miss Theresa ate a cake with egg cream after her lunch. In the afternoon, she started to vomit and to have diarrhoea. She visited a doctor and he made the anal swab. Several days later, officers of public health administration called her. Finally, although she did not believe, that cake was not guilty.

Who is guilty now, then?

- Bacterial culprit is *Salmonella enterica* serovar Enteritidis, briefly *Salmonella* Enteritidis
- Cream cake cannot be the culprit! Incubation time does not correspond.
- Another cake, consumed two days before, was later proven to be the infection source.
- As to humans, probably someone in the „Hysterical Maid Cafee“ failed to do something. Public health officers do their monitoring just now.

Salmonella endocarditis

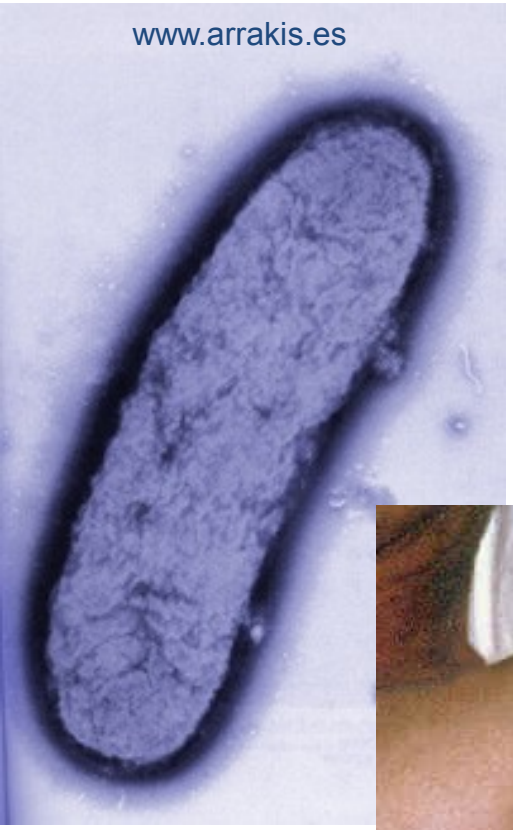


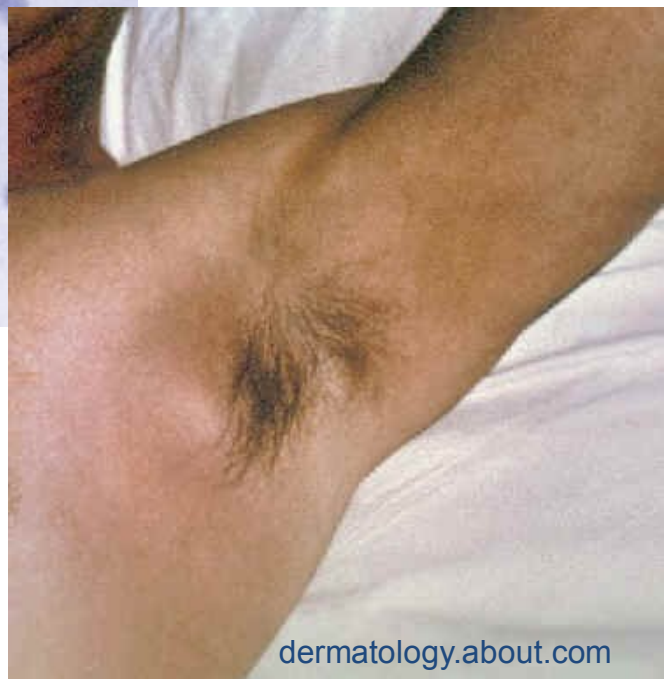
http://www.som.tulane.edu

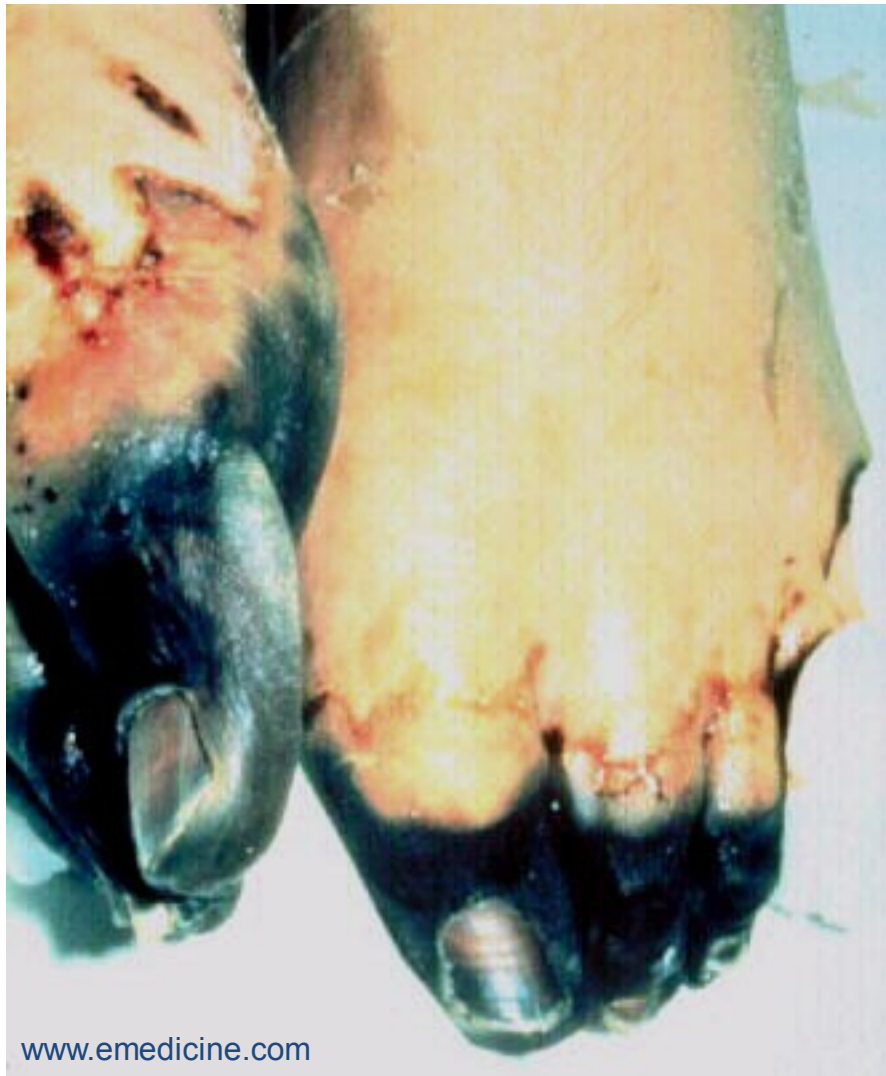
Primary pathogens among enterobacteria

- *Enterobacteriaceae* is the clinically most important family among Gram– rods (not only clinically)
- The worst pathogens perform systemic infections: it is *Yersinia pestis* (causing plague) and so named anthropopatogenous serovars of *Salmonella* (serovar Typhi, Paratyphi A, Paratyphi B and C – causing typhoid fever – septicaemia with high fever and headache)
- Obligatory pathogens causing usually intestinal infections only are important, too. Even here, sometimes a systemic infection may occur.
- We are speaking about genera *Salmonella*, *Shigella* and *Yersinia*

Black plague *(Yersinia pestis)*







Note to *Salmonella* and *Shigella*

- The fact, that there are differences even between intestinal pathogens, can be shown on the example of *Salmonella* and *Shigella*.
- **Salmonella** needs high infectious dose. They have to multiply in a food. So the infections are almost food-borne.
- **Shigella,** on the contrary, has a small infectious dose, so it is easily transmitted by dirty hands, WC handle or contaminated water.
- There exist also **clinical differences** (different character of diarrhoea etc.). For example, shigelosis has its specific name – **bacillar dysentery** (do not confuse with amoeba dysentery)

Story Two

- Mrs. Wet is a patient of a urological clinic.
- She has repeated problems with urination. After three children, he has damaged muscles of pelvic region, she was treated even for urine incontinence. The doctors warned her, that she is in an elevated risk of infection. And really – now, she got infected again.

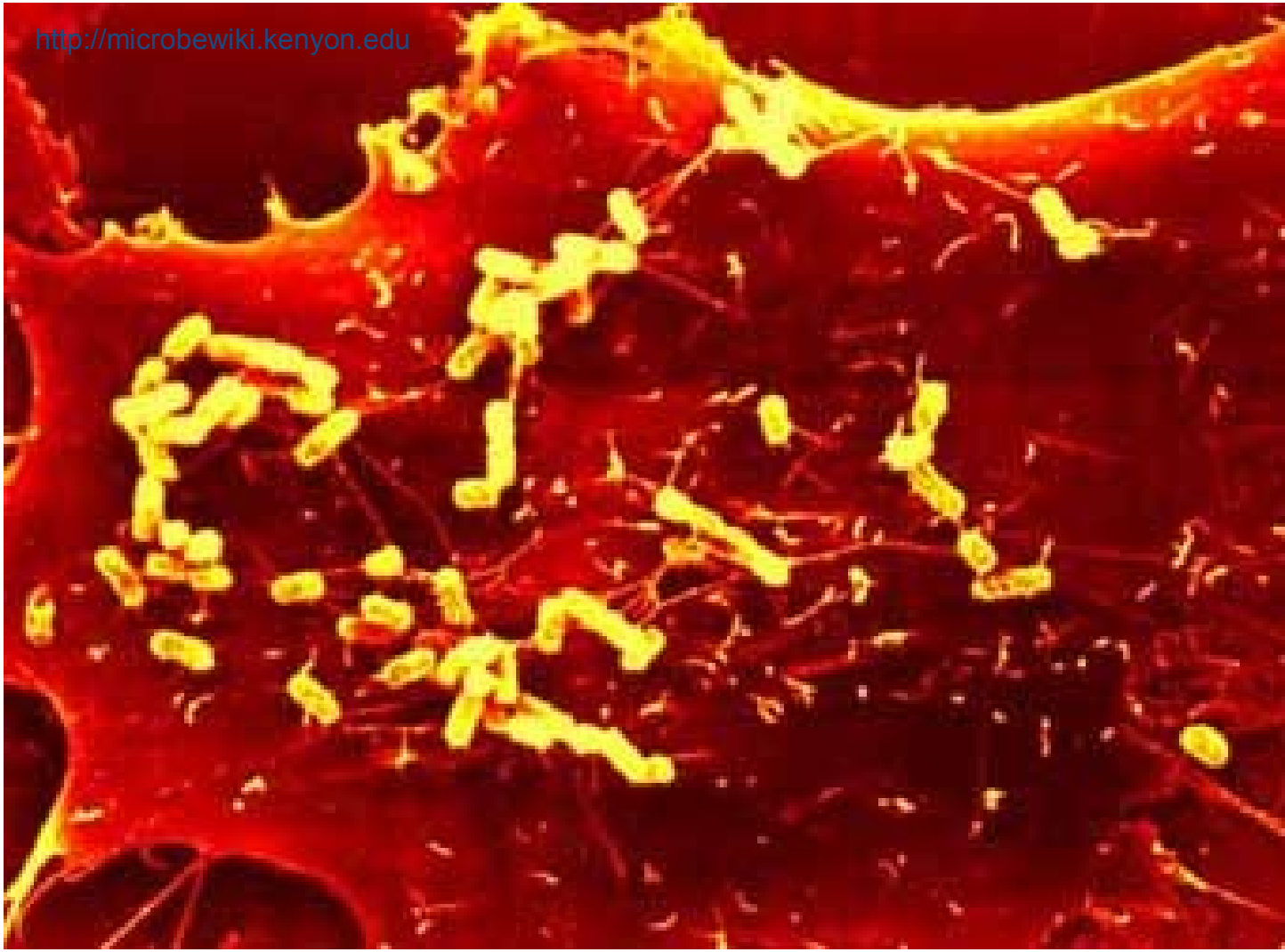
Who is guilty now?

