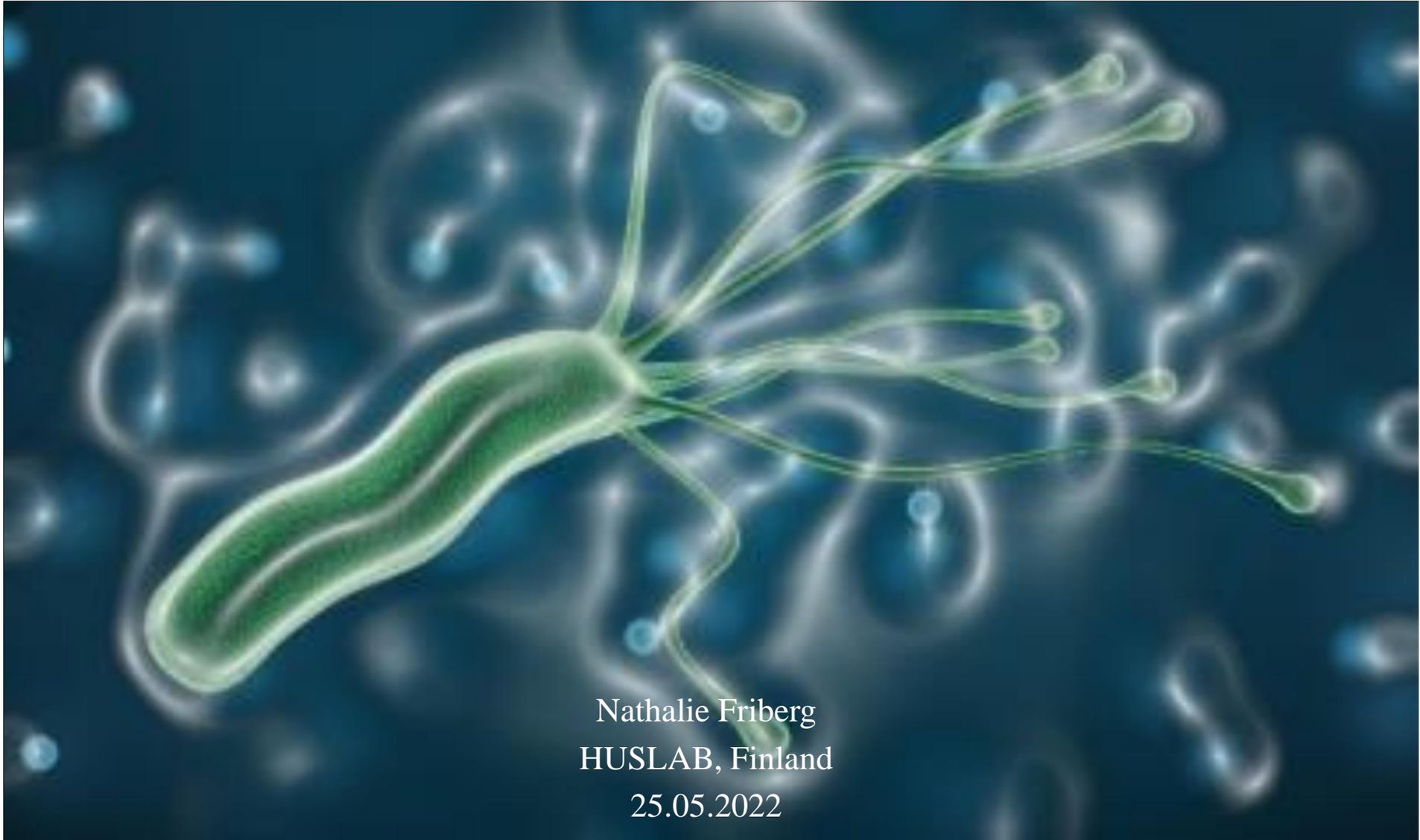


***Helicobacter pylori*:**
clinical picture, AST, PCR and treatment

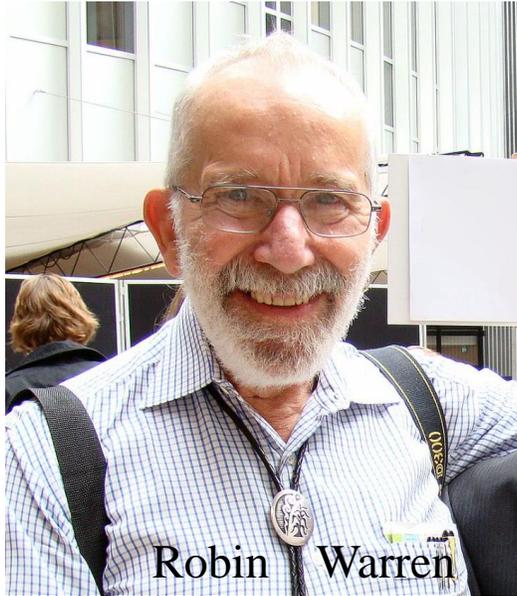


Nathalie Friberg
HUSLAB, Finland
25.05.2022

Clinical picture

Helicobacter pylori

- subdivision of *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*
- **microaerophilic spiral-shaped gram-negative rod**
- **cocoid shapes** could be observed after prolonged culture or antibiotic treatments (dead cells or viable non-culturable state?). May enable the organism to survive outside the human host (in feces or in drinking water)
- In 1893 Bizzozero reports for the first time the presence of bacteria in human stomach
- Efforts to culture bacteria failed
- No link to disease was made



- In the 80's Warren and Marshall were the first to address Koch's postulate:
 - First they managed to **isolate** and **cultivate** bacteria from human stomach
 - Later Marshall and Morris practiced **self-ingestion** and **infection of healthy volunteers** and observed that *Helicobacter pylori* can colonize human stomach and induce inflammation of gastric mucosa
 - Morris developed a persistent gastritis, which resolved after **treatment** with doxycycline and bismuth subsalicylate

Transmission and epidemiology

- worldwide, all ages
- 50% of world's population might be infected
- Exact transmission mechanisms still unknown
- *H. pylori* is a strict human pathogen and probably only **human-to-human transmission**
- oral-oral OR fecal-oral OR both
- acquisition occurs during early childhood
- **Large geographical variations:** in developing countries > 80% population is *H. pylori* positive (also in children), in industrialized countries generally < 40% (low rates in children)
- Correlation with **socioeconomic status** and **living conditions in childhood**

