

Practicals 5WT enterobacteriaceae

- Enterobacteriaceae – characteristic, structure, types of diseases,
- sampling – *E. coli*, *Proteus sp.*, *Klebsiella sp*
- Microscopy – *E. coli* - Gram,
- capsule detection *K. pneumoniae* - Burri
- Cultivation – *E. coli* and *K.pneumoniae* on blood agar and Endo agar -
- *P. mirabilis* – Rauss phenomenon - motility
- Biochemical properties - Enterotests, - reading of Enterotest *E. coli*, *Kl. Pneumoniae*, *P. vulgaris.*, - biochemical diagnostic cultivation medium -TSI agar (Hajn)
- Serology – antigenic structure detection of unknown strain polyvalent and monovalent sera – slide agglutination

G- rods

- 1) Facultatively anaerobic : _____ **Enterobacteriaceae - E. coli, Salmonella, Schigella**, Enterobacter, Citrobacter, Serratia, **Klebsiella, Proteus**, Morganella, Providencia
- Vibrionaceae - Vibrio, Aeromonas, Plesiomonas
Campylobacter, Helicobacter
- Widest and most heterogenous group of medically important bacteria
- 27 genus, 102 species (95% medically important ones belong to 25 species) - differentiation based on DNA homology, biochemical properties, antigen characteristics, ATB susceptibility
- Commonly present in nature (soil, water, plants), part of physiological flora of colon.
- Obligatory pathogenic (Yersinia pestis, salmonella, schigella) **oportunistic pathogens (Klebsiella, E. coli, Proteus)**
- Transmission from animals (Salmonella), from human carrier (S. typhi), **endogenous infection (E.coli)**

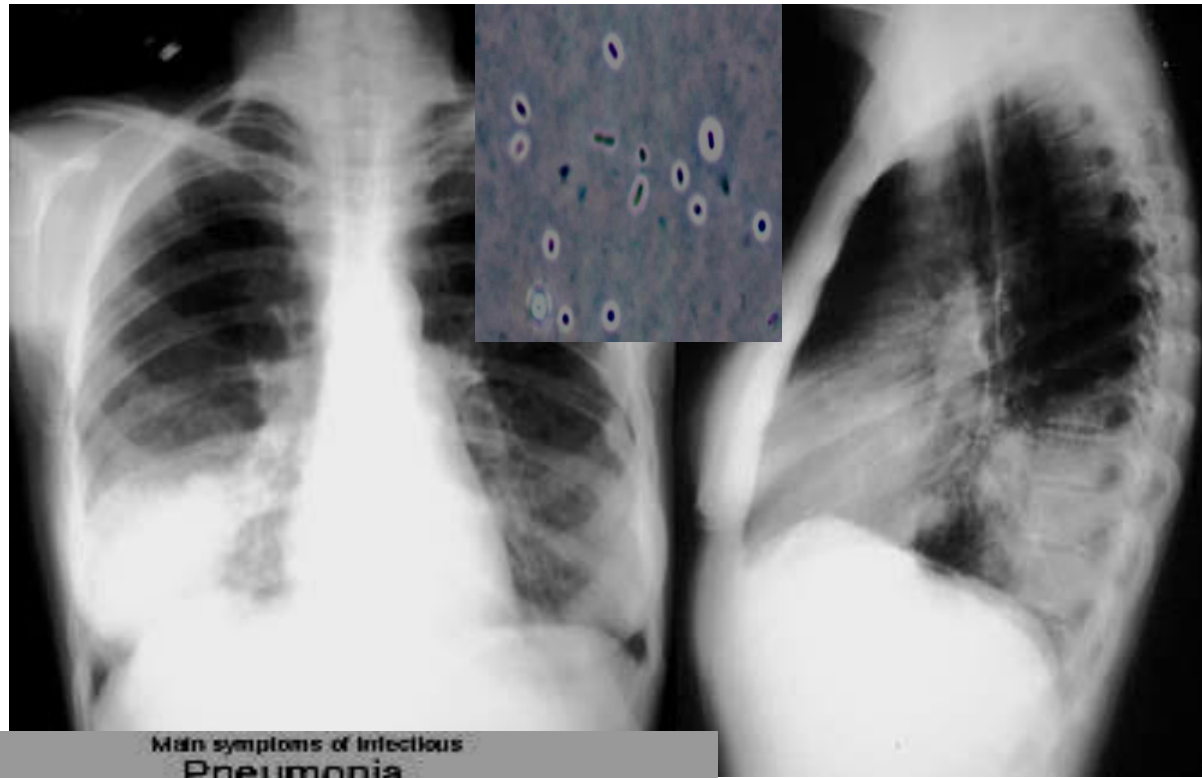
Physiology and structure

- G-rods, usually motile (flagella)- besides Klebsiella, non spore - forming, facultative anaerobe, poor nutrition requirements, biochemical active,
- catalase +,
- cytochromoxidase COX negat = dif.dg.from other G - rods
- Fermentation of lactose + or – (pathogenic Salmonella, Shigella, Yersinia, a Proteus are lactose negative), capsule (Klebsiella)
- 3 groups of antigens:
 - - somatic O antigen – most important antigen of cell wall, heat resistant, part of LPS – composed of 3 parts - O polysachride, core polysacharide and lipid A – with endotoxin activity
 - - capsular K antigen – heat susceptible, In Salmonella typhi known as Vi antigen.
 - - flagellar H antigen - termosusceptible,

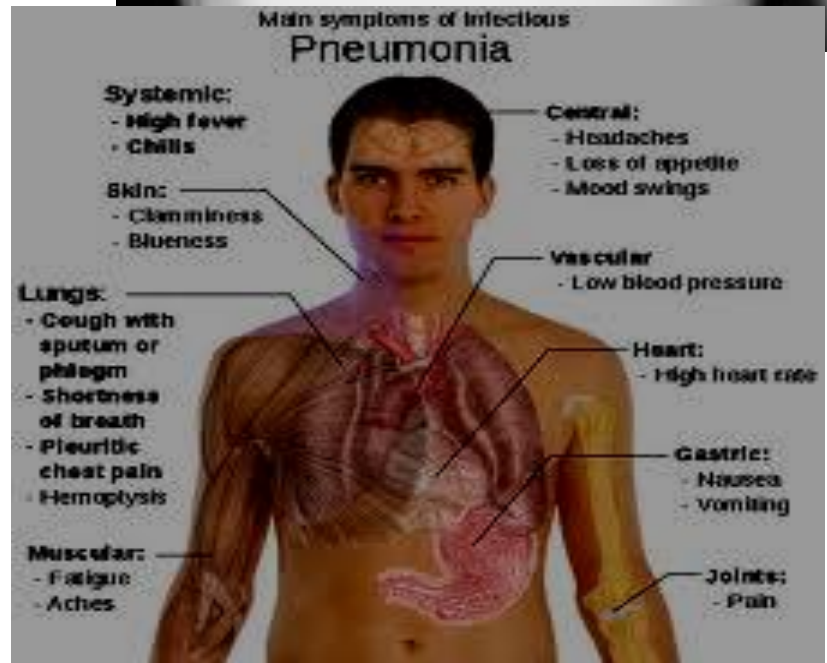
Klebsiella sp.