- It is *Escherichia coli*, more precisely, its uropathogenous strain (uropathogenous *E. coli* – UPEC)
- But the same problems might be due to other opportune pathogenic enterobacteria (sometimes even obligatory pathogens, like *Salmonella*)
- *Escherichia coli* is one of the most important components of intestinal microflora, and it is protective – it produces bacteriocins that do not enable other bacteria to colonize the mucous membrane. *Escherichia coli* even supplies our body by some vitamins (mostly E and K).
- *Escherichia coli was found by German-Austrian professor Theodor Escherich (died 1911 – 100 years)*

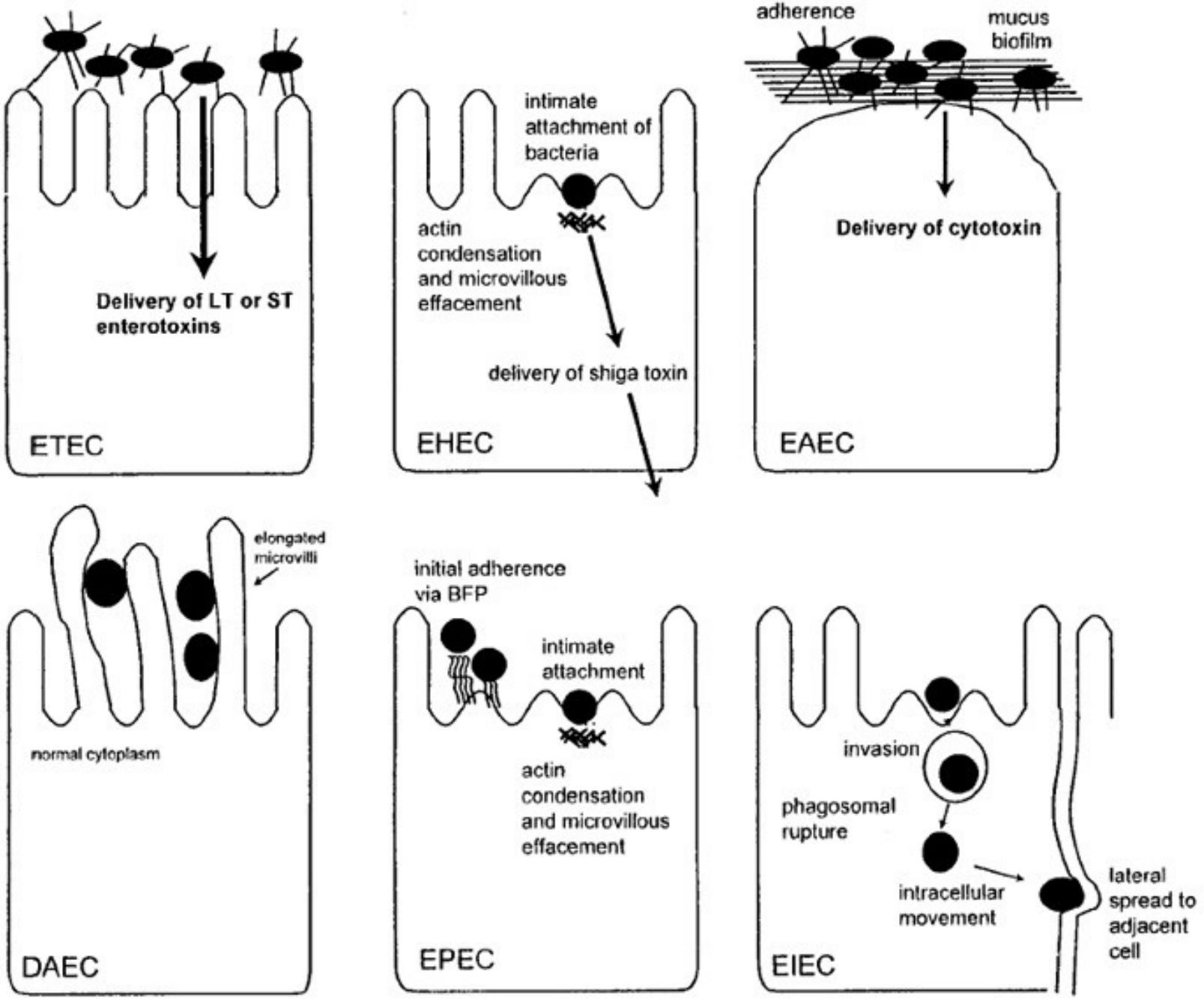
Pathogenicity of *Escherichia coli*

- Intra-intestinal pathogenicity:
  - EPEC (enteropatogenous *E. coli*) – mostly newborns, babies
  - ETEC (enterotoxic *E. coli*) – mostly travellers
  - EIEC (enteroinvasive *E. coli*)
  - STEC (shiga toxigenous *E. coli*; this group also contains EHEC – enterohaemorhagic *E. coli*)
  - EAggEC (enteroaggregative *E. coli*)
  - Combinations (STEC + EAggEC = strain O:104H:4, that caused a severe epidemic in Germany 2011)
- Extraintestinal pathogenicity:
  - UPEC (uropathogenous *E. coli*)
  - Strains causing respiratory infections
  - Strains causing sepsis, wound infections, etc.