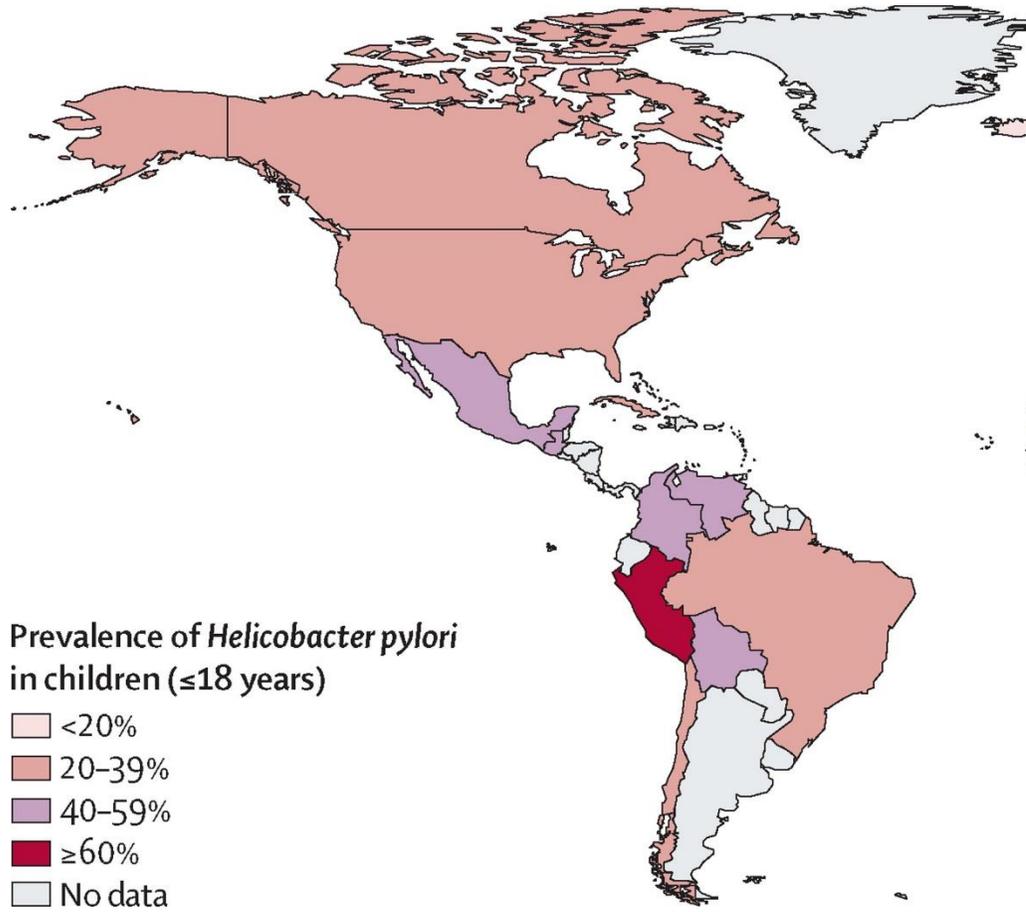
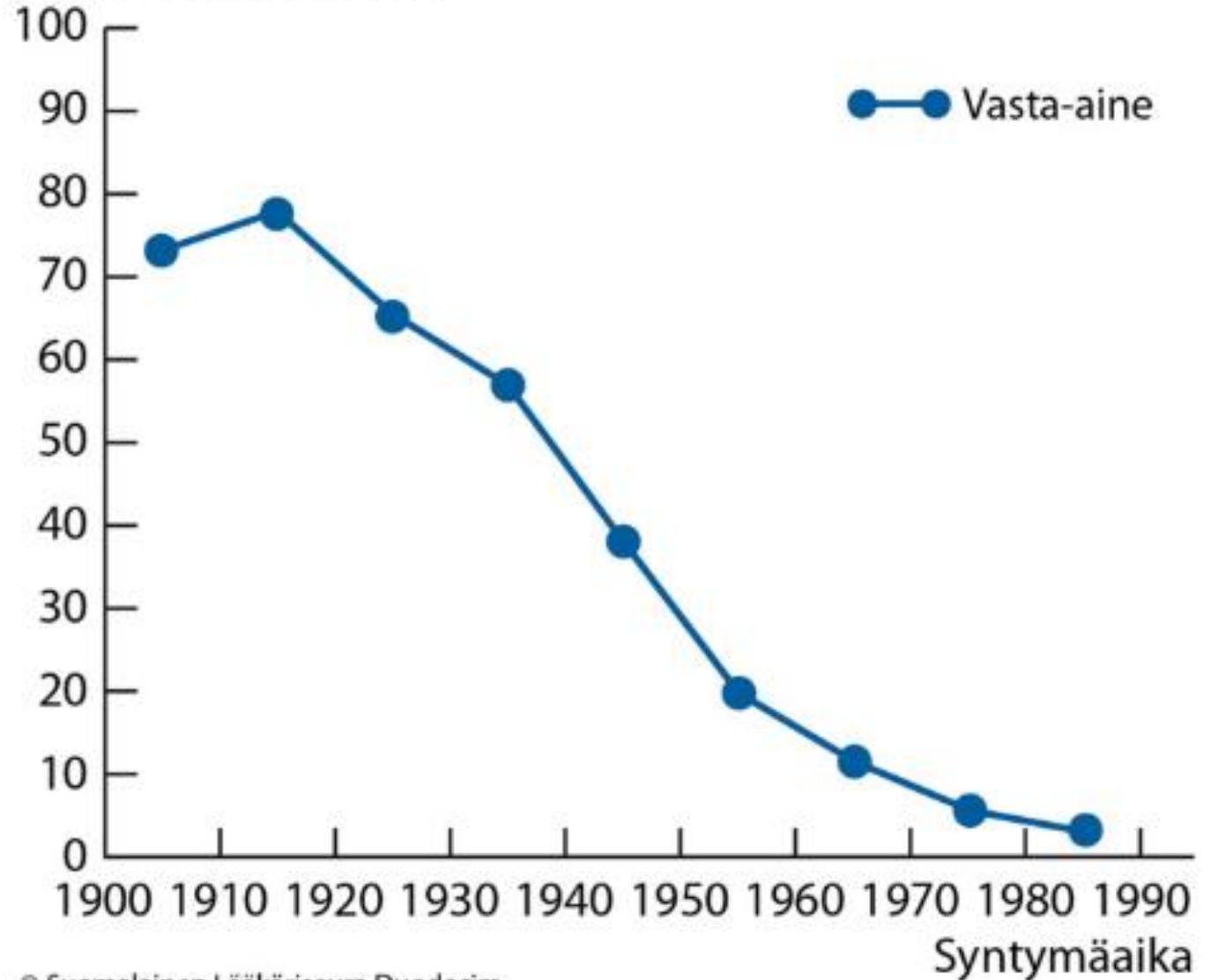