- Encapsulated – mucous colonies, increased virulence, non motile
- *Klebsiella pneumoniae* –
 - - pneumonia (necrotic destruction of alveolar spaces, bloody sputum).
 - - Infections of wounds, soft tissues and urinary tract.
- *K. rhinoscleromatis* - scleroma
- *K. ozaenae* – atrophic disease of nasal epithelium

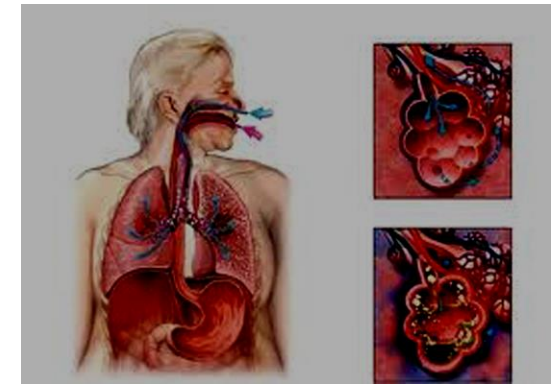
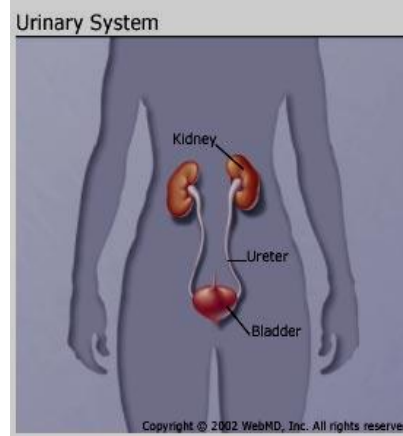
klebsiella...



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Proteus



- Motile G- rod, Rauss phenomenon
- *P. mirabilis*, *P. vulgaris* –
- infections of urinary tract,
- production of urease (lysis of urea, alcalinisation, increased possibility for calculi formation, toxicity for epithelium
- *Proteus vulgaris*



Salmonella

Salmonella infection

Almost any kind of food or beverage can carry the bacteria that causes salmonella infection, although meat and eggs the most are common sources.

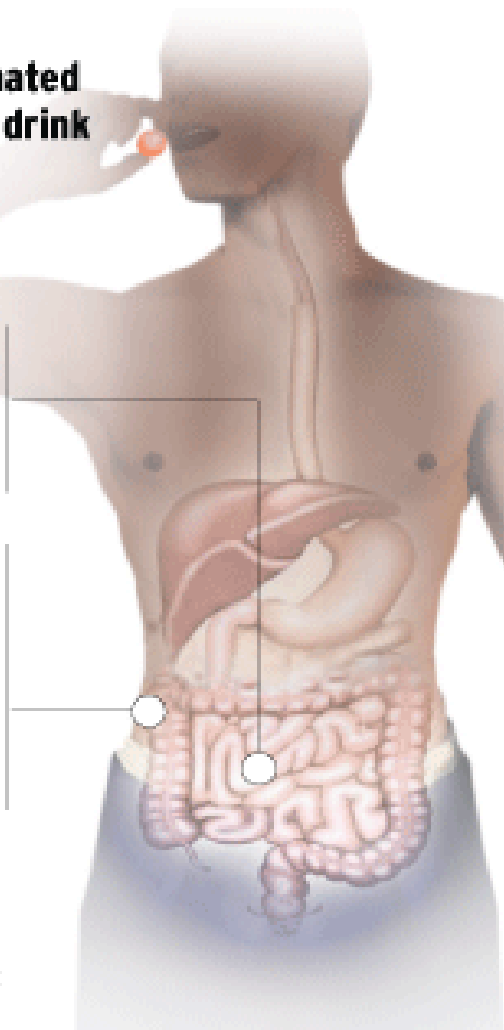
**Contaminated
food or drink**

How salmonella progresses

Bacteria travel to small intestine, adhere to lining; begin life cycle

In severe cases, bacteria break through intestinal wall to bloodstream; can be deadly if not properly treated

Source: U.S. Food and Drug Administration, Current Medical Diagnosis & Treatment, Mayo Clinic



Symptoms

Within 12-72 hours

Nausea, vomiting, fever, diarrhea abdominal cramps

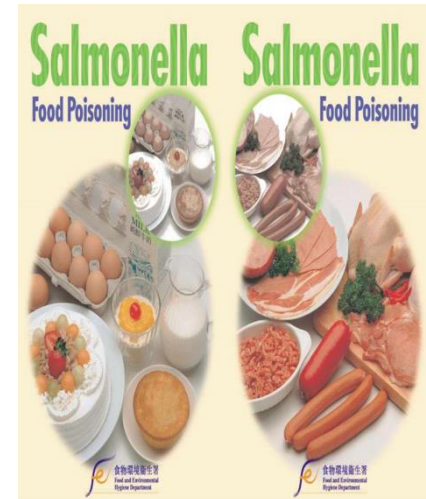
4-7 days Illness ranges from mild to severe; most people recover without treatment

Severe cases More likely with infants, elderly, people with impaired immune systems

Treatment

Oral or injected antibiotics, usually for 2 weeks

McClatchy-Tribune



Laboratory dg



- growing well on non selective media
- Selective media for detection of pathogens - lactose negative strains Salmonella, Shigella, Yersinia
- Biochemical identification – TSI agar (Hajn) medium, Enterotests – group of biochemical tests
- Serodiagnosis – detection of antigenic structure – Salmonella, Shigella, E.coli
- Interpretation of results
 - in stool sample – Salmonella, Shigella, Yersinia – pathogens, other enterobacteriaceae – according the the state of eubiosis, when overgrowth - from
 - physiologically sterile materials: urine – UTI, ascendent infection, blood – most improtant etiological agens, wounds, sputum – in monoculture, nose and throat sample - monoculture