Urinary bladder with adhered escherichias

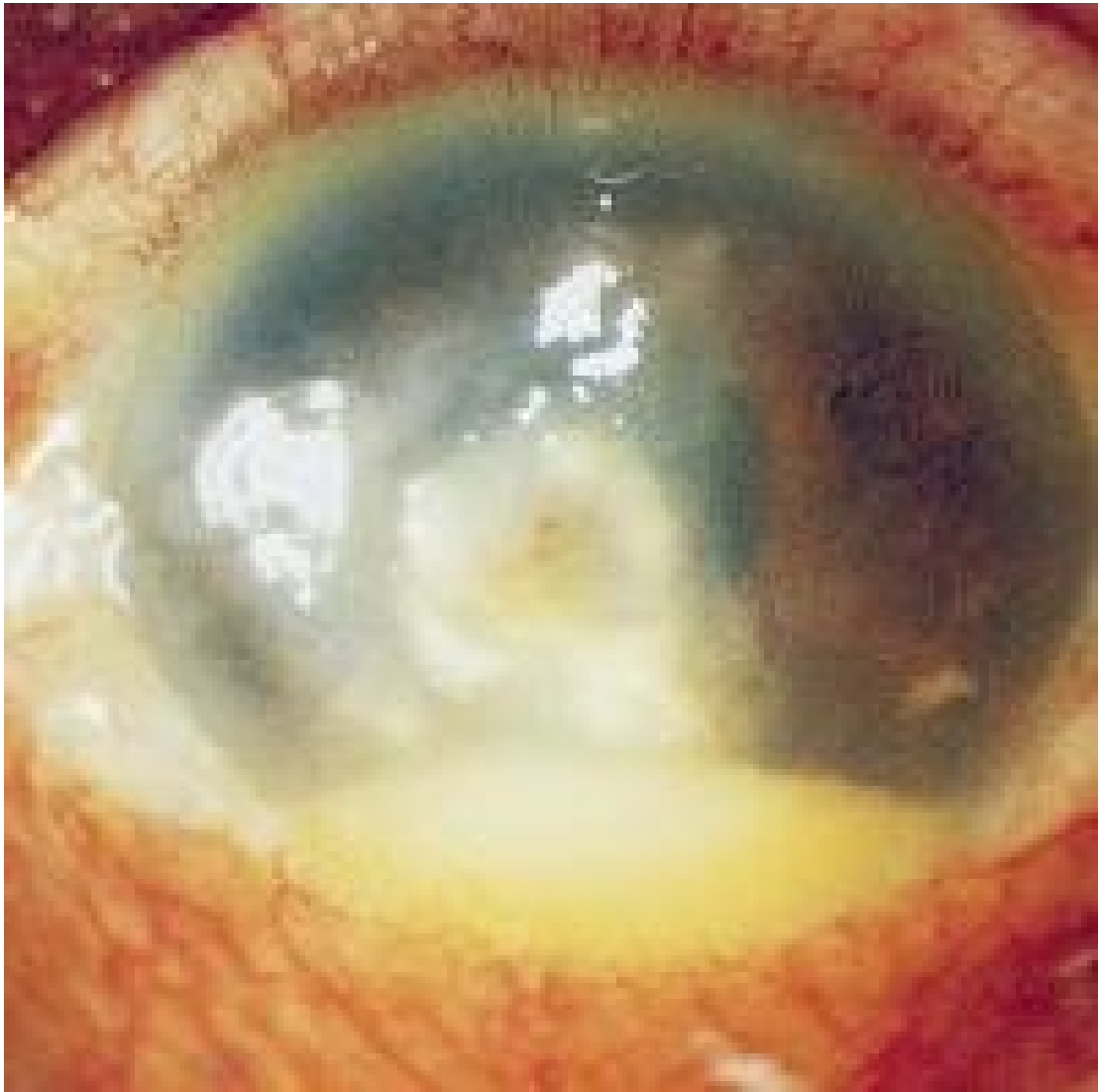


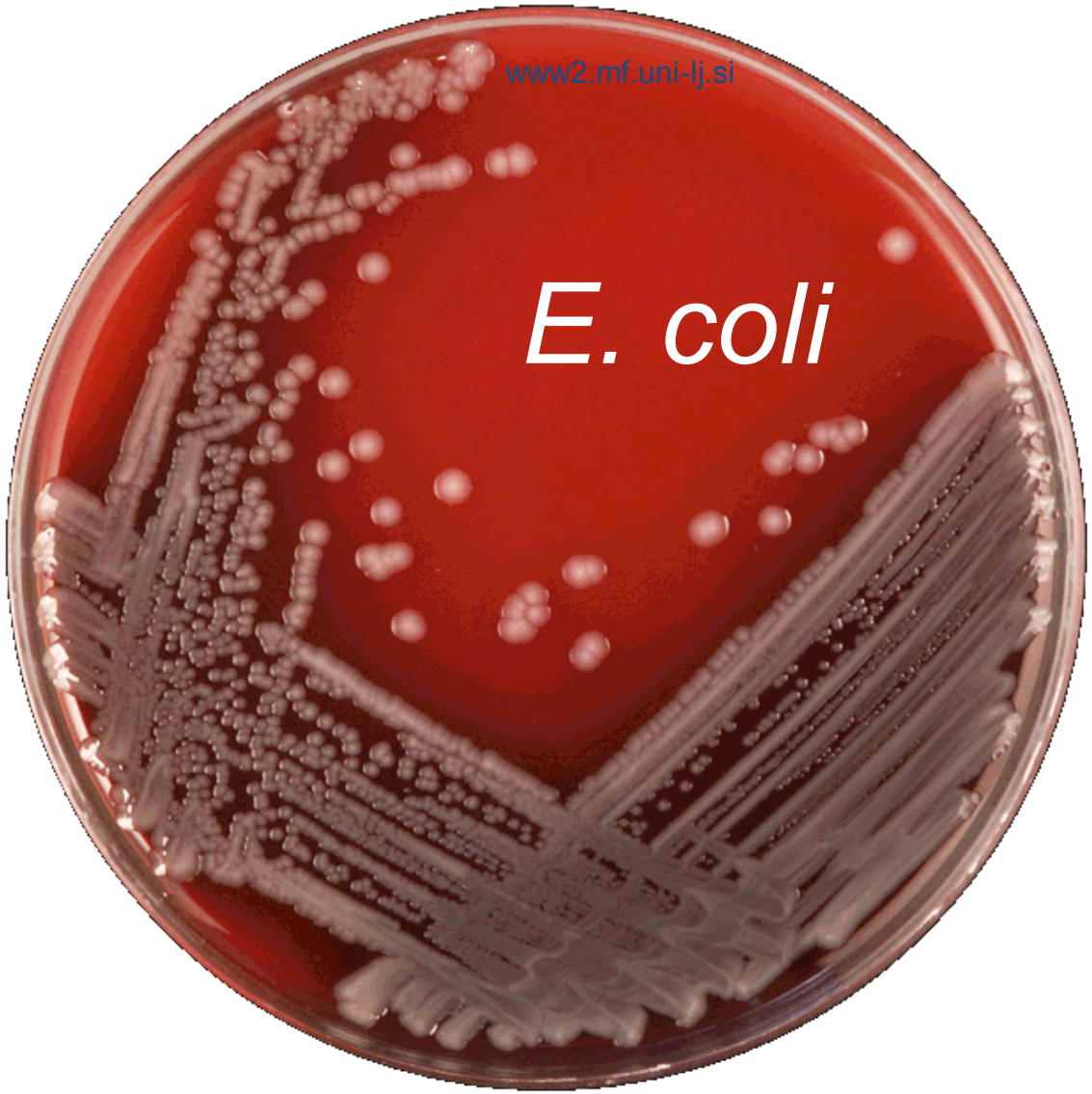
http://microbewiki.kenyon.edu



Even corneal ulcer may be caused by *Escherichia coli*

www2.mf.uni-lj.si



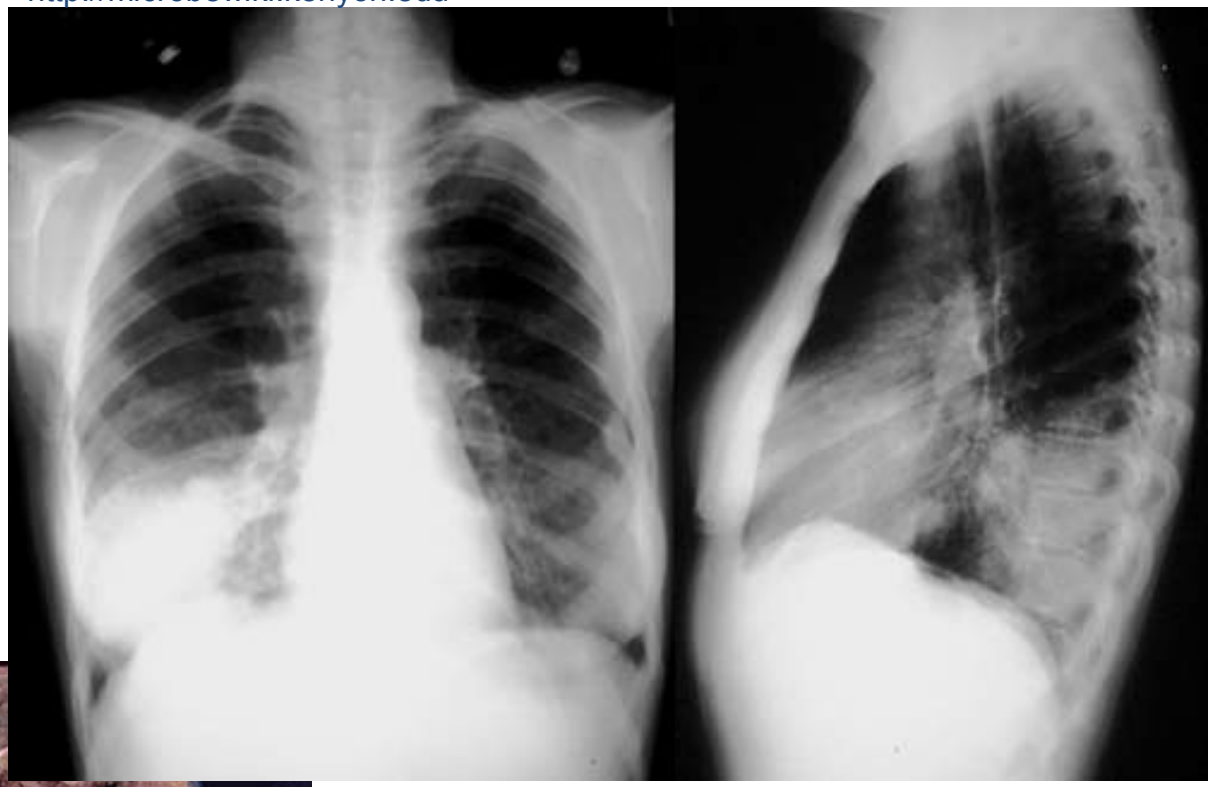


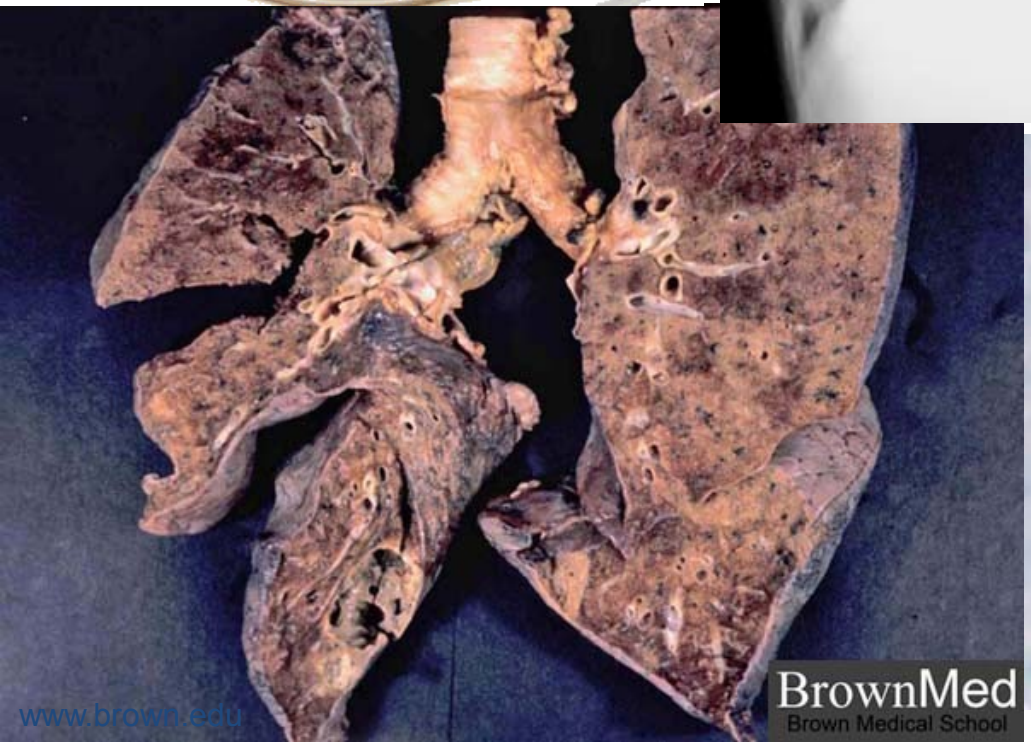
Some more oportune-pathogenous enterobacteriae

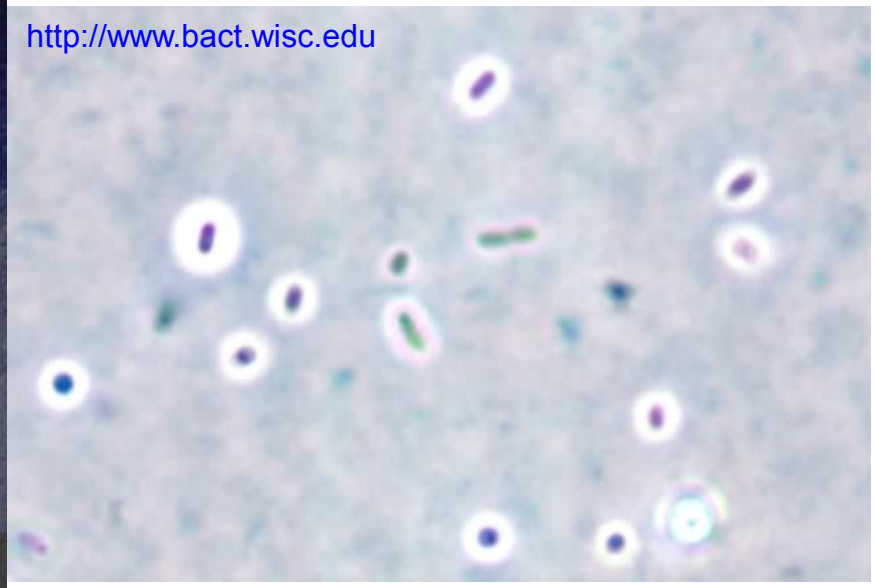
- *Enterobacter, Klebsiella, Pantoea* – often encapsulated, mucous colonies. Especially *Klebsiella* is a common pathogen causing hospital infections (respiratory ways, UTI)
- *Proteus, Providencia, Morganella* – proteolytic bacteria (in diagnostic typical bad smell of their colonies). Occasionally causing UTI and different other infections
- *Citrobacter* – biochemically similar to *Salmonella*, but with positive ONPG test
- *Hafnia* – primary bee pathogen

Action of *Klebsiella*

http://microbewiki.kenyon.edu







Survey of enterobacteria

A red pigmented strain of *Serratia*

Story	Pathogen.	Examples
–	Systemic	*Y. pestis*, AP** *Salmonella*
1.	Intestinal	ZP* *Salmon., shigella, Yersinia*
2.	Opportune	*E. coli, Klebsiella, Enterobacter, Serratia, Proteus, Providencia, Morganella, Citrobacter,* etc.
–	Nearly zero	Many, e. g. *Pragia fontium* and *Budvicia aquatica*

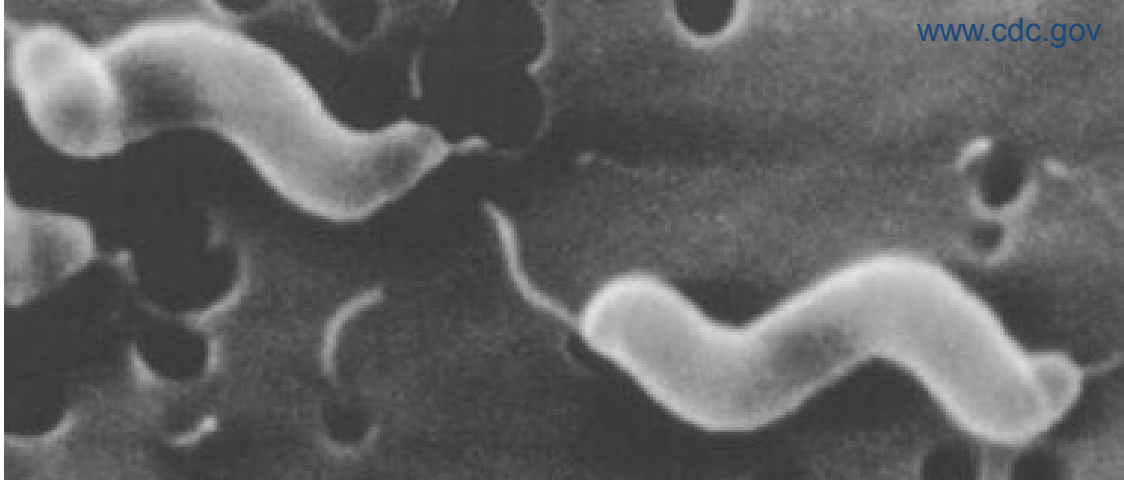
*zoopathogenenous **antropopathogenous

Clinical description: *Campylobacter*, *Helicobacter* and *Vibrionaceae*

Story three

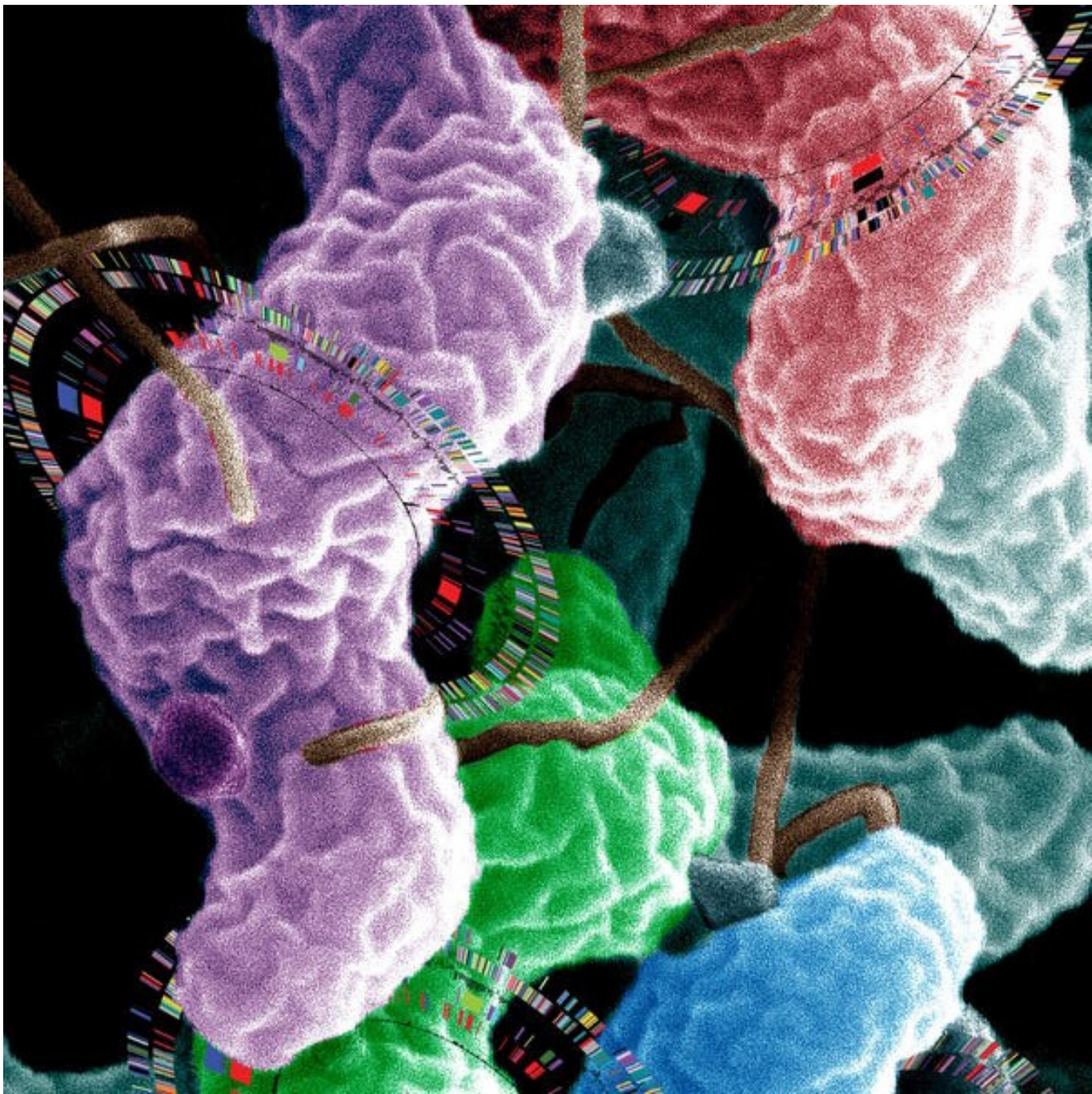
- Student Francis often visits fast-foods. Especially, he likes chicken meat.
- Thus, even public health officers were not able to source the food responsible for his diarrheic problems. Francis thought about salmonelosis. But it was not this. Salmonella comes mostly from eggs, this one rather from chicken meat.