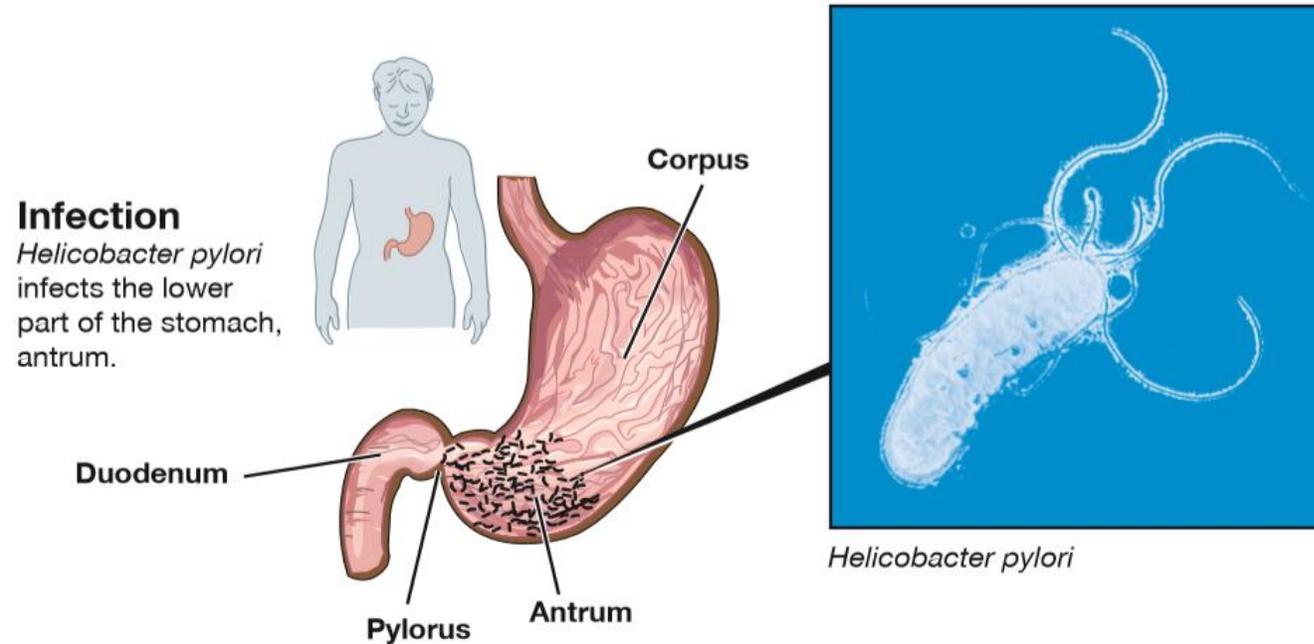
Figure 3: Estimated prevalence of *Helicobacter pylori* infection among children in 60 countries

Lancet Child Adolesc Health. 2022 Mar;6(3):185-194.

Seropositiivisuus (%)



Gastric adaptation of *Helicobacter pylori*



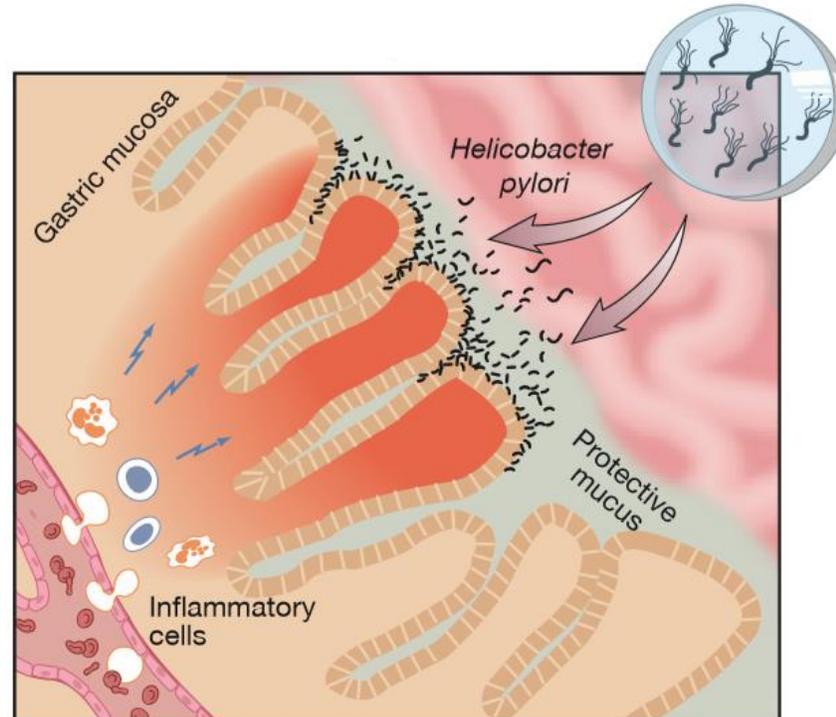
© The Nobel Committee for Physiology or Medicine

- **urease**: neutralises gastric acid by hydrolysing gastric luminal urea to ammonia
- **spiral shape** , **flagella** and **mucolytic enzymes**: facilitate passage through mucus
- surface **adhesins**: attachment to gastric epithelial cells
- others virulence factors: cagA protein, VacA vacuolating cytotoxin, LPS etc...

- *H. pylori* lives within or beneath the gastric mucous layer
- Once acquired, *Helicobacter pylori* persists on gastric mucosa
- Renders the underlying mucosa more vulnerable to acid peptic damage by disrupting the mucous layer, liberating enzymes toxins and adhering to gastric epithelium
- Chronic inflammation (gastritis) with altered gastric secretion
- In most patients stays asymptomatic and no progression of the disease