CULTIVATION

- Blood agar
- Endo agar
- DC (dezoxycholat-citrate) agar

Blood Agar

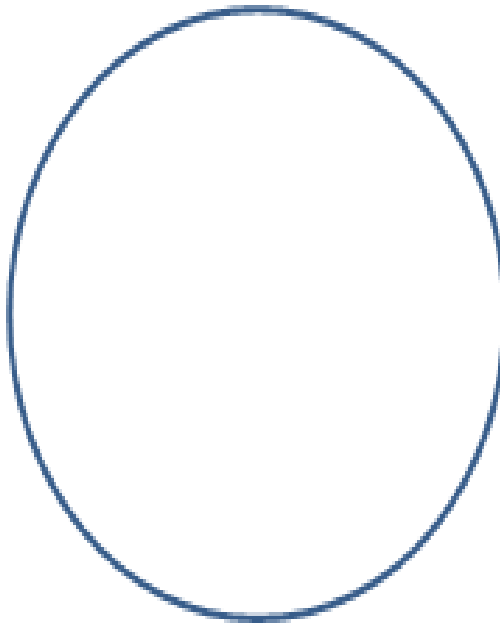
4. Cultivation - *E. coli*, *K. pneumoniae*, *Salmonella sp.*, *Shigella sp.* dan *P. vulgaris* – blood agar, Endo agar (EA), Deoxycholate citrate agar (DCA).

Blood agar	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Shigella sp.</i>	<i>Salmonella sp.</i>	<i>P. vulgaris</i>	<i>P. mirabilis</i>
<u>colony</u>						

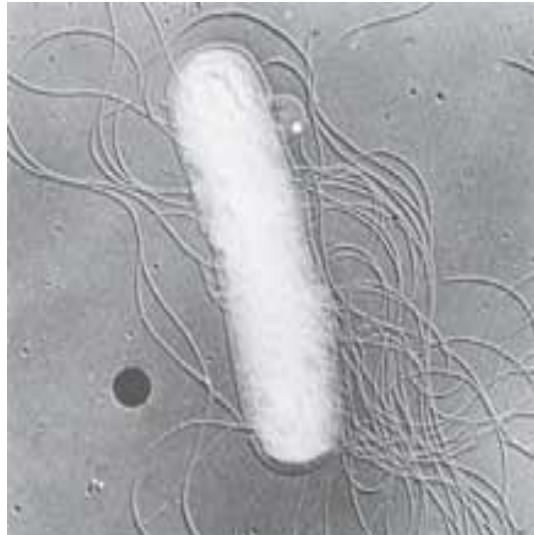
Enteric organisms cultivated on blood agar usually reveal large, smooth, shiny, circular, raised colonies which may or may not be hemolytic or pigmented.

Proteus often exhibit swarming.

RAUSS phenomenon

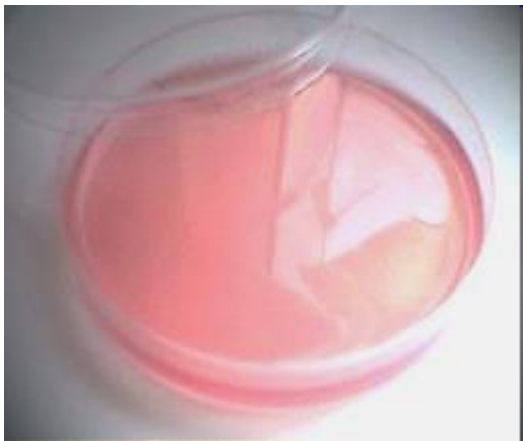


Proteus sp. on blood agar
-for most strains of *P. mirabilis* and *P. vulgaris* is typical their ability to swarm (**RAUSS phenomenon**) over the surfaces of solid cultivation media (the spreading growth covers other organisms in the culture and thus delays their isolation).



ENDO agar

- Endo Agar is a differential and slightly selective culture medium
- for the detection of coliform and other enteric microorganisms.
- culture medium for the differentiation of **lactose fermenters from lactose non-fermenters**
- Inhibition of gram-positive microorganisms achieved by the sodium sulfite and **basic fuchsin** contained in the formulation.



Deoxycholate-citrate agar(DCA)

- selective medium
- isolation of **enteric (G negative)** pathogens
- Differentiation of *Salmonella* and *Shigella* species
- medium is selective for enteric pathogens ... increased concentrations of both citrate and deoxycholate salts.
- Sodium deoxycholate and Citrate salts are inhibitory for Gram-positive bacteria.
- Lactose
 - lactose fermenters produce red colonies
 - lactose non-fermenters produce colourless colonies

***Salmonella* may produce H₂S –**

colorless colonies with black centers

Klebsiella a escherichia

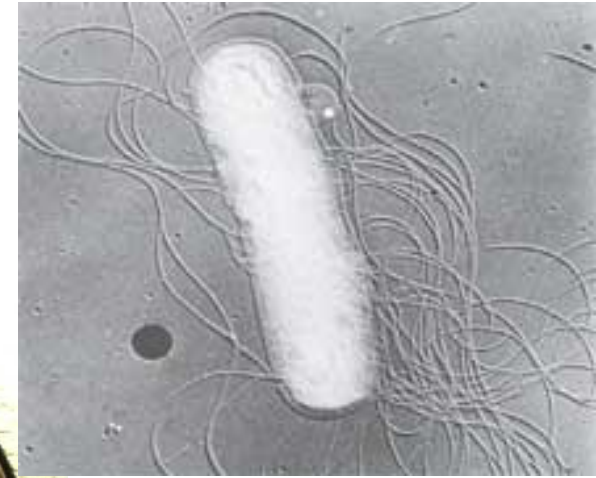


Salmonella

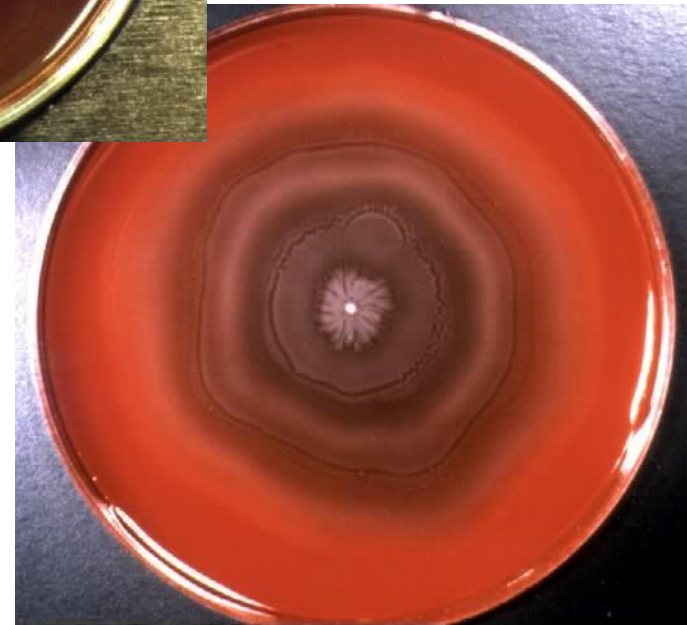
Shigella



Proteus mirabilis, *P. vulgaris*



RAUSS phenomenon



- Endo agar.