Culprit‘s name



- *Campylobacter jejuni*, Gram-negative curved rod. It does not belong in family of enterobacteria, but the infection is similar that caused by *Salmonella*
- Number of cases in Czechia is about the same as that of salmonelosis. Formerly it was not so common, but maybe only the diagnostic was not so much developed.

Campylobacters

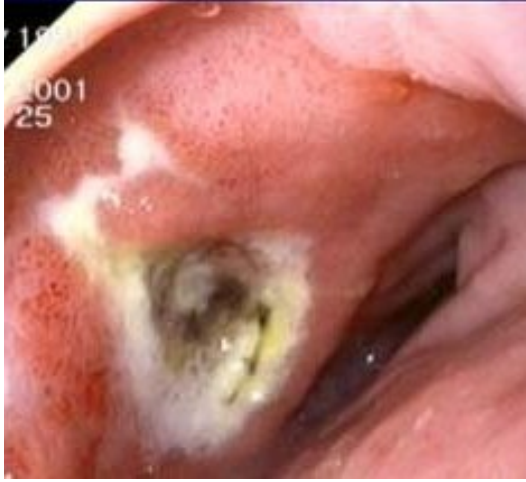


http://biology.plosjournals.org

Important notice: stool sampling

- Unlike parasitology and virology, where a bit of stool is necessary, in bacteriology we are used to send an anal swab (but a bit of stool is not a mistake).
- Today's method of sampling is use of a cotton swab with transport medium (usually Amies). This is especially because of *Campylobacter* – *Salmonella* would survive even on a swab without transport medium.

Story Four



- Mr. Acid has a problem: pyrosis. This is not the only problem – sometimes he even vomited blood. He is likely to have a peptic ulcer
- He comes very often to gastroenterology, and a fibroscope is often present in his oesophagus.
- At last fibroscopy, the doctors took two samples endoscopically – one for histological, another for microbiological examination.
- Both methods showed the same: *it is there.*

Only half-culprit…

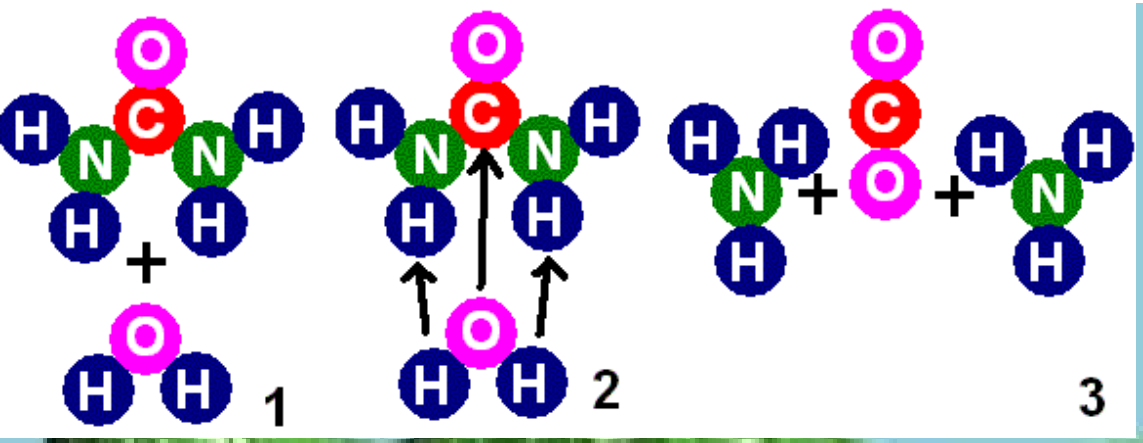
- Peptic (= gastric / duodenal) ulcer is caused by more causes. Such diseases are called multifactorial diseases.
- The part of *Helicobacter pylori* on ulceral disease is still discussed, not only among GPs, but even among specialists. Even healthy persons may have a helicobacter in their stomach. Nevertheless, certain and not negligeable role of this pathogen is sure.

How can it survive at low pH?

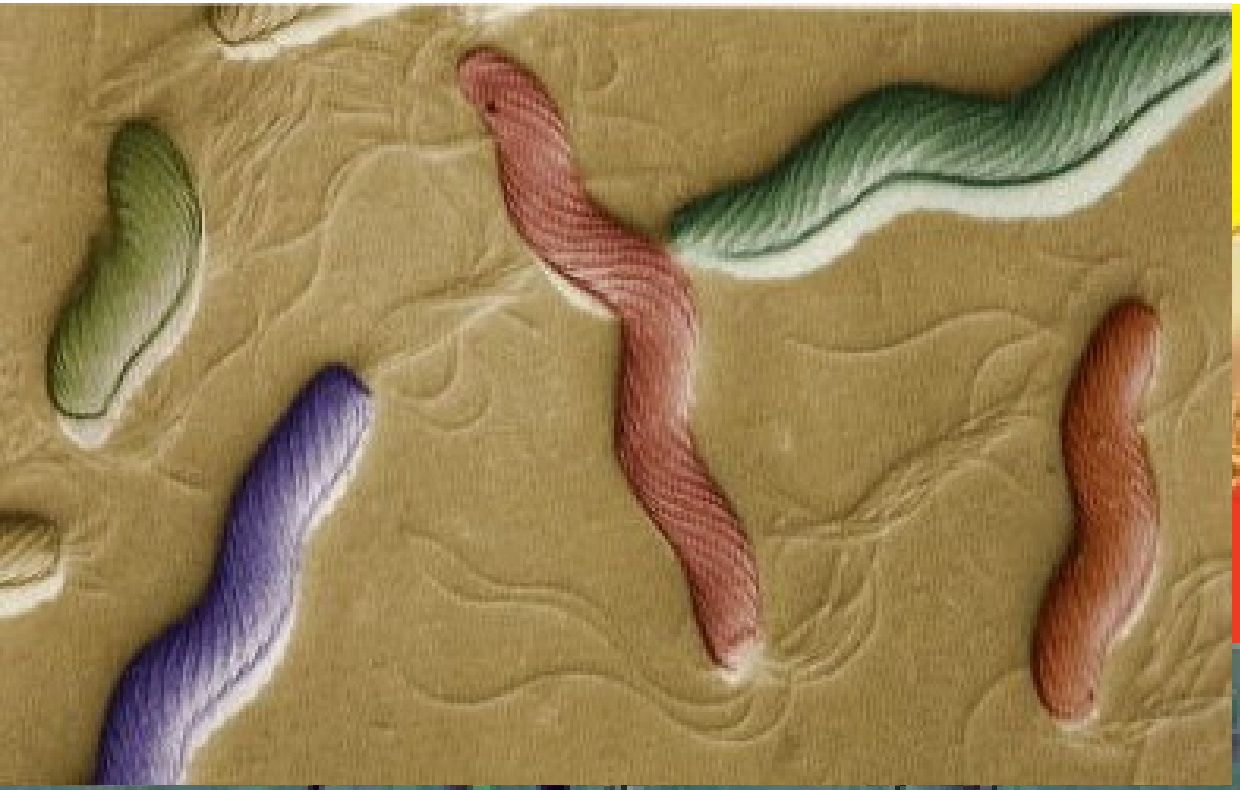
- It adapts its microenvironment – in alkalises it, by splitting urea
- Urea is split into acid carbon dioxide, that is breathed out, and alkali ammonia, that remains and alkalizes the environment:

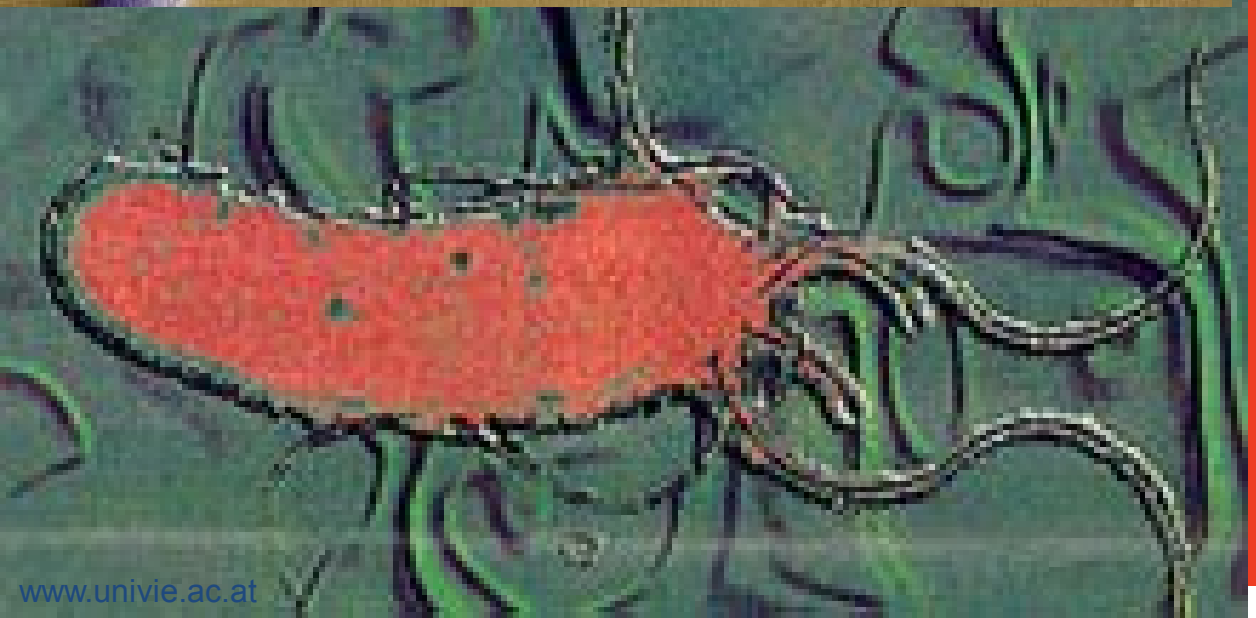
$NH_2\text{-}CO\text{-}NH_2 + 3\ H_2O \rightarrow CO_2 + 2\ NH_4OH$