Inflammation

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



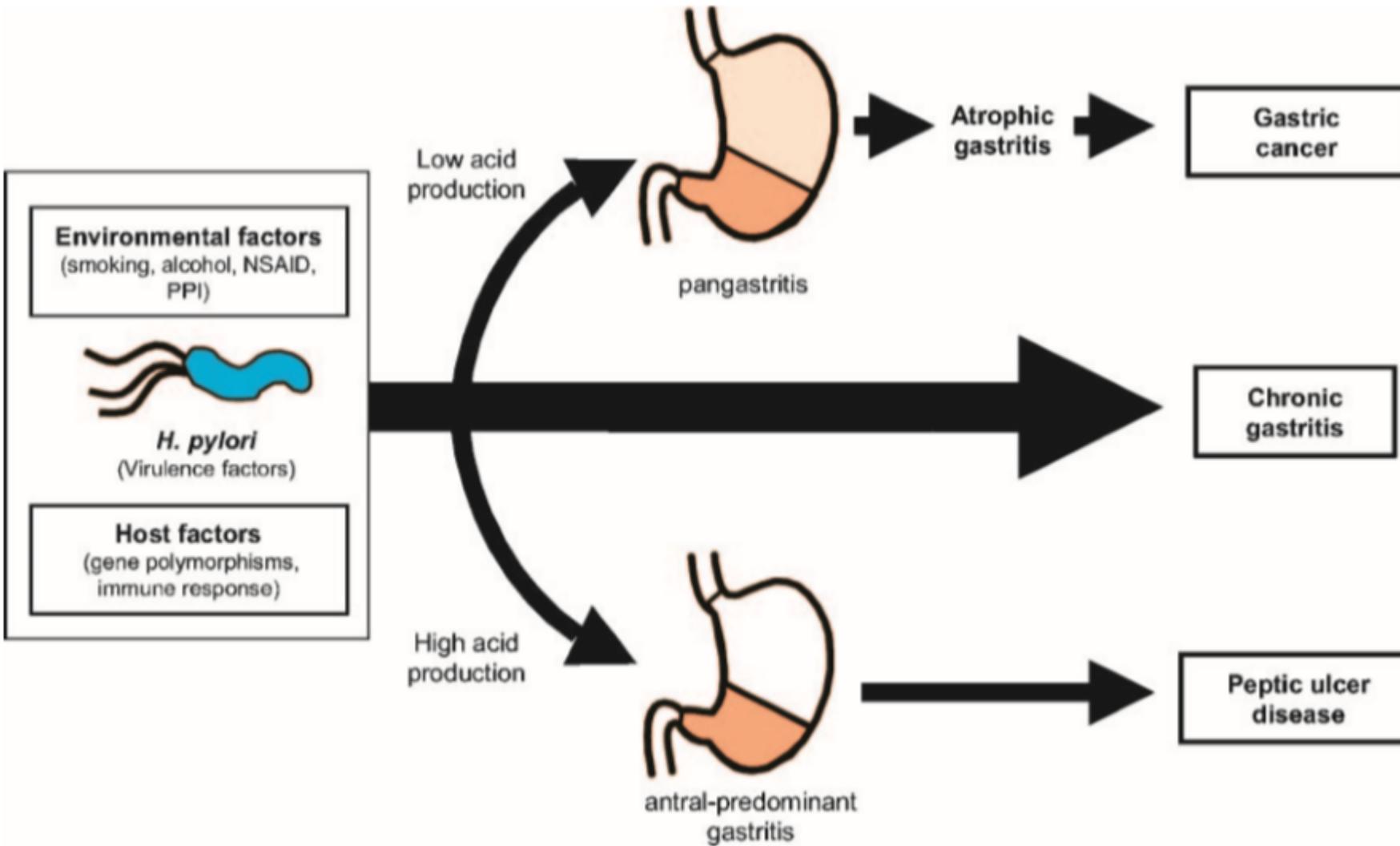


FIG. 2. Schematic representation of the factors contributing to gastric pathology and disease outcome in *H. pylori* infection.

Helicobacter pylori infection can cause

- acute gastritis
- chronic gastritis
- dyspepsia
- peptic ulcers
- atrophic gastritis
- gastric adenocarcinoma
- MALT (mucosa-associated lymphoid tissue) lymphoma

H. pylori eradication cures gastritis and can prevent the progression to long-term complications, or recurrence of disease

Other implications?

- Unexplained iron deficiency anemia (IDA)

proven in adult and children with normal gastro- and coloscopy

- Idiopathic thrombocytopenic purpura (ITP)

treatment might be beneficial for adults, controversial for children

- Vitamin B12 deficiency

possible link

- Extra-gastrointestinal conditions:

- atherosclerosis
- stroke
- Alzheimer disease
- idiopathic Parkinson disease
- obesity
- asthma

possible link? no sufficient proofs to make a clear causal or therapeutic link

Diagnosis

Who should be tested for *Helicobacter pylori*?

- **absolute indications:**

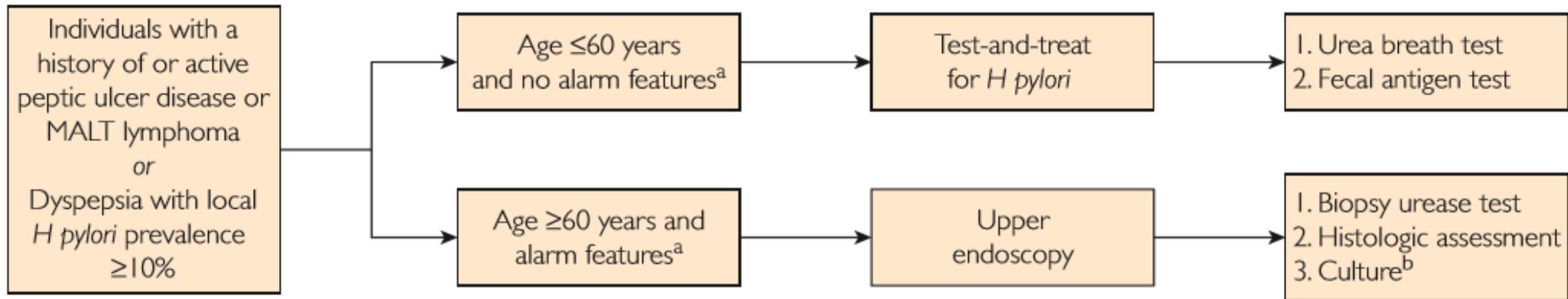
- uncomplicated or complicated active ulcer disease or history of peptic ulcer
- MALT lymphoma
- gastric cancer

- **relative indications:**

- dyspepsia (test and treat strategy)
- atrophic gastritis
- unexplained iron deficiency
- 1st degree relatives of patient with ventricular cancer
- previous long term treatment with NSAIDs

Tests	Sensitivity*	Specificity*	Remarks
Histology Culture biopsy Rapid urease test			Invasive tests
Urea breath test Fecal antigen Serology			Non-invasive tests

* World J Gastroenterol. 2019 Aug 28;25(32):4629-4660. doi: 10.3748/wjg.v25.i32.4629.



^aAlarm features include fevers, weight loss, anemia, gastrointestinal bleeding, dysphagia, and personal or family history of malignant neoplasm.

^bThis test is rarely used in clinical practice.