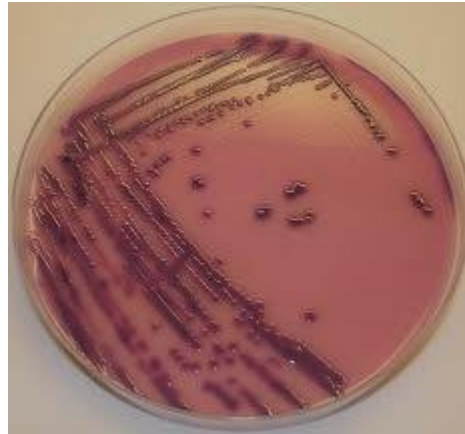


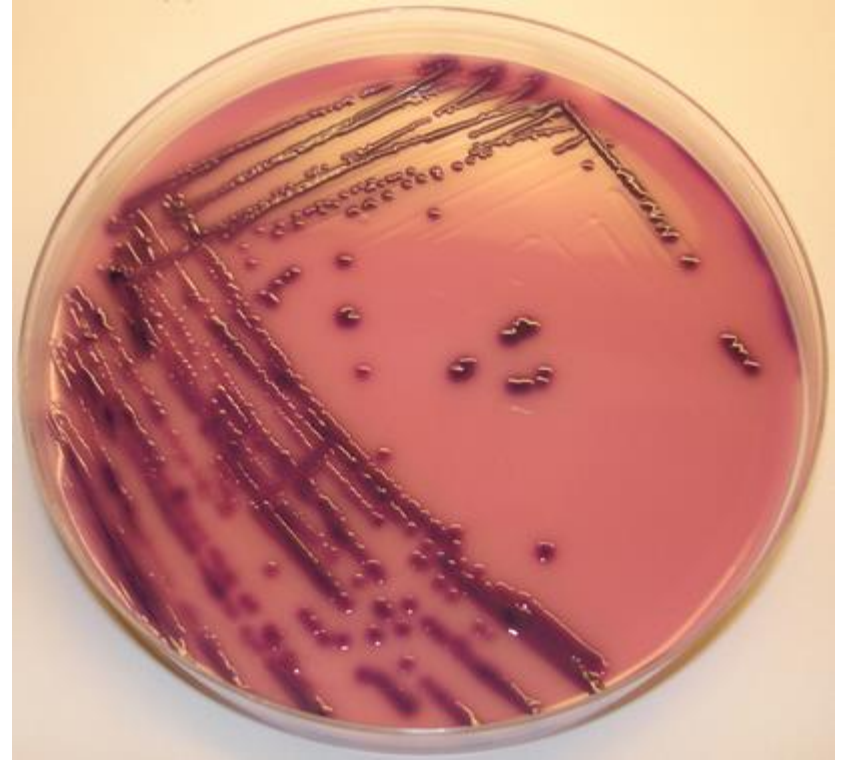
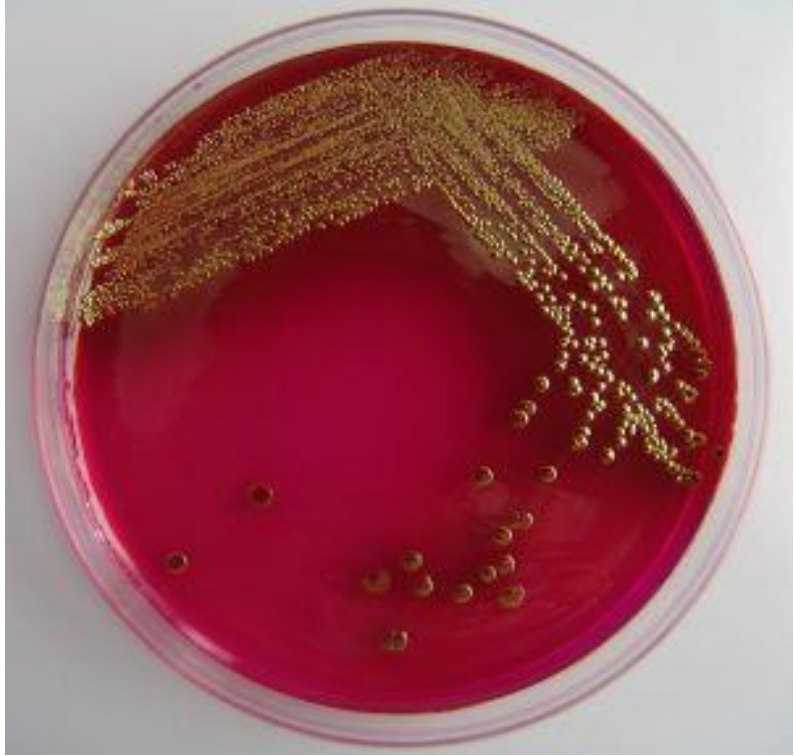
Endo

Salmonella

E.coli

Shigella





DCA



Tests for biochemical properties and metabolic activity testing

Aim – final identification

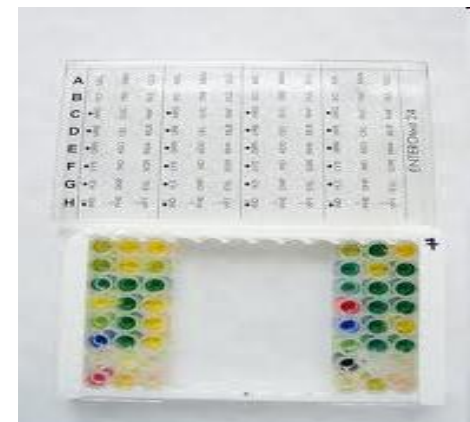
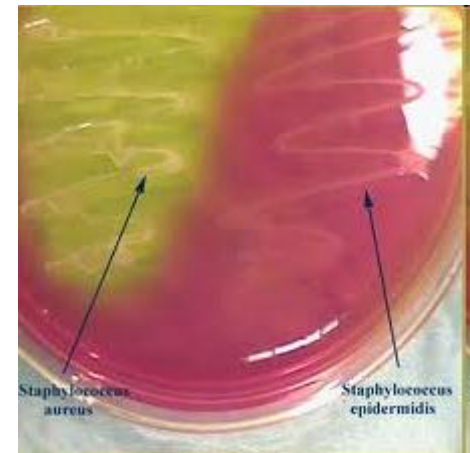
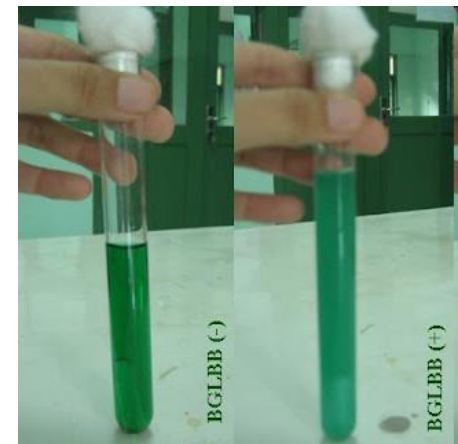
Method – subcultivation on the series of testing diagnostic media