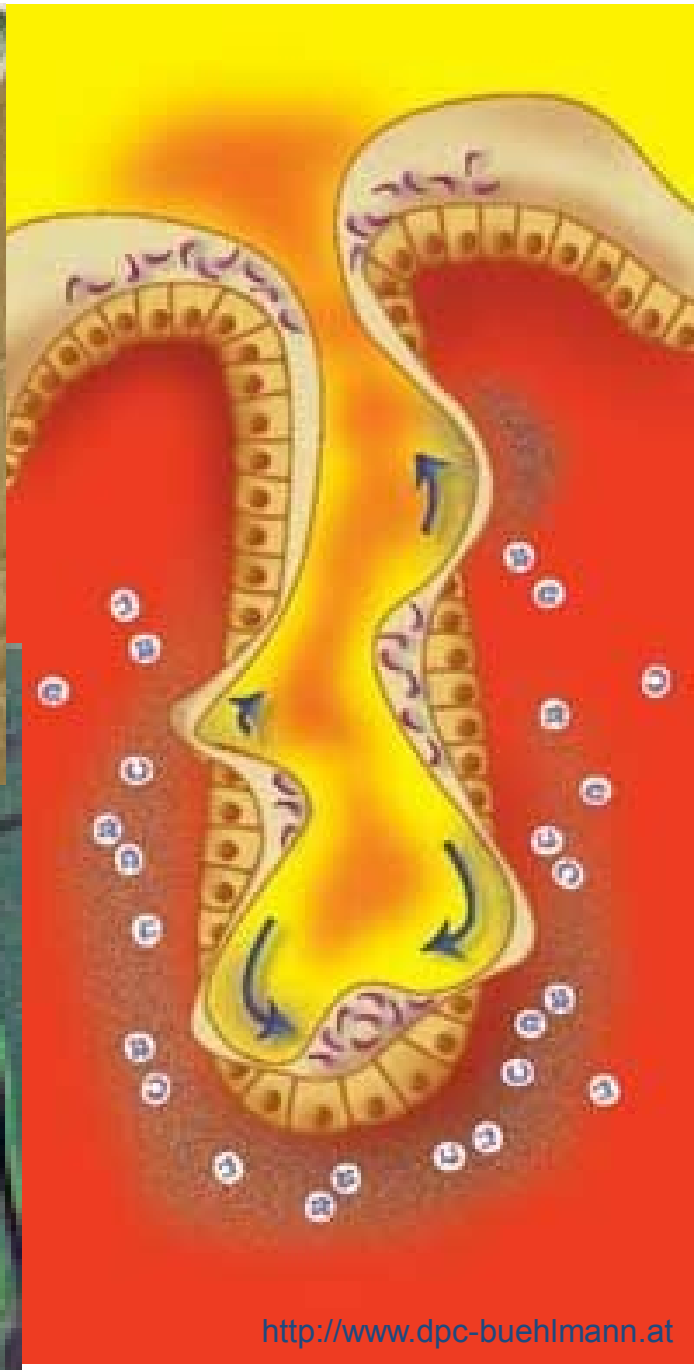
Once more the same



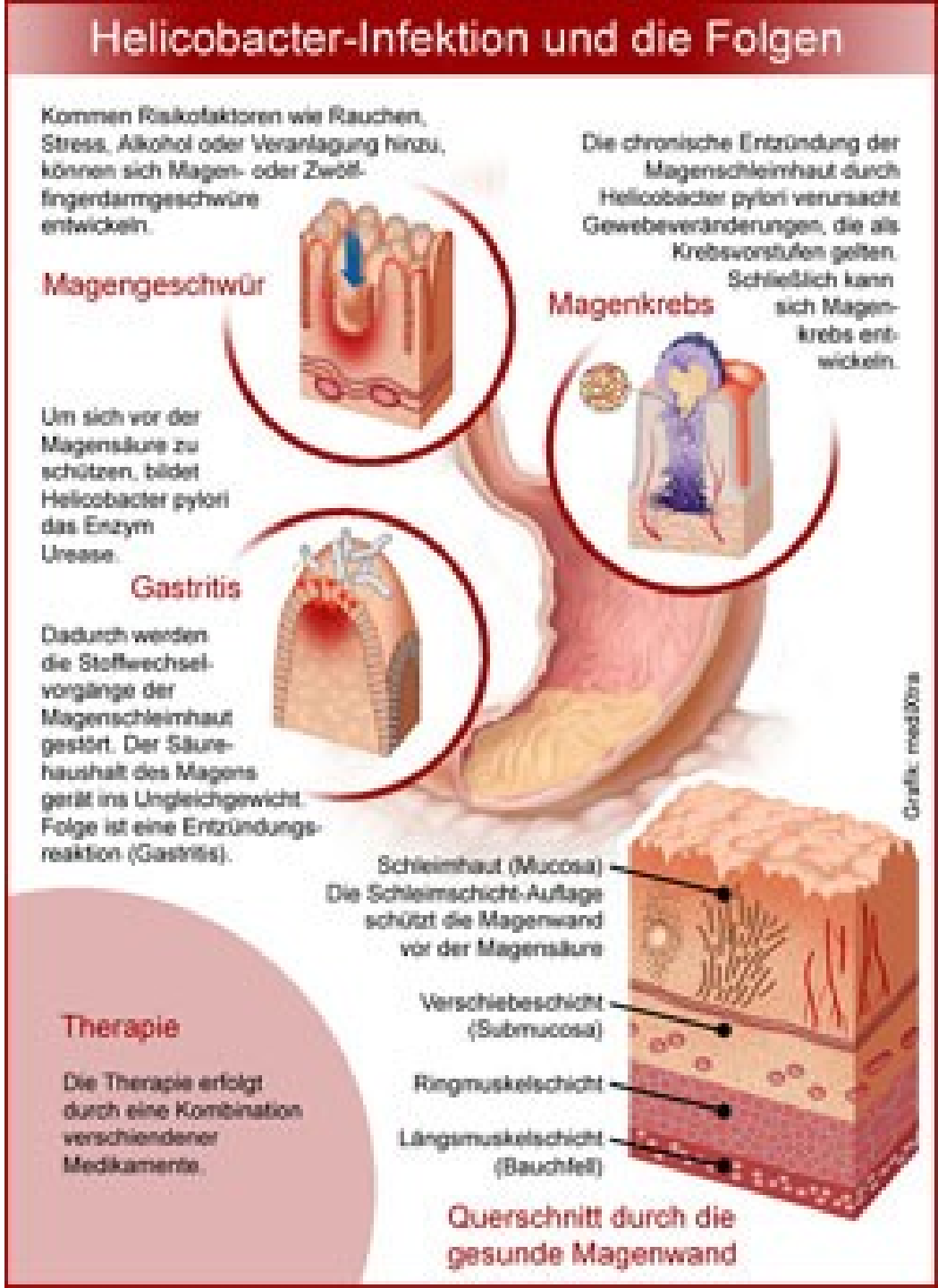
(the difference between writing ammonia as NH_4OH or NH_3 is only formal)







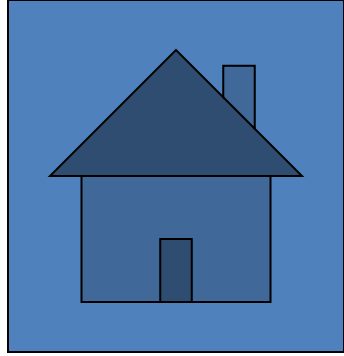
Complications of helicobacter disease



Story Five

- Mr. Exotic was on his exotic holiday. He was used to drink **water from local sources**
- So, no surprise, he was attacked by a very severe watery **diarrhoea**.
- This one, nevertheless, was more heavy than usually. It was watery and very profound
- **Oral ingestion of water was insufficient**. Only infusion supplementation of missing liquids brought a help to him.

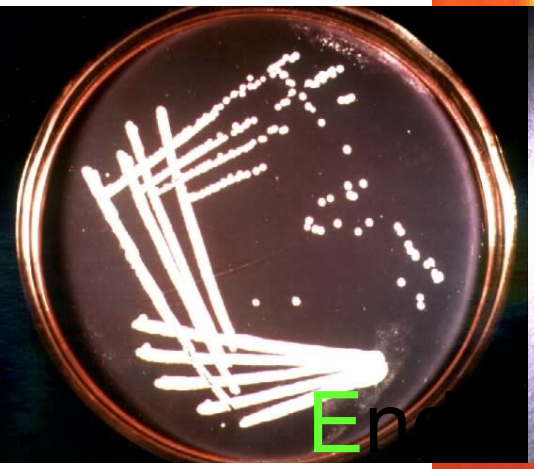
Vibrionaceae

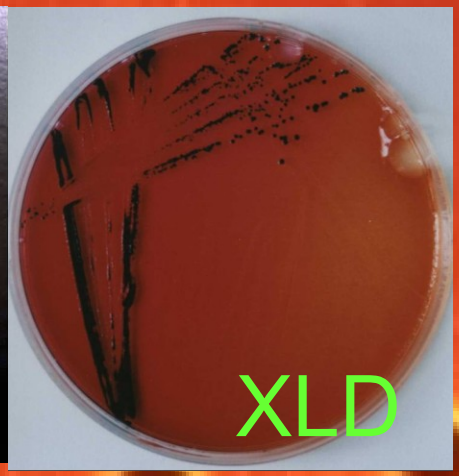


- ***Vibrio cholerae*** causes cholera, a profound diarrhoeic disease in subtropic and tropic countries
- **Other members of genus *Vibrio*** may accidentally perform either diarrhoea, too, or wound infections. They are called „halophilic vibrios“, as they prefer elevated NaCl concentrations
- ***Aeromonas***, the second important genus, also causes wound infections, e. g. at preparing meals from fishes and seafood.

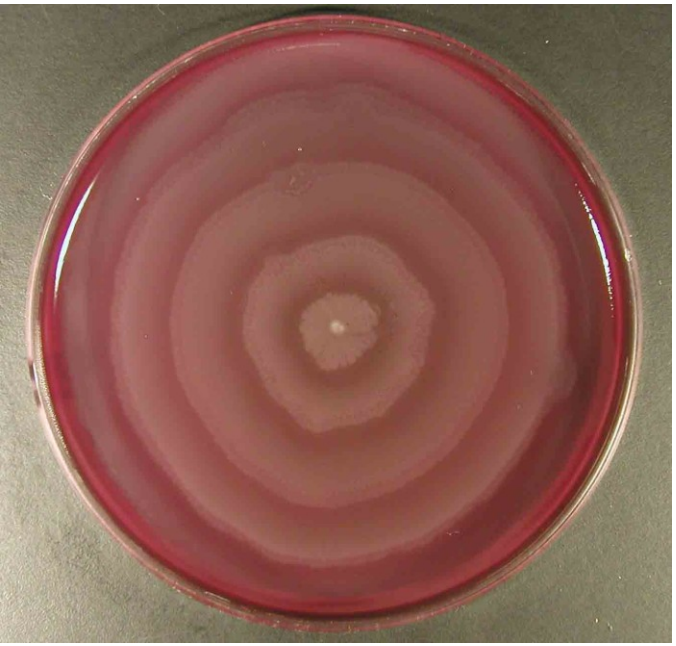
Pictures of bacteria

Photographs from culprit database: *Salmonella*

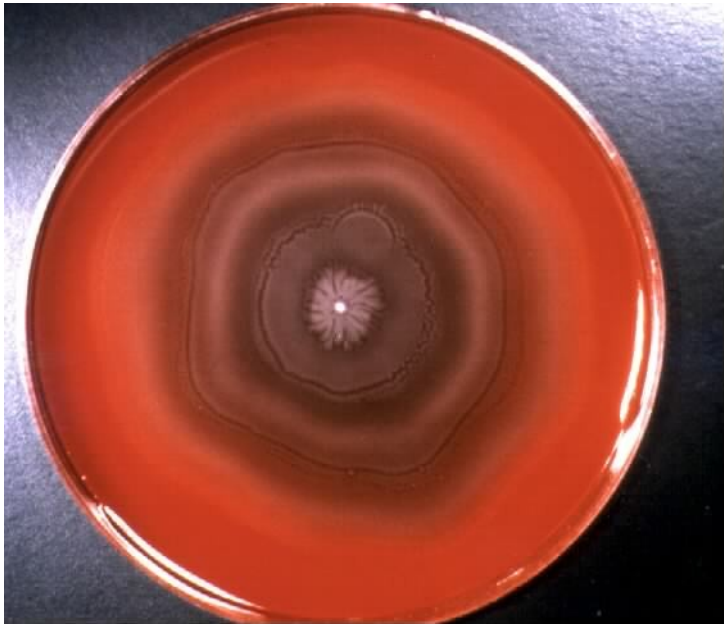




Proteus mirabilis, P. vulgaris (below)