FIGURE. Indications for testing and diagnosis of *Helicobacter pylori*. MALT = mucosa-associated lymphoid tissue.

Culture

- *H. pylori* is a fastidious microorganism
- can be cultivated from gastric biopsy samples (antrum, corpus or bulbus)
- transportation to laboratory with adapted transport media
- samples are streaked on selective or not selective medium but supplemented with blood or serum
- incubation in microaerophilic atmosphere at 35° C for at least 3 to 4 days
- *H. pylori* forms small translucent smooth colony
- catalase +, oxidase +, urease +, identification also possible by MALDI-TOF
- only diagnostic test that allows AST

AST

Helicobacter pylori
Expert Rules and Intrinsic Resistance Tables

EUCAST Clinical Breakpoint Tables v. 12.0, valid from 2022-01-01



Disk diffusion criteria for antimicrobial susceptibility testing of *Helicobacter pylori* have not yet been defined and an MIC method should be used. If a commercial MIC method is used, follow the manufacturer's instructions.

Penicillins	MIC breakpoints (mg/L)			Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	ATU	
Amoxicillin oral	0.125	0.125		

Fluoroquinolones	MIC breakpoints (mg/L)			Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	ATU	
Levofloxacin	1	1		

Macrolides	MIC breakpoints (mg/L)			Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	ATU	
Clarithromycin	0.25	0.5		

Tetracyclines	MIC breakpoints (mg/L)			Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	ATU	
Tetracycline	1	1		

Miscellaneous agents	MIC breakpoints (mg/L)			Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	ATU	
Metronidazole	8	8		
Rifampicin	1	1		

The breakpoints are based on epidemiological cut-off values (ECOFFs), which distinguish wild-type isolates from those with reduced susceptibility

MIC distributions for Helicobacter pylori, 2022-05-06

Species: Helicobacter pylori (Method: MIC)

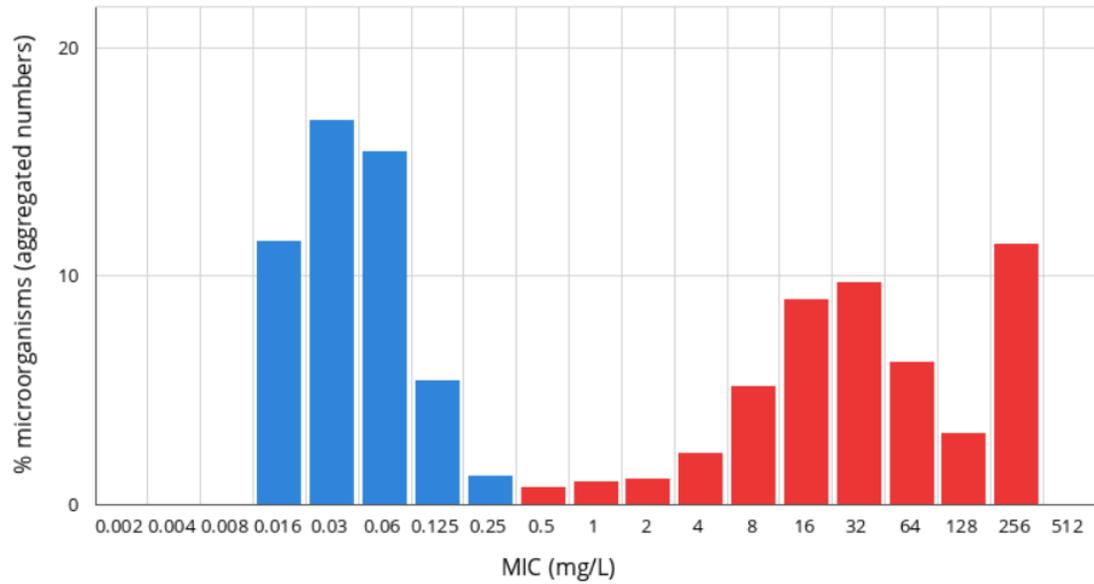
	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	Distributions	Observations	(T)ECOFF	Confidence interval
Amoxicillin	4	0	14	1075	150	68	38	19	13	7	5	10	32	19	44	0	0	0	0	2	1498	ID	
Ciprofloxacin	5	7	5	51	228	712	1268	769	157	32	27	78	93	81	427	0	0	0	0	2	3940	-	
Clarithromycin	0	0	0	1021	1491	1372	481	107	61	88	96	199	459	793	862	551	276	1008	0	5	8865	0.25	0.125 - 1
Levofloxacin	3	0	4	28	107	303	788	539	162	64	57	134	101	68	246	0	0	0	0	3	2604	(0.5)	
Metronidazole	0	0	0	93	136	218	500	597	1089	1786	1781	336	133	224	324	421	384	3024	122	11	11168	8	
Rifampicin	0	0	0	1	6	7	14	30	21	17	14	1	1	0	0	0	0	0	0	2	112	ID	
Tetracycline	1	0	0	533	1583	2603	1804	387	136	53	13	7	3	7	11	79	0	0	0	5	7220	0.25	

EUCAST

HUSLAB

Clarithromycin / *Helicobacter pylori*
International MIC distribution - Reference database 2022-05-06
Based on aggregated distributions

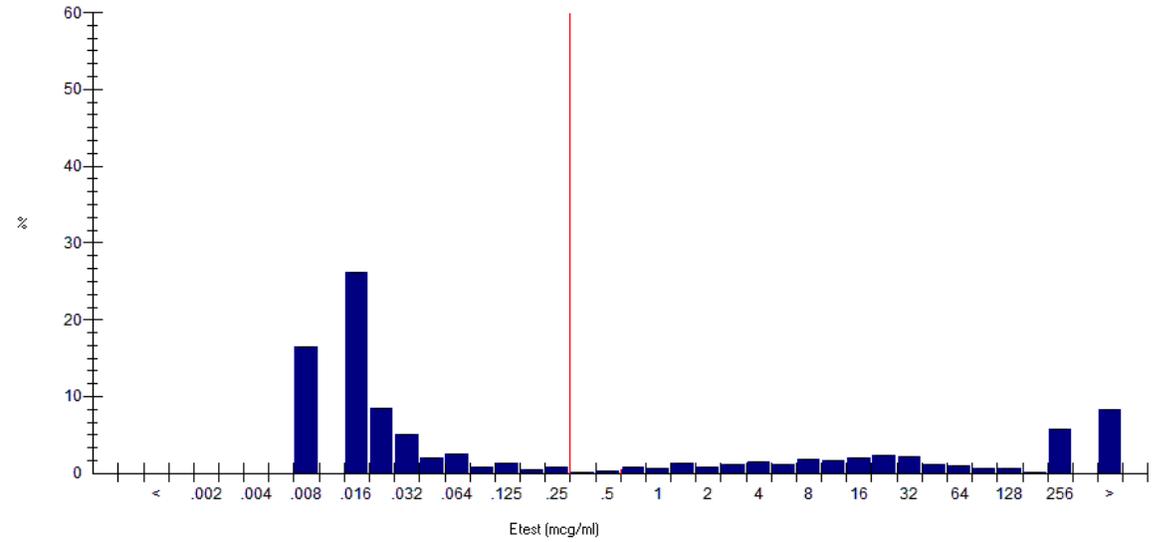
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 0.25 mg/L
Wildtype (WT) organisms: ≤ 0.25 mg/L