-liquid media – with chemical structure – substrate and indicator,

-solid media with biochemical – metabolic substrate and indicator

-diagnostic disc with substrate, micromethods
– liquid media with substrate and indicator in microwells

Algorithm – of chosen procedures



Tests for biochemical properties and metabolic activity testing

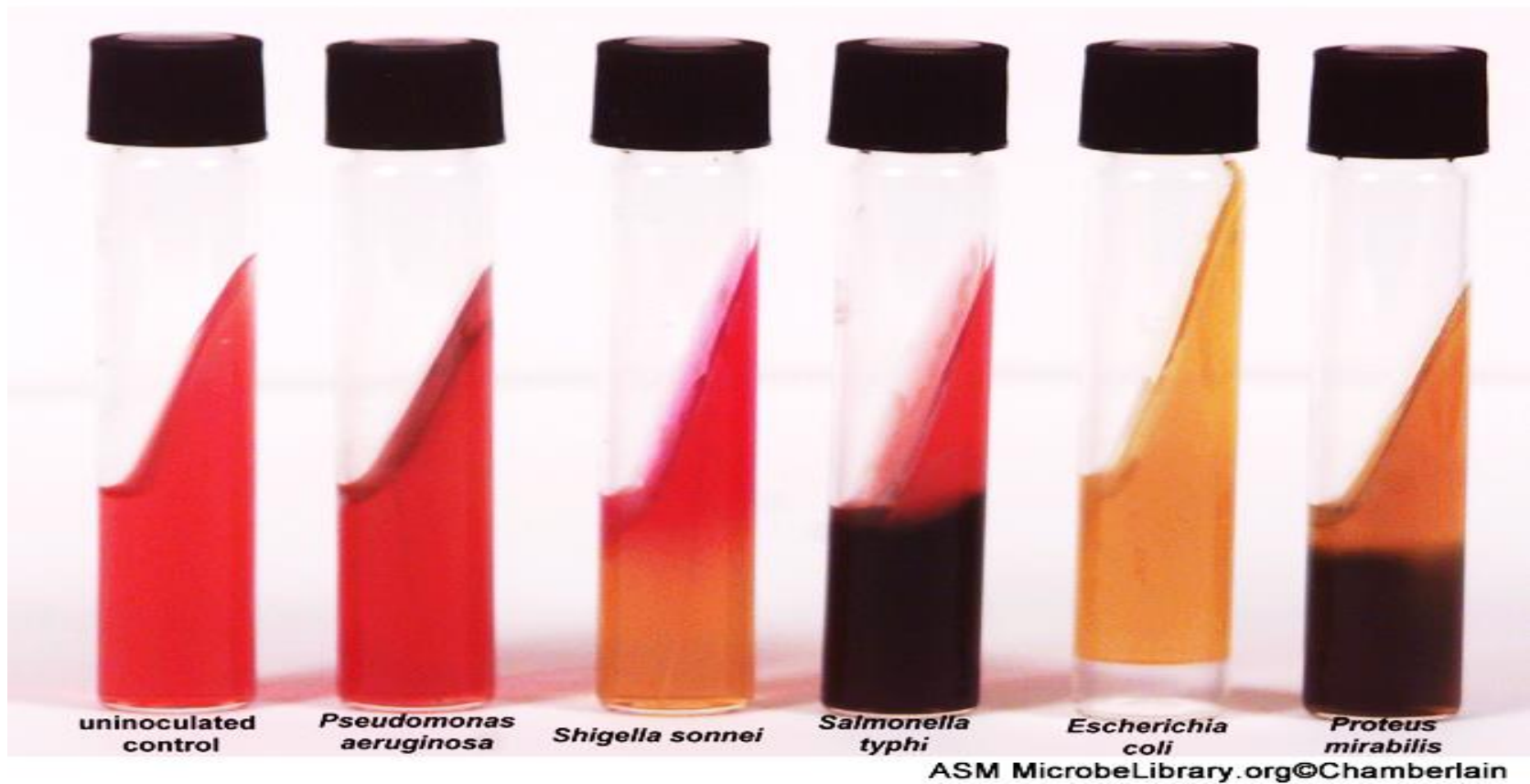
ENTEROBACTERIACEAE

- TSI (HAJN) agar
- ENTEROTEST

TSI agar (Hajn)

- The **Triple Sugar Iron** or **TSI**
- to test microorganism's ability to ferment sugars and to produce H₂S [hydrogen sulfide](#).
- It is often used in the selective identification of enteric bacteria including Salmonella and Shigella.
- test tube contains:
 - agar
 - pH-sensitive dye (phenol red),
 - 1% lactose, 1% sucrose, 0.1% glucose,
 - Sodium thiosulfate and ferrous sulfate





Surface of tube medium – aerobe environment, lactose negative bacteria do not ferment, it is alkaline, red

Lower part - in anaerobe environment, enterobacteria ferments glycid – acidic – yellow or black H₂S .