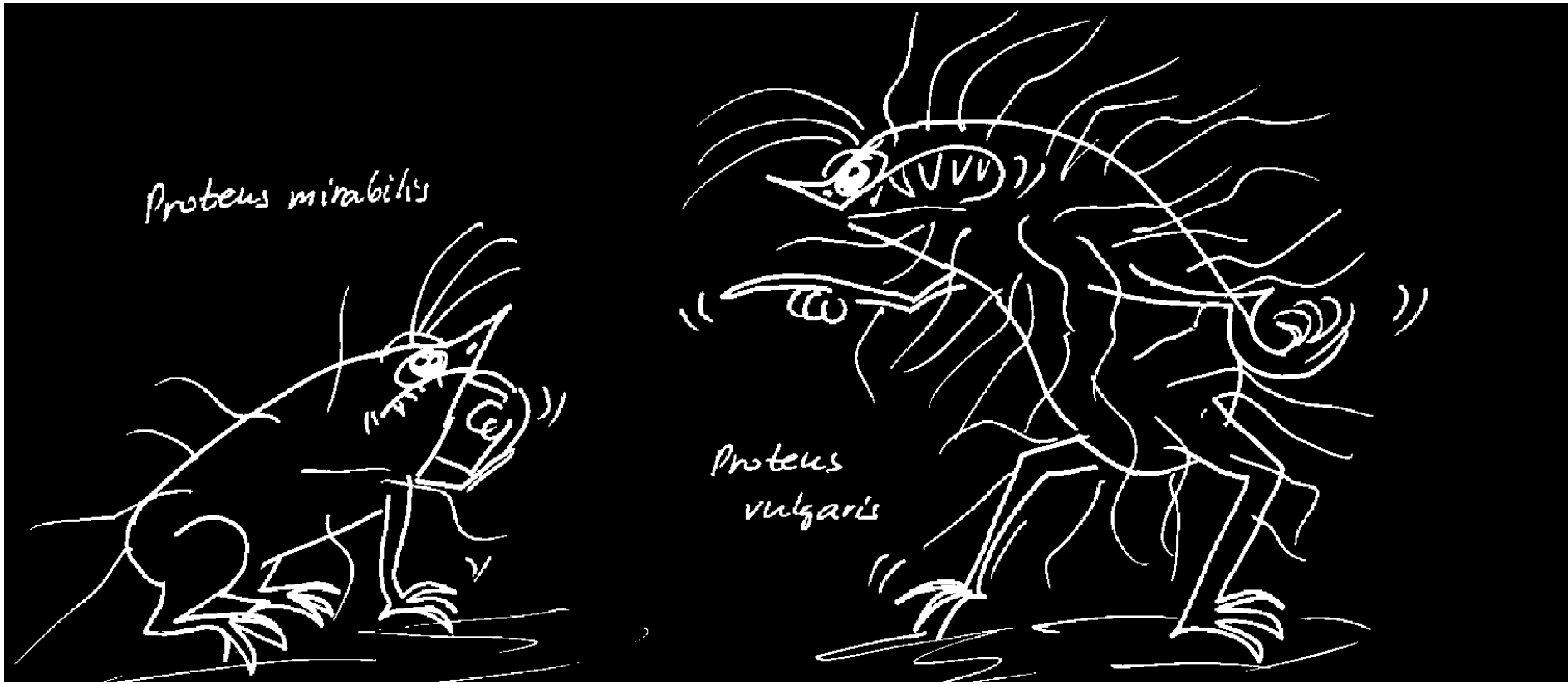


www.medmicro.info



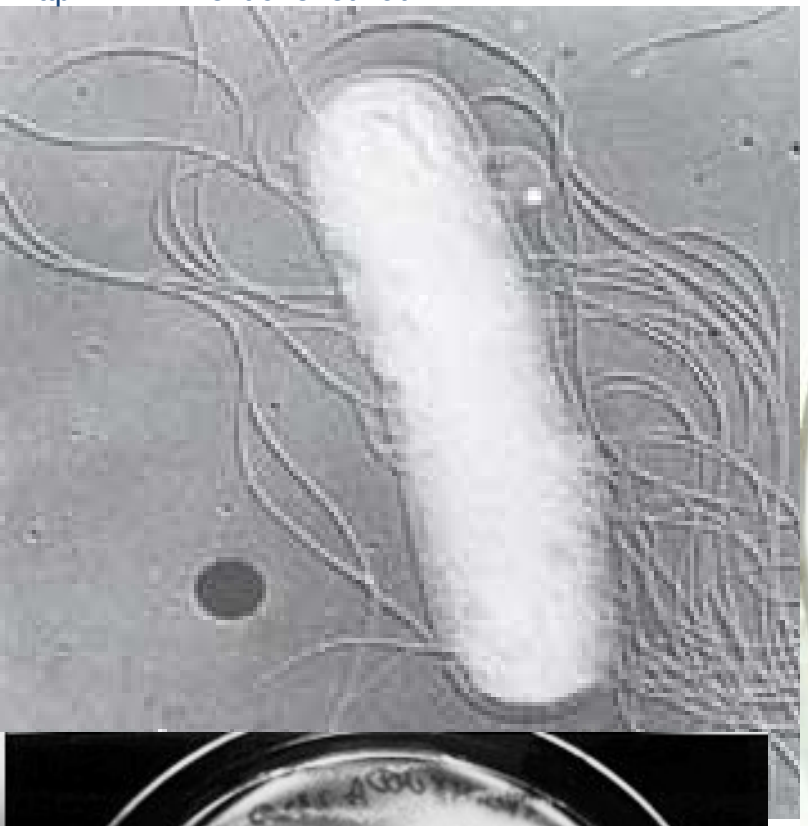
- Typical for Protei: they do not grow only in inoculation place, but they spread on the surface of agar (Rauss phenomenon)

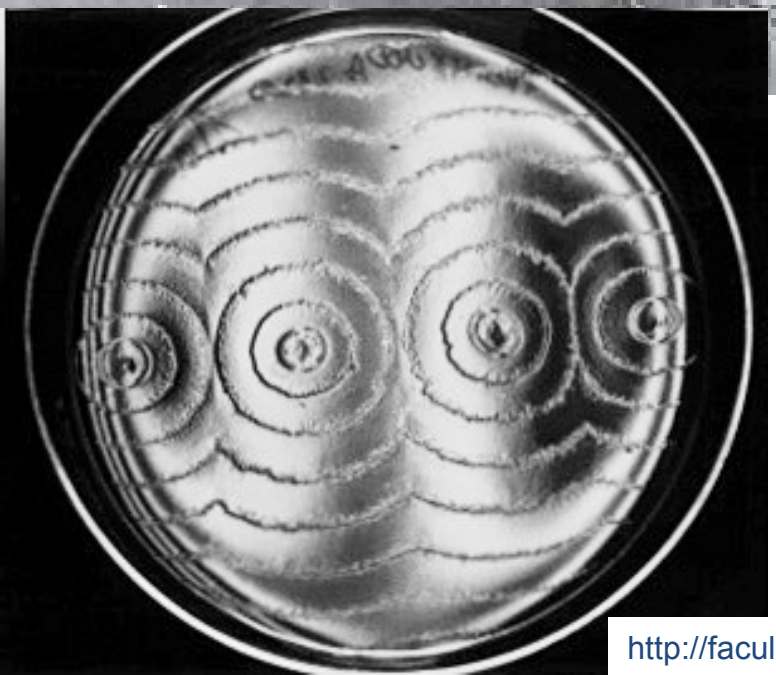
Proteus according to P. Ondrovčík



„It is nice, colleague, that you are able to dekarboxylate ornithin; much more sad is, that you are not able to perform Rauss phenomenon as well as I do!“

http://www.infektionsnetz.at



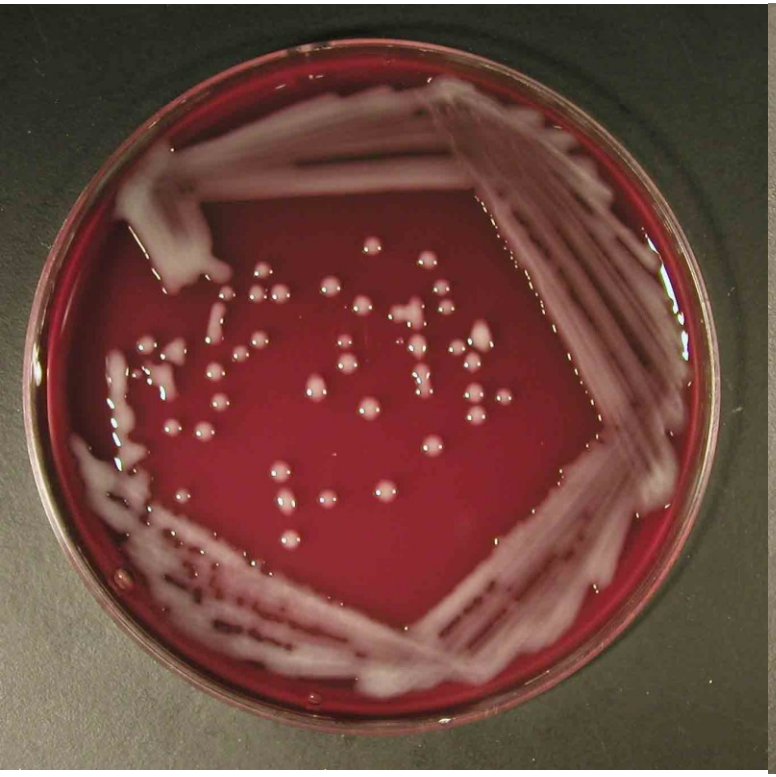


http://faculty.smu.edu

http://www.icbm.de

Proteus – typical swarming growth

Klebsiella & Escherichia



www.medmicro.info

Colonie of Klebsiella on blood agar are more mucoid and more white than those of *E. coli*…

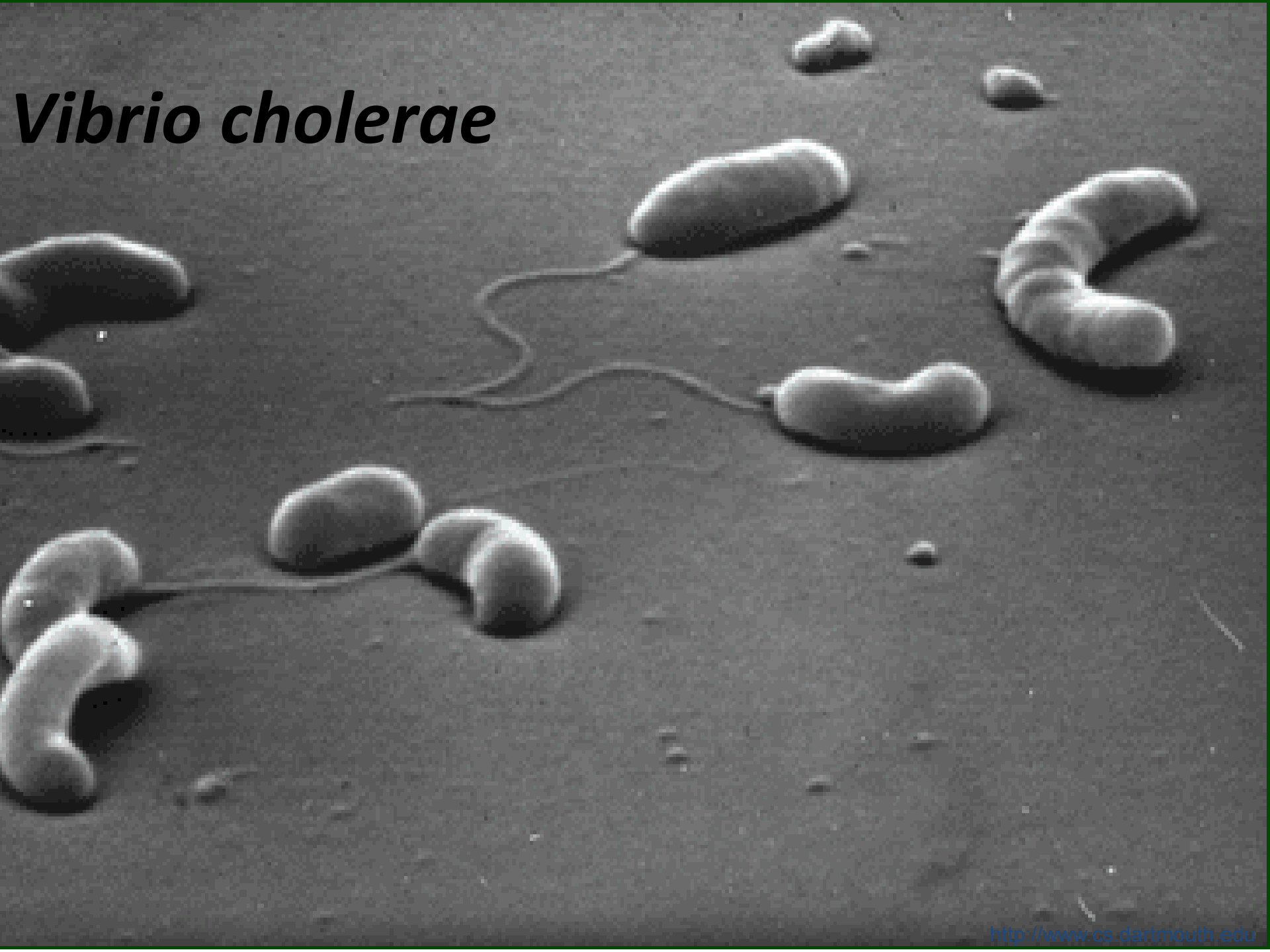
… but just THIS strain of *E. coli* is quite white and mucoid, too ☺