Confidence interval: 0.125 - 1
8865 observations (5 data sources)

Clarithromycin



n=1715

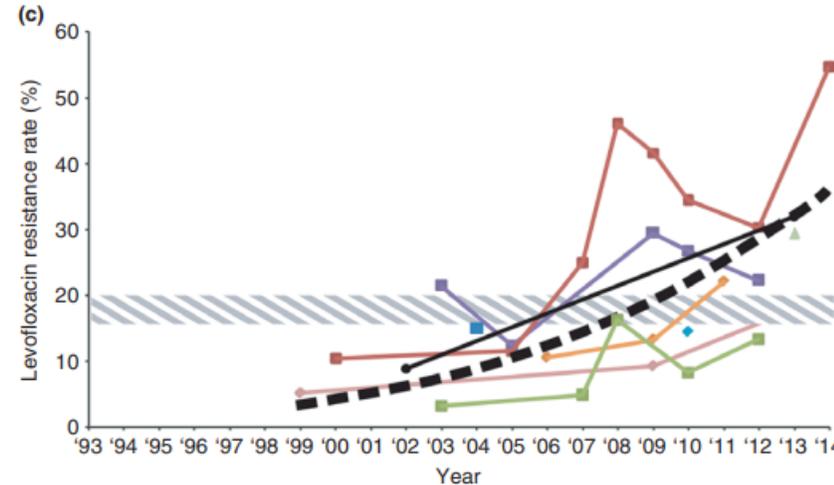
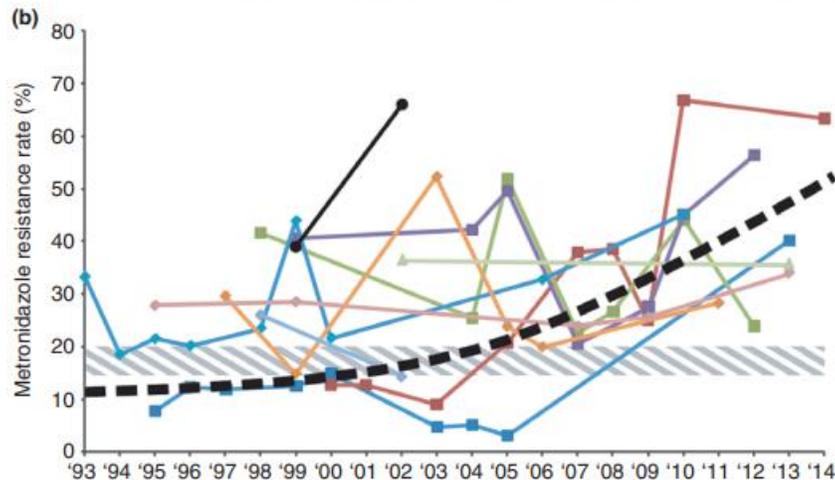
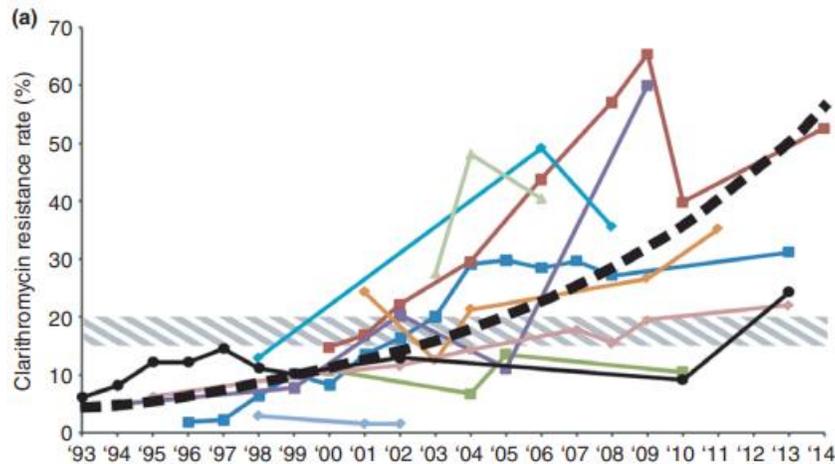
- Agar dilution is considered to be the gold standard method
- Most used method in routine laboratory is gradient test
- Helicobacter grows slowly
- AST reading is challenging
- Quality control: no EUCAST control strains, external quality control?
- Do we need AST for Helicobacter pylori?
 - biopsy is mandatory for AST but not for proving *H. pylori* infection
 - treatment mainly empiric and could be conducted without AST
 - indicated in case of treatment failure (1st and second line)?
 - epidemiological purposes

Is *Helicobacter pylori*'s resistance an issue?

AP&T Alimentary Pharmacology and Therapeutics

Review article: the global emergence of *Helicobacter pylori* antibiotic resistance

I. Thung^{*†}, H. Aramin^{*†}, V. Vavinskaya^{*}, S. Gupta[†], J. Y. Park[†], S. E. Crowe[†] & M. A. Valasek^{*}



■ Japan ■ China ■ Taiwan ■ Korea ■ Spain
■ Italy ■ Sweden ■ Bulgaria ■ Turkey ■ USA

Figure 2 | (a) Global prevalence of clarithromycin antibiotic resistance by country and year.^{22, 48, 70–85}
(b) Global prevalence of metronidazole antibiotic resistance by country and year.^{62, 65, 71–77, 79, 81–84, 87–106}
(c) Global prevalence of levofloxacin antibiotic resistance by country and year.^{22, 67, 72–74, 77, 81–83, 85, 87, 88, 90, 95, 96, 106, 117–121} Hashed band indicates threshold for altering therapeutic intervention.

The prevalence of bacterial antibiotic resistance is regionally variable and appears to be markedly increasing with time in many countries.