1 negative control 2 Ps.aeruginosa : net fermenting - red

3 Shigella sonnei: H₂S - negat., gas – negat., TSI – acid/alcalic red/yellow

4 Salmonella typhi: H₂S – pozit., gas–negat., TSI – acidic/alcalic red/yellow

5 Escherichia coli: H₂S – negat., gas -posit., TSI – acid/acidic red/red

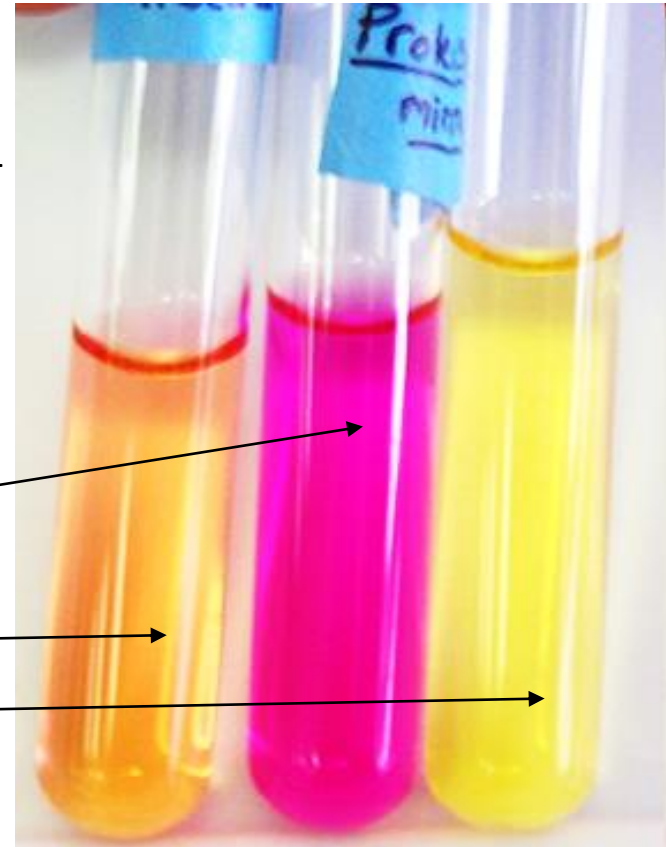
6 Proteus mirabilis: H₂S – posit, gas - negat., TSI – acid /acid red/red

Urease test

- This test is used to identify bacteria capable of hydrolyzing urea using the **enzyme urease**.
- The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and **the pH indicator, phenol red, turns from yellow to pink.**
- *Proteus mirabilis* is a rapid hydrolyzer of urea (center tube pictured here).
- The tube on the far right was inoculated with a urease negative organism and
- the tube on the far left was uninoculated.

Helicobacter pylori

has urease activity that hydrolyse urea (making so a good environment– NH₄ – for surviving in acidic stomach)



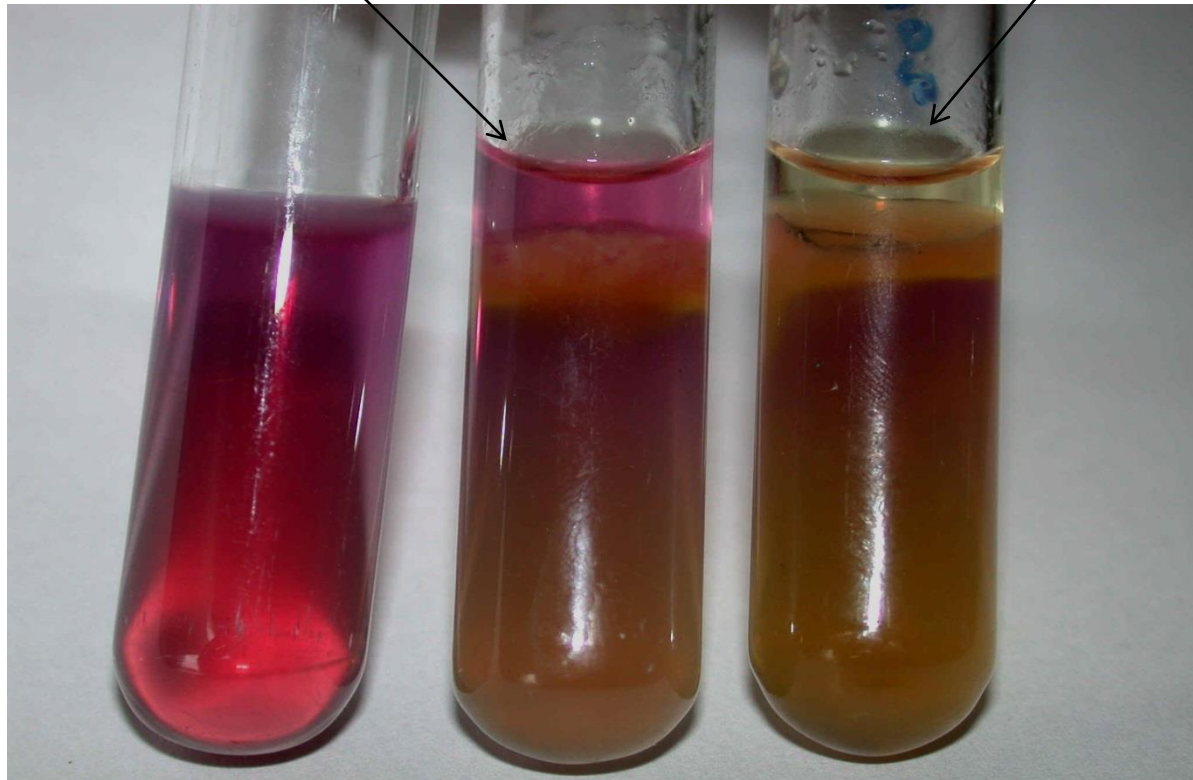
Simmon's Citrate Agar



- This is a defined medium used to determine if an organism can use citrate as its sole carbon source.
- It is often used to differentiate between members of *Enterobacteriaceae*. In organisms capable of utilizing citrate, the **enzyme citrase** hydrolyzes citrate into oxaloacetic acid and acetic acid.
- If CO₂ is produced, it reacts with components of the medium to produce an alkaline compound.
- **The alkaline pH turns the pH indicator (bromthymol blue) from green to blue.**
- This is a positive result, the tube on the right is **citrate positive**.
- *Klebsiella pneumoniae* and *Proteus mirabilis* are examples of citrate positive organisms.
- *Escherichia coli* and *Shigella dysenteriae* are citrate negative.