Escherichia

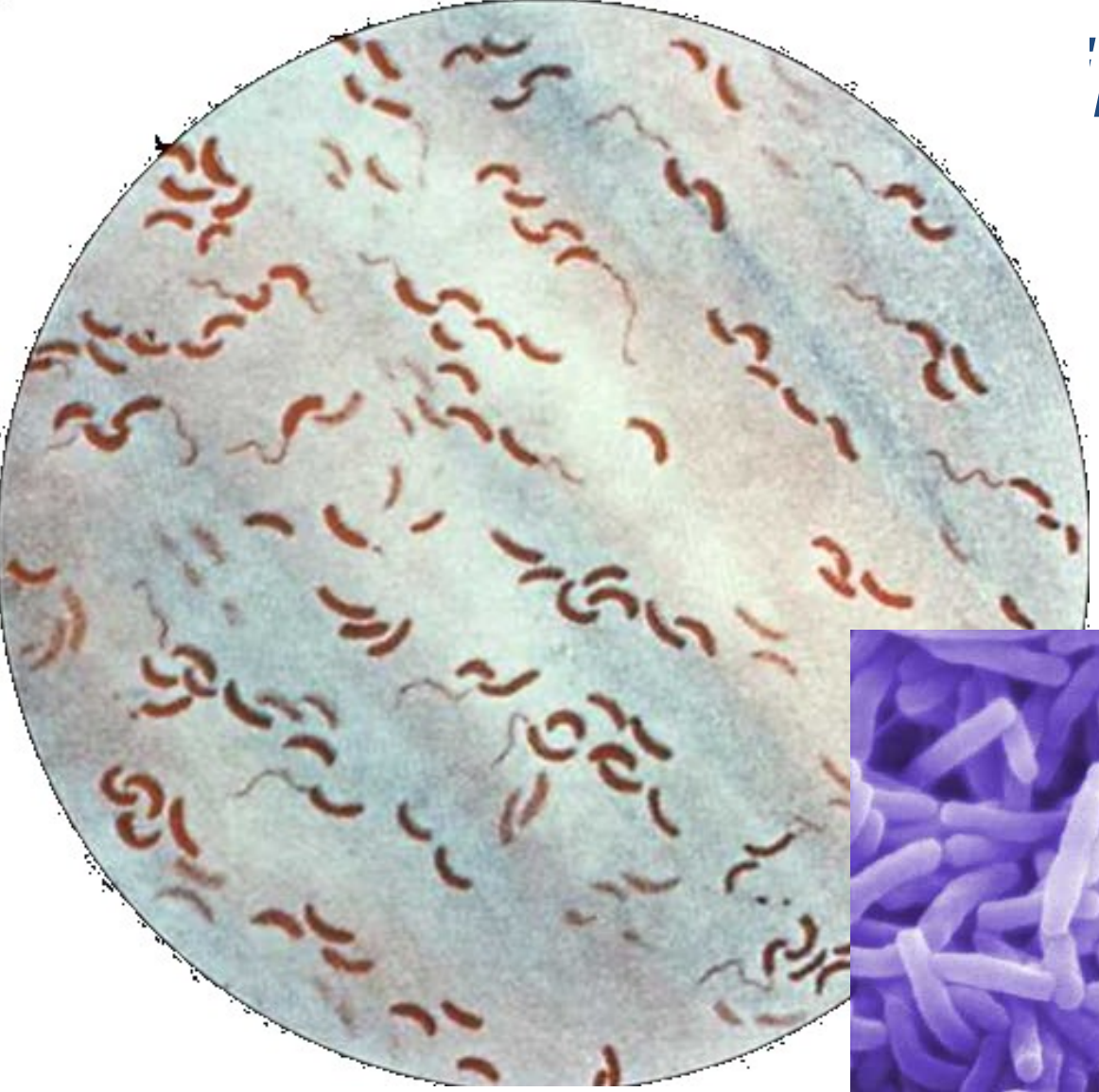
Haemolysis of *Escherichia* on blood agar is sometimes present, but it is not important for its diagnostics.

One less common *Helicobacter*

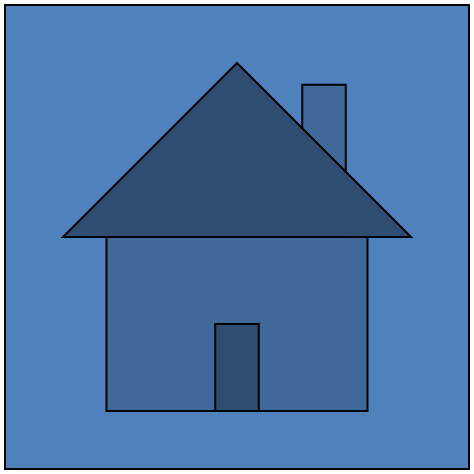
Helicobacter cinaedi

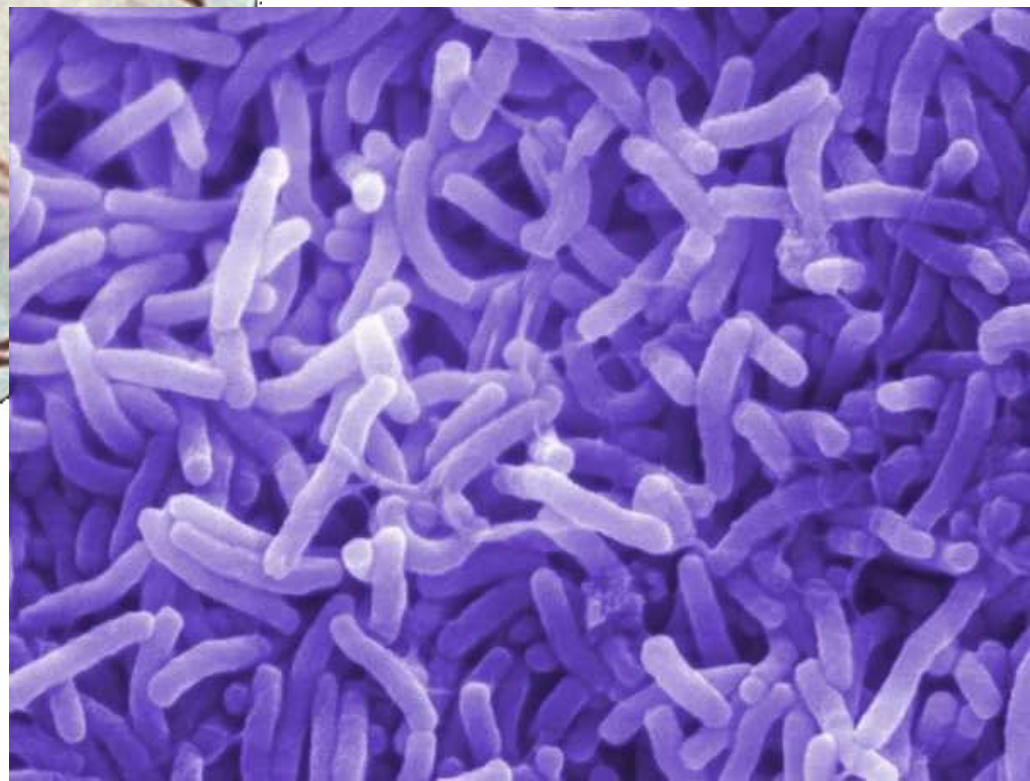

Vibrio cholerae
http://www.cs.dartmouth.edu

'brio cholerae



http://bepast.org





Diagnostics of *Enterobacteriaceae*

Enterobacteria – methods

- Direct
  - Microscopy – not very important in real diagnostic, but we will use it in our practicals
  - Culture – many various media
  - Biochemical identification – very important
  - Antigen analysis – *Salmonella, Shigella*, EPEC
- Indirect methods
  - Widal reaction in typhoid fever, antibodies against yersinia

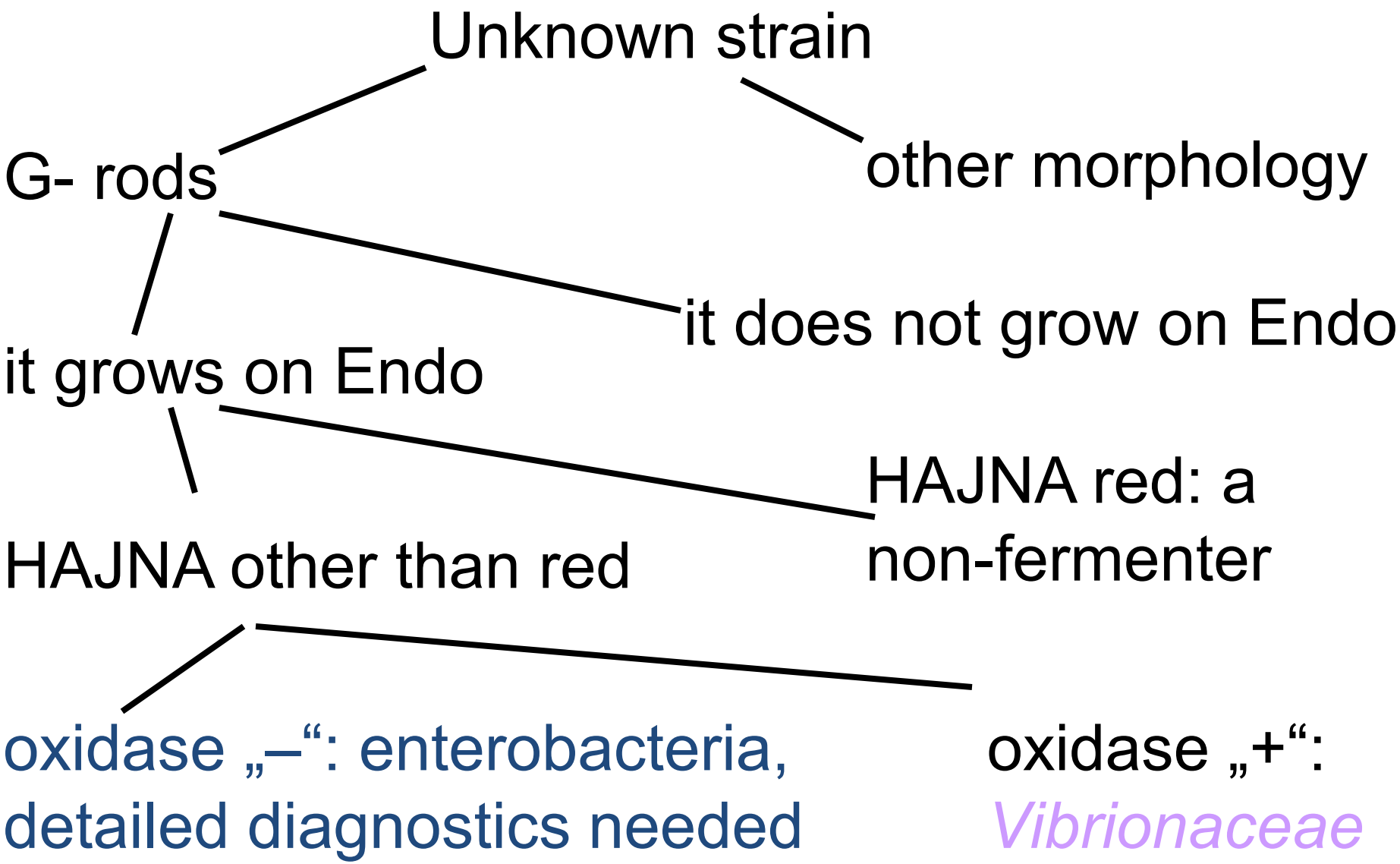
Differential diagnostics

- Gram stain differentiates Gram– rods from other bacteria
- Endo agar (I): among clinically important, only enterobacteria, *Vibrionaceae* and Gram negative non fermenters do grow
- Non fermenters may be differentiated by not fermenting glucose (Hajna medium remains red, no change of colour).
- *Vibrionaceae* are oxidase positive, unlike enterobacteria

Gram– rods: group differentiation of the three groups able to grow on Endo agar

- Enterobacteria are oxidase negative and glucose fermenters
- Vibrionaceae are glucose fermenters, too, but always oxidase positive
- Gram– non-fermenters never ferment glucose. Oxidase may be positive or negative. They are sometimes coccobacilli.