Clarithromycin resistance has been rapidly increasing in many countries over the past decade, with rates as high as approximately 30% in Japan and Italy, 50% in China and 40% in Turkey; whereas resistance rates are much lower in Sweden and Taiwan, at approximately 15%;

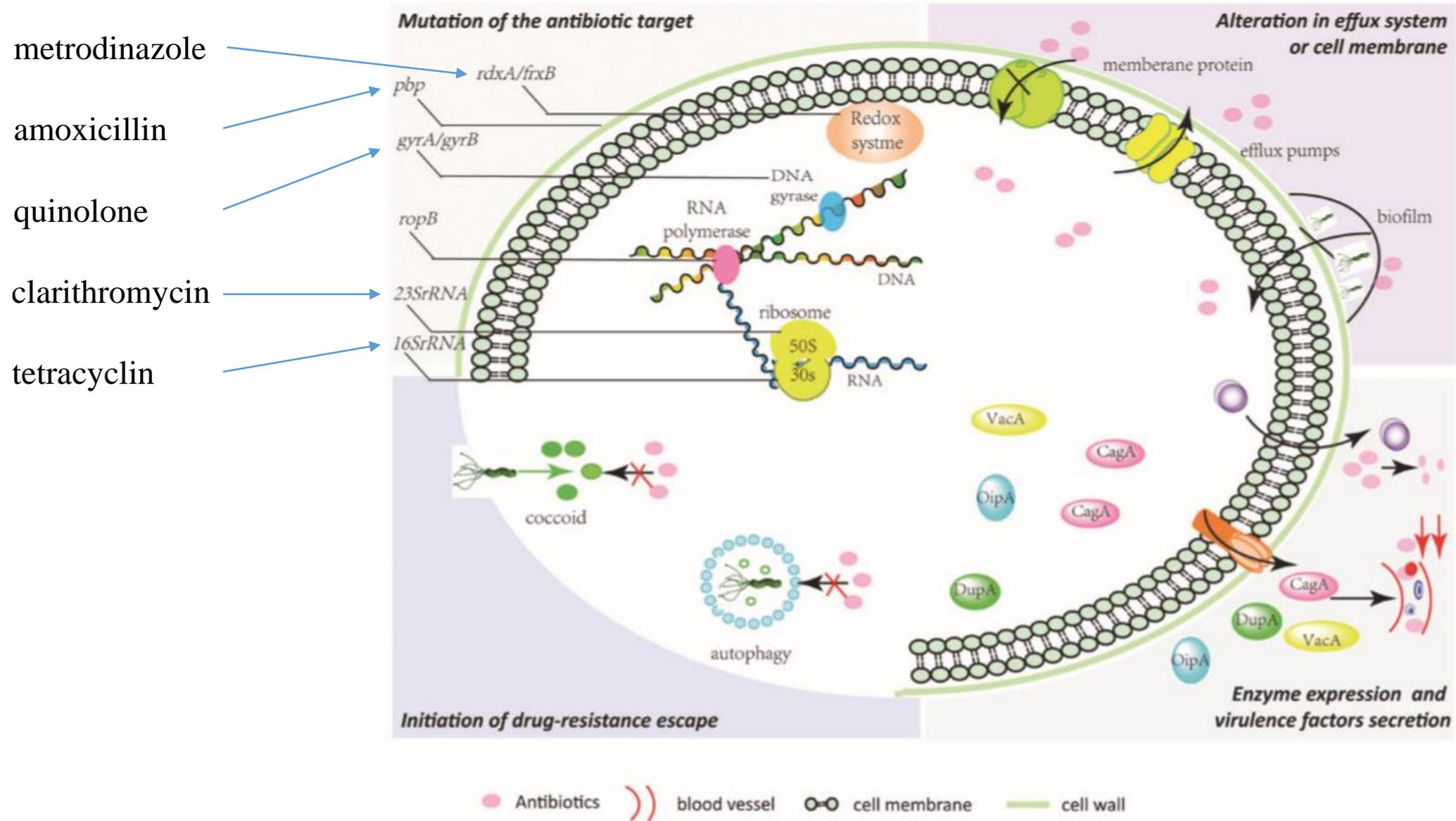


Figure 1. Pathogen-based *H. pylori* resistance mechanism. The image depicts the *H. pylori* resistance mechanism from the perspective of the pathogen including mutation of cellular targets to evade antibiotic activity, alterations in the efflux system or cell membrane to reduce intracellular accumulation of antibiotic compounds, expression of enzymes to inactivate antibiotic compounds, secretion of virulence factors affecting antibiotic activity, and initiation of drug-resistant escape mechanisms to prevent antibiotic clearance.

Table 1. Mutation of the cellular target to evade antibiotic activity in *H. pylori*.

Mutation classification	Genes	Amino acid changes and position	Antibiotics	References
Genes associated with nucleic acid synthesis	gyrA	87(N to K, to Y, to I) 91(D to G, to N, to Y)	Quinolone	(Lee et al. 2011; Seck et al. 2013; Kao et al. 2014; Trespalacios et al. 2015; Sanchez et al. 2016; Trespalacios-Rangel et al. 2016)
		M57Y, V77A D91H, A88P V199L, V199A N87A, A88N, V65I D86N, T87I S63P, P188S, D99V, V172L, R130K A88V, D155N A129T, R140K, D192N, D34N, D161N, D34Y, R140K		(Chang et al. 2012) (Garcia et al. 2012) (Teh et al. 2014) (Phan et al. 2015) (Trespalacios et al. 2015) (Miftahussunur et al. 2016) (Hanafi et al. 2016) (Miftahussunur et al. 2016)
	gyrB	D481E, B484K D484K, F438S S429T E463K		(Chung et al. 2012; Teh et al. 2014) (Teh et al. 2014) (Hanafi et al. 2016) (Rimbara et al. 2012)

Genes associated with protein translation

Domain V 23S rRNA

A2143G, A2142C, A2142G

Clarithromycin

G2111A, A2115G, A2144G

A2143C
A2116G, T2182C

T2243C
G2172T

G2111A, A2115G, A2144G

(Mansournani et al. 2012; Chung et al. 2014; Caliskan et al. 2015)

A2143C

(Gazi et al. 2013)

A2116G, T2182C

(Rasheed et al. 2014; Teh et al. 2014; Phan et al. 2015; Gehlot et al. 2016)

T2243C

(Khademi et al. 2014)

G2172T

(Miftahussunur et al. 2016)

16S rRNA

AGA926-928A1C

Tetracycline

(Wueppenhorst et al. 2009)

AGA926-928AGC

(Ontsika Ngyoyi et al. 2015)

AGA926-928TTC, A928C, AG926-

(Toledo and Lopez 2010)

927GT, A926G/A928C

AGA 926-928GGG/AGC, A939C

(Dardashtzadeh et al. 2014)

T556S

Amoxicillin

(Matteo et al. 2008; Rasheed et al. 2014; Zerbetto De Palma et al. 2016)

N562Y

(Rimbara et al. 2008; Kim and Kim 2013; Zerbetto De Palma et al. 2016)

A369T, V374L, S414R, L423F, T593A

(Rimbara et al. 2008)

V16L, V45L, S414R, T593A, G595S,

(Kim and Kim 2013)

A599T

D535N, S543R

(Rasheed et al. 2014)

A296V, S494H, A541M, E572G

(Rimbara et al. 2008)

A499V, E536K

(Rimbara et al. 2008)

pbp2
pbp3

PCR

New kid in the block: RT-PCR!