Indol

P. vulgaris + *P. mirabilis*-



Group of biochemical tests aligned so that they allow numeric identification based on statistical probability of the result of one test

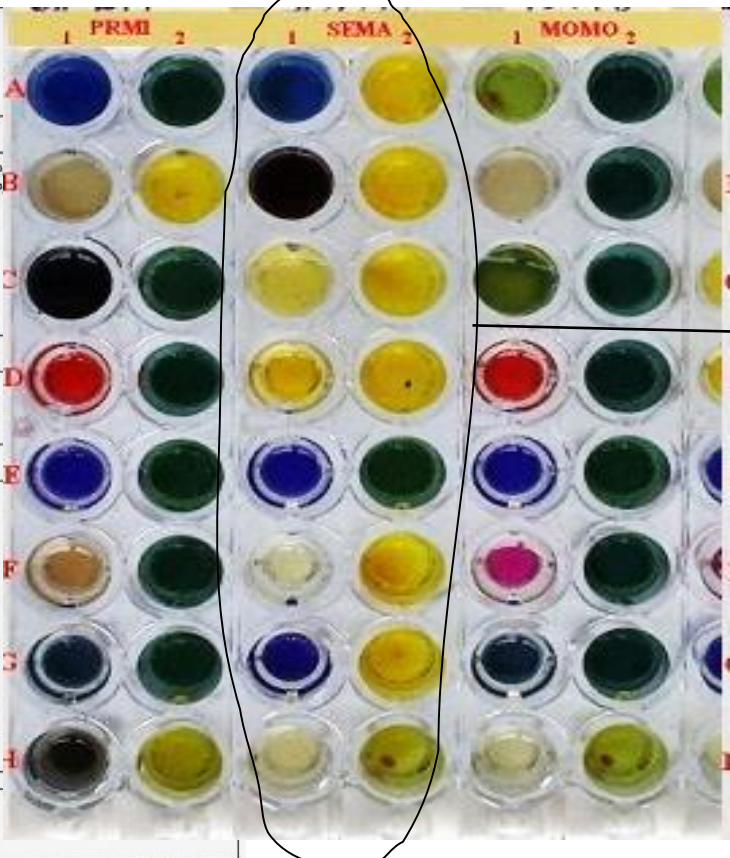
In the positive result the well is attributed the chifer according to the position in the triplet.

(1 2 or 4)

Addition of chifers in triplet gives the number and each result of the triplet gives a subsequent one position of the code that is the combination of numbers of tested triplets.

This code is corresponding to one bacteria (526663 – Serratia marcescens)

Sloupec Column	Zkratka Code	Reakce Reaction			
		Pozitivní Positive		Negativní Negative	
Rádek 1 - Row 1					
H	H ₂ S	●	●	○	○
G	LYS	●	●	●	●
F	IND	●	●	●	●
E	ORN	●	●	●	●
D	URE	●	●	●	●
C	PHE	●	●	●	●
B	ESL	●	●	●	●
A	SCI	●	●	●	●
Rádek 2 - Row 2					
H	MAL	●	●	●	●
G	IND	●	●	●	●
F	ADO	●	●	●	●
E	CEL	●	●	●	●
D	SUC	●	●	●	●
C	SOR	●	●	●	●
B	TRE	●	●	●	●
A	MAN	●	●	●	●
OXI test	OXI	●	●	○	○
ONP test	ONP	●	●	○	○



MIKRO-LA-TEST® Datum/Date: Jara Sprac./Sprac.: Ref./Refer. spolec. PLIVA - Lachema a.s.
Karásek 1
621 33 Brno
CZECH REPUBLIC

ENTEROtest 16

Kmen č. Kmeň č. Strain No./No. analýza Poznámky/Notes/Примечания

Příloha Strip Пролога	ENTEROtest 16																
	Rádek/Radok/Strip/Строчка 1						Rádek/Radok/Strip/Строчка 2										
	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	A	
OXI	ONP	ILS	LYS	IND	ORN	URE	PHE	ESL	SCI	MAL	IND	ADO	CEL	SUC	SOR	TRE	MAN
	1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
		+	-	+	-	+	-	+	+	-	+	+	-	+	+	+	+
		5			2			6		6			6			3	

Profil/Profile/Профиль

Dodatek testy/Additional tests/Дополнительные тесты

Identifikace/Identifikácia/Identification/Идентификация
SERRATIA MARCESCENS

3/01

Slide agglutination - serotyping

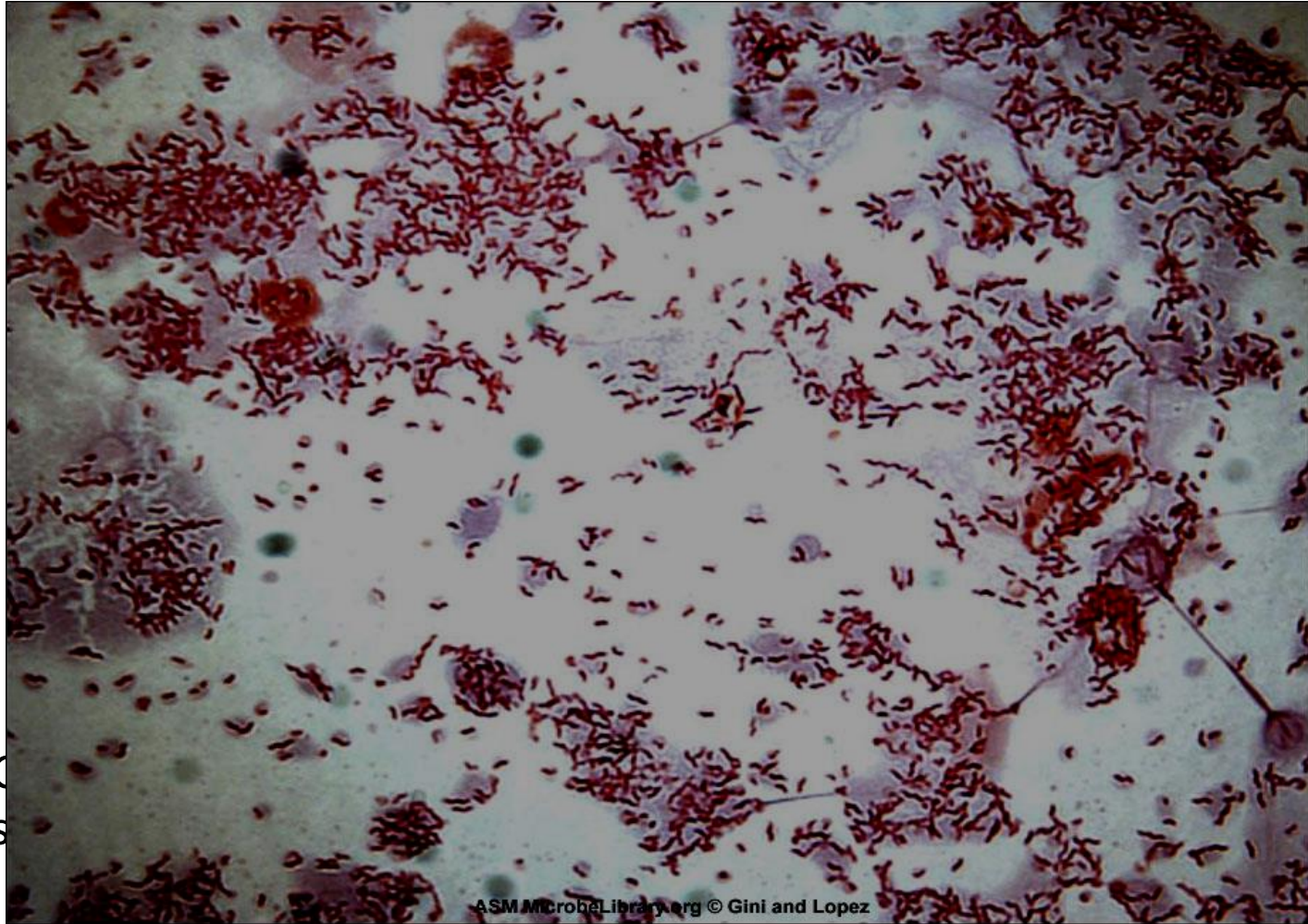
- On slide – suspension with FR
- 1) Suspension + drop of polyvalent serum
 - suspension + poly ABC = aggl+ S+ poly DEF = aggl–
- 2) Suspension + drop of monovalent serum
 - susp + mono A = aggl.+ susp + mono B = aggl –
 - susp + mono C = aggl.–
- result – tested strain is type A