Diagnostic algorithm



Mutual differentiation of *Enterobacteriaceae*

- Endo agar (II): orientation differentiation of obligatory pathogens (usually L-) and opportune pathogens (usually L+, L = lactose)
- More media: XLD, MAL, DC, WB and more for Salmonella, CIN for Yersinia, etc.
- Biochemical tests: Hajna medium, MIU test, Švejcar plate, ENTEROtests etc.
- Antigen analysis usually using slide agglutination

Diagnostics of Campylobacter, Helicobacter and Vibrio will be discussed in a separate part.

Lactose splitting

Lactose positive bacteria have dark red surroundings on Endo agar. Lactose negative bacteria have pale colonies on the same medium.

Photo O. Z.

Culture characteristics of several enterobacteria

- On XLD agar:
  - ***Salmonella*** has pale colonies with black centre (the centre is like a yolk in a fried egg)
  - other bacteria do not grow at all, or grow in colonies of different morphology
- On MAL agar the results are similar to those on XLD agar (slightly different colours of some colonies etc.)
- On CIN agar ***Yersinia*** would grow in tiny, dark pink colonies. If no bacteria do grow on the medium, then no one of your strains is a *Yersinia*.

Biochemical testing of enterobacteria

- For biochemical testing of Enterobacteriaceae, we use various tests. In Czechia, the most common ones are ENTEROtest 16 and ENTEROtest 24. We will use the first of them.
- The first reaction is ONPG test (a test tube with reagent strip, like in VPT test in StaphyTest and StreptoTest). First row in your panel is 2nd to 9th reaction, second row is 10th to 17th reaction.

Antigen analysis

- Antigen analysis is used in some situations only, so not very commonly.
- There are two main situations like this:
  - In obligatory pathogens (*Salmonella, Shigella, Yersinia*) to make the diagnose more sure, and for epidemiological reasons
  - In intestinal isolates *E. coli* in case of suspicion for EPEC or STEC (but usually not the other groups of *E. coli*)
- Both cases are demonstrated by examples

E. coli agglutination for EPEC

- We try to detect any of 12 main serovars belonging to EPEC
- **If nonavalent serum (I, II, III) is positive**
  - we continue with three trivalent sera (I, II and III)
  - when one of them is positive, we continue with corresponding monovalent sera
- **If trivalent serum IV is positive**, we continue with monovalent sera belonging the „IV“ group
- Understand: there exist hundreds of serovars in *E. coli* species. So, the result „*E. coli*, EPEC excluded“ means „it is one of remaining cca 200 serovars“

Salmonella agglutination

- At agglutination of any motile enterobacterium, we evaluate two types of antigens: body = O antigens, and flagellar = H antigens (exceptionally also capsular K antigens).
- So, each *Salmonella*, too, has its specific antigenic structure. E. g. *Salmonella* of serovar Enteritidis has body (O) antigens type **9, 12** and flagellar (H) antigen type **m**.
- **So, if we have a *Salmonella* Enteritidis, we should find presence of agglutination both for body and flagellar antigens.**

atb = antibiotic

Tests of atb susceptibility

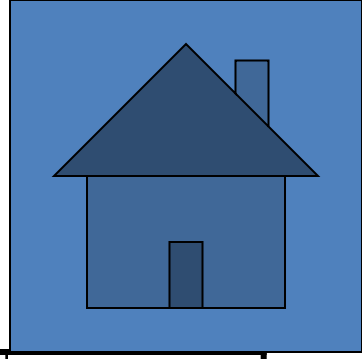
- We do not perform atb tests for stool strains. *(Usually, use of antibiotics in bacterial diarrhoea leads paradoxically to longer time of presence of the pathogen in stool; dietary treatment and probiotics in reconvalescence are then recommended rather than anitibotics.)*
- So, usually we test it in UTI origin strains, therefore also some of antibiotics are anti-UTI (like nitrofurantoin)

More to antibiotic susceptibility tests

- The test used in this task is so called „GNTM“ test – one of tests used in our laboratory:
  - ***For non-urinary infections**, we have G1 (basic test with mostly orally administered antibiotics),G2 and G3 (rather intravenous and broad spectre antibiotics).*
  - ***For UTI** the tests are adapted: GNTM (basic test) and G2M; G3 is the same as for non-urinary infections*

  Although the tests together contain 21 antibiotics in both cases, there exist beta-lactamase-producing strains susceptible to not more than 4–6 of them. For carbapenemase producing strains it is even worse.

Susceptibility zones table – example



Antibiotic	Abbrev.	„S“ if (mm)	„R“ if (mm)
Ampicillin (aminopenicillin)	AMP	≥ 14	< 14
Cefazolin (CS 1 gener.)	KF	≥ 15	< 14
Ko-trimoxazol (mixture)	SXT	≥ 16	< 13
Nitrofurantoin (nitrofuran)	F	≥ 11	< 11
Tetracyclin (tetracyclin)	TE	≥ 15	< 12
Cefuroxim (CS 2 gener.)	CXM	≥ 18	< 18
Norfloxacin (quinolone)	NOR	≥ 22	< 19

**also valid for doxycycline*

Neither S, nor R → intermediate („I“)

Diagnostics of *Campylobacter, Helicobacter* and *Vibrionaceae*

Diagnostics of *Campylobacter*

- *Campylobacter* does not grow on common media; together with its typical morphology (curved rod) the diagnostic is possible
- Look at culture results of campylobacter and write down its description
- Look at oxidase tests result (the test will be performed by your teacher as a demonstration)

Some notes to *Campylobacter* diagnostics

- They need four special conditions:
  - Their special black medium – its name CCDA is not used commonly, so it is simply „Campylobacter medium“
  - Temperature elevated to 42 °C. (Primarily, it is a bird pathogen, and bird body temperature is elevated if compared with others)
  - Elevated pCO_2
  - Prolonged culture period – not 24, but 48 hrs

Urease test in diagnostics of *Helicobacter*

- *Helicobacter,* too, does not grow on common media. It requires its own special medium, and it needs approx. five days until any growth is visible.
- Urea splitting is one of very specific test for Helicobacter. Unlike other biochemical tests used in microbiology, it can be used directly with the specimen (gastric tissue) and not with a strain. In task 8, you will see a difference between positive and negative result.

Urea breath test

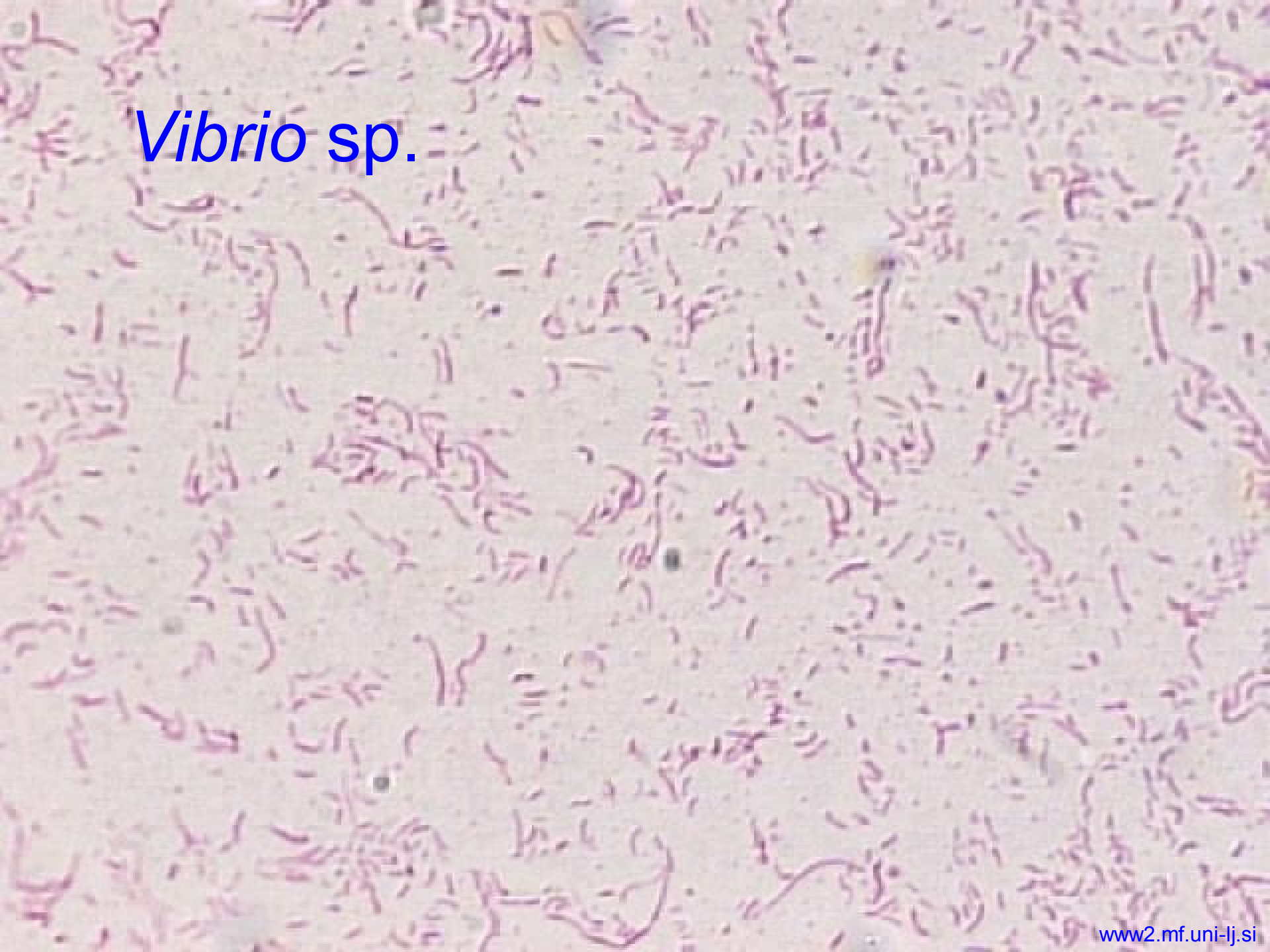
- The patient gets a mixture with urea with heavy carbon isotope (^{13}C) or radioactive carbon isotope (^{14}C)
- In healthy person urea comes to the bowel and comes out of the body with stool
- In presence of helicobacter, it is split in the stomach and labelled CO_2 is found in the air. The more CO_2, the more helicobacter

Vibrionaceae – diagnostics

- Similar to *Enterobacteriaceae*, but they are oxidase positive.
- Microscopically: motile, curved rods
- We use special media like alkali pepton water or TCBS (Thioglykolate, cystein, bile salts)
- We use similar biochemical tests as for enterobacteria
- But we have to choose „oxidase positive“ in computer system (there is no codebook for them)

Differential dg. of*Vibrionaceae*

- In **microscopy**, *Vibrio* is a curved rod (see next picture, draw it to your laboratory report)
- For **culture**, we use TCBS medium (a solid medium) and alkali pepton water (a liquid medium)
- For **biochemical identification**, we use the same Enterotest 16 as for enterobacteriaceae, but we need another codebook (or another programme in PC)
- By **antigen analysis**, we can find the major serotypes of Vibrio cholerae: O1 and O139.
- **More precise differentiation inside O1 serotype** (into biotypes Classic and El Tor) would require more biochemical tests


Vibrio sp.
www2.mf.uni-lj.si

The End

Drawn using *Proteus* and *Escherichia*