- (in house PCR)
- several commercial kits, real time PCR (RT-PCR)
- can be run from fecal samples and gastric biopsy
- Can be used for primary diagnosis
- Antibiotic and PPI treatments might affect the result (false negative)
- good specificity but some cross reactions described (i.e campylobacter)
- detection of most common point mutations of the 23S rRNA leading clarithromycin resistance (A2143G, A2142C, A2142G)
- rapid (2h)
- cost effective

But

- sensitivity might be lower compared to other non invasive tests
- differences between tests and studies (64%-84%-93%-96%)
- importance of DNA extraction
- no other resistance information than the 3 point mutations of clarithromycin
- need of quality control (external quality control available)
- In Helsinki: 25-30% resistance to clarithromycin (stool RT-PCR)

[World J Gastroenterol. 2019 Aug 28;25\(32\):4629-4660. doi: 10.3748/wjg.v25.i32.4629.](#)

[Helicobacter. 2020 Sep;25 Suppl 1:e12735. doi: 10.1111/hel.12735.](#)

[J Clin Microbiol. 2020 Mar 25;58\(4\):e01787-19. doi: 10.1128/JCM.01787-19.](#)

and also

- Commercial line blot:
 - from gastric biopsy
 - detects clarithromycin and levofloxacin resistance
 - time consuming
- Next generation sequencing: promising results but not yet available in routine laboratory

Treatment

Principals of treatment

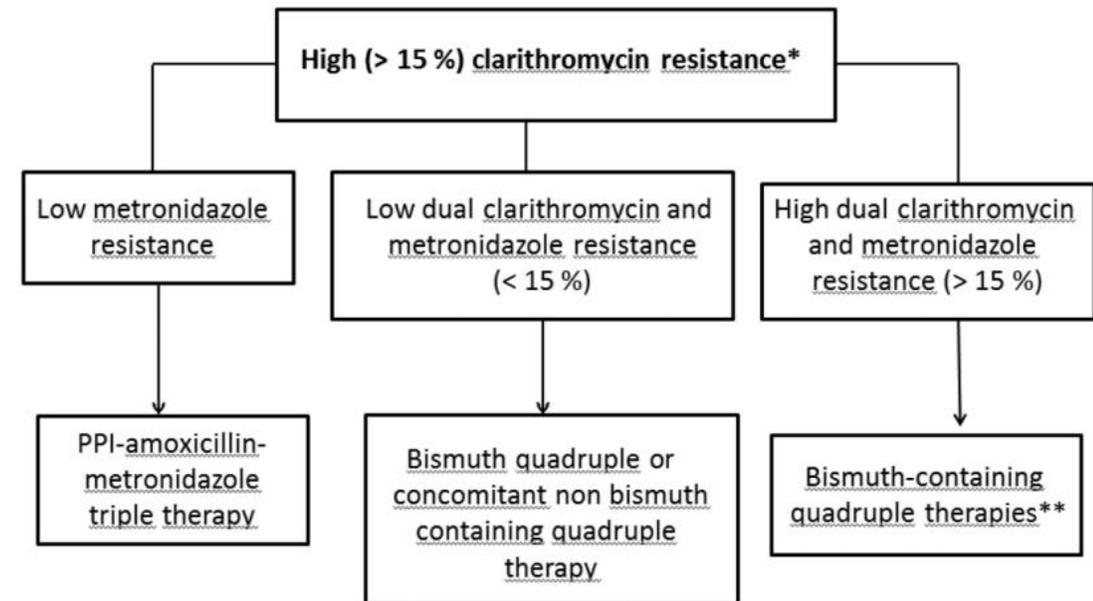
- empirical treatment
- always a combination of drugs: 2 antibiotics + PPI (+ bismuth salt)
- the choice of antibiotics depends on local resistance rates (if available), patient allergies and previous eradication failures
- in most nordic countries first line therapy:
 - **PPI+ clarithromycin (500mgx2)+ amoxicillin (1gx2) or metronidazole (500mgx2), 7days**
- in case of failure different regiments are possible
 - PPI+ amoxicillin (1gx2) or tetracyclins (500mgx4)+ levofloxacin (500mg) for 10 days
 - PPI+ amoxicillin (1gx2) or tetracyclins (500mgx4)+ metronidazole (500mgx2) for 14 days
 - PPI+ bismuth+ tetracyclin (250 – 500 mgx4) + metronidazole (250 mg x 4)

- treatment failure is not uncommon
- follow up after eradication treatment is necessary preferably by urea breath test or fecal tests
- follow up tests should be done 4 weeks after the end of treatment
- Reinfections are theoretically possible but are unusual. More likely recrudescence of original strains

Guidelines

Management of *Helicobacter pylori* infection—the Maastricht V/Florence Consensus Report

P Malfertheiner,¹ F Megraud,² C A O'Morain,³ J P Gisbert,^{4,5} E J Kuipers,⁶ A T Axon,⁷ F Bazzoli,⁸ A Gasbarrini,⁹ J Atherton,¹⁰ D Y Graham,¹¹ R Hunt,^{12,13} P Moayyedi,¹⁴ T Rokkas,¹⁵ M Rugge,¹⁶ M Selgrad,¹⁷ S Suerbaum,¹⁸ K Sugano,¹⁹ E M El-Omar,²⁰ on behalf of the European Helicobacter and Microbiota Study Group and Consensus panel



* Regardless of their population expectations, individuals who have previously taken clarithromycin and/or metronidazole should be considered high risk patients for dual resistance.

** If bismuth is not available, levofloxacin, rifabutin and high dose dual (PPI + amoxicillin) therapies might be considered. If tetracycline is not available, bismuth-containing quadruple therapy combining furazolidone-metronidazole or amoxicillin-metronidazole can be considered.

Thank you
for your attention!