Campylobacter jejuni

- Colonies are flat, droplet-like,
- Older colonies become white to salmon-coloured.
- The colonies have a very characteristic odour.
- This culture has been incubated for 48 hours in an atmosphere containing 5% oxygen and 10% carbon dioxide.



Campylobacter jejuni

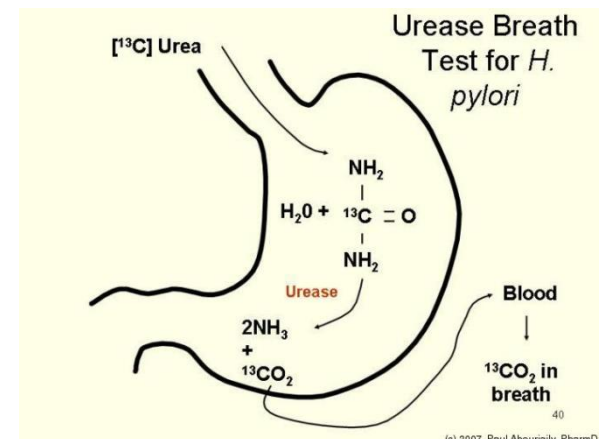


• C
S

ull or

Helicobacter pylori

- gram negative,
- microaerophilic,
- curved bacillus.
- It is motile, has flagellae
- affinity for human gastric mucosa
- ability to produce **urease**

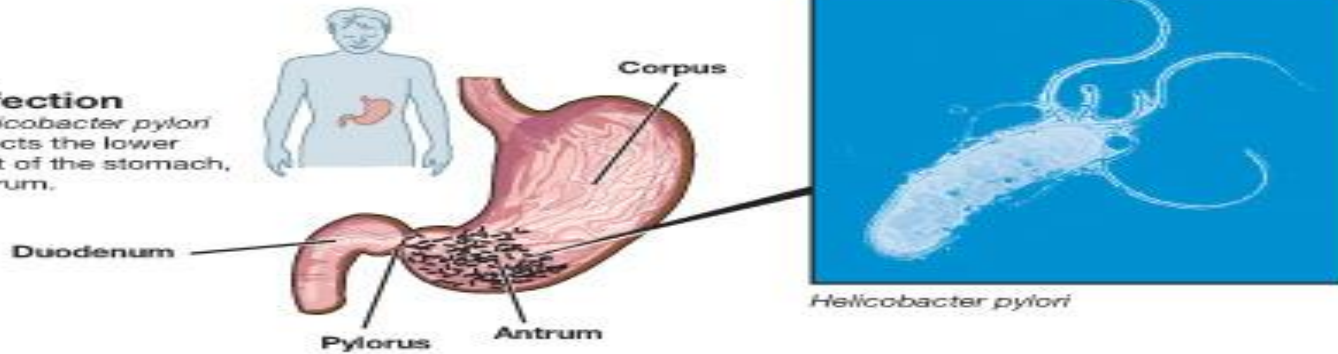


Helicobacter pylori

— the bacterium causing peptic ulcer disease

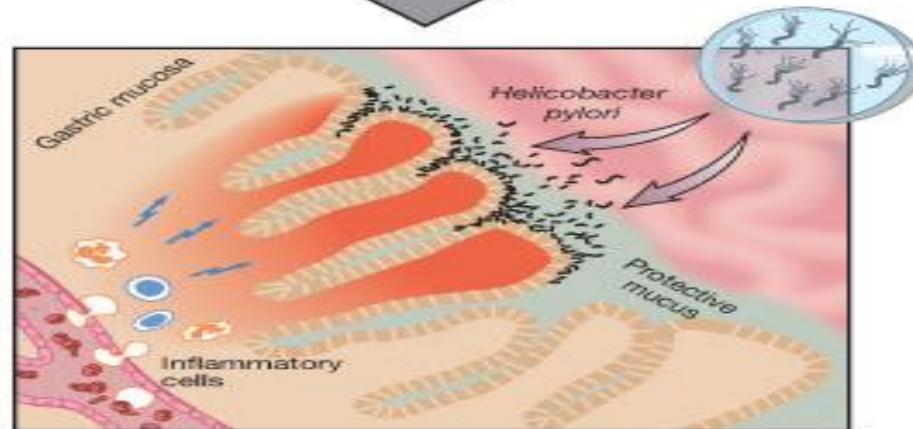
Infection

Helicobacter pylori infects the lower part of the stomach, antrum.



Inflammation

Helicobacter pylori causes inflammation of the gastric mucosa (gastritis). This is often asymptomatic.



Ulcer

Gastric inflammation may lead to duodenal or gastric ulcer. Severe complications include bleeding ulcer and perforated ulcer.



Helicobacter pylori - dg

Urea breath tests - simple and noninvasive, diagnosis of *H. pylori* infection.

- *H. pylori* produces large quantities of the enzyme urease
- Patients ingest a solution containing labeled urea and an exhaled breath is sampled for isotope-labeled CO₂ released by intragastric *H. pylori* urease activity.
- The test can be completed within 20 minutes and is highly sensitive and specific

1. Patient drinks HN-¹⁴C-NH₂.
In the stomach, HN-¹⁴C-NH₂ is broken down by urease into H¹⁴CO₃ and NH₄.
2. H¹⁴CO₃ travels to the lung and is...
3. ...expired...
4. ... as ¹⁴C₂ into...
5. ... a 0.5 mM hyamine solution, where a scintillation cocktail is added to test for ¹⁴C.

