



# Antimicrobial resistance in *Helicobacter pylori*, a neglected problem

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## Background

- *Helicobacter pylori* (HP) infection is one of the most common chronic bacterial infections in humans, affecting approximately 4.4 billion individuals worldwide.
- Prevalence varies among geographic regions. Highest levels developing countries. Relationship with socioeconomic status and hygiene conditions.
- HP infection causes gastritis, peptic ulcer, MALT lymphoma and gastric cancer (IARC, 1994 and 2009, as group 1 carcinogen). Also extragastric diseases.
- Gastric cancer: is the fifth most common malignancy, the third leading cause of cancer-related morbidity globally, 9% of all cancer-related mortality.

**Savoldi *et al*, 2018, Gastroenterology; Hooi *et al*, Gastroenterology, 2017; Malfertheiner *et al*, 2017, Gut; IARC Working Group, 2012**

## Antibiotic resistance in *H. pylori*

- The efficacy of the HP eradication treatment has decreased dramatically because of antibiotic resistance.
- Low treatment success (i.e., below 80%) in the world, especially with CLA.
- In 2017, WHO published a list of 16 antibiotic-resistant bacteria that pose the greatest threat to human health (*H. pylori* CLA-RES)
- Efficacy of available alternatives: such as quadruple, sequential, concomitant, and levofloxacin-containing triple regimens) has varied greatly
- The most recent international consensus reports strongly recommend the selection of treatment based on local resistance patterns, HP testing is rarely performed.

**Kim *et al*, 2015, World J Pharmacol; Arslan *et al*, 2017, WJG; Thung *et al*, 2016, Aliment Pharmacol Ther; Dang & Graham, 2017, Nat Rev Gastroenterology**

## Susceptibility testing for *H. pylori*

### Culture-based techniques

**Agar dilution**  
**Breakpoint testing**  
**E-test**  
**Disc diffusion**

Slow growing of *H. pylori* cultures  
Easy contamination of cultures

### Nucleic acid-based techniques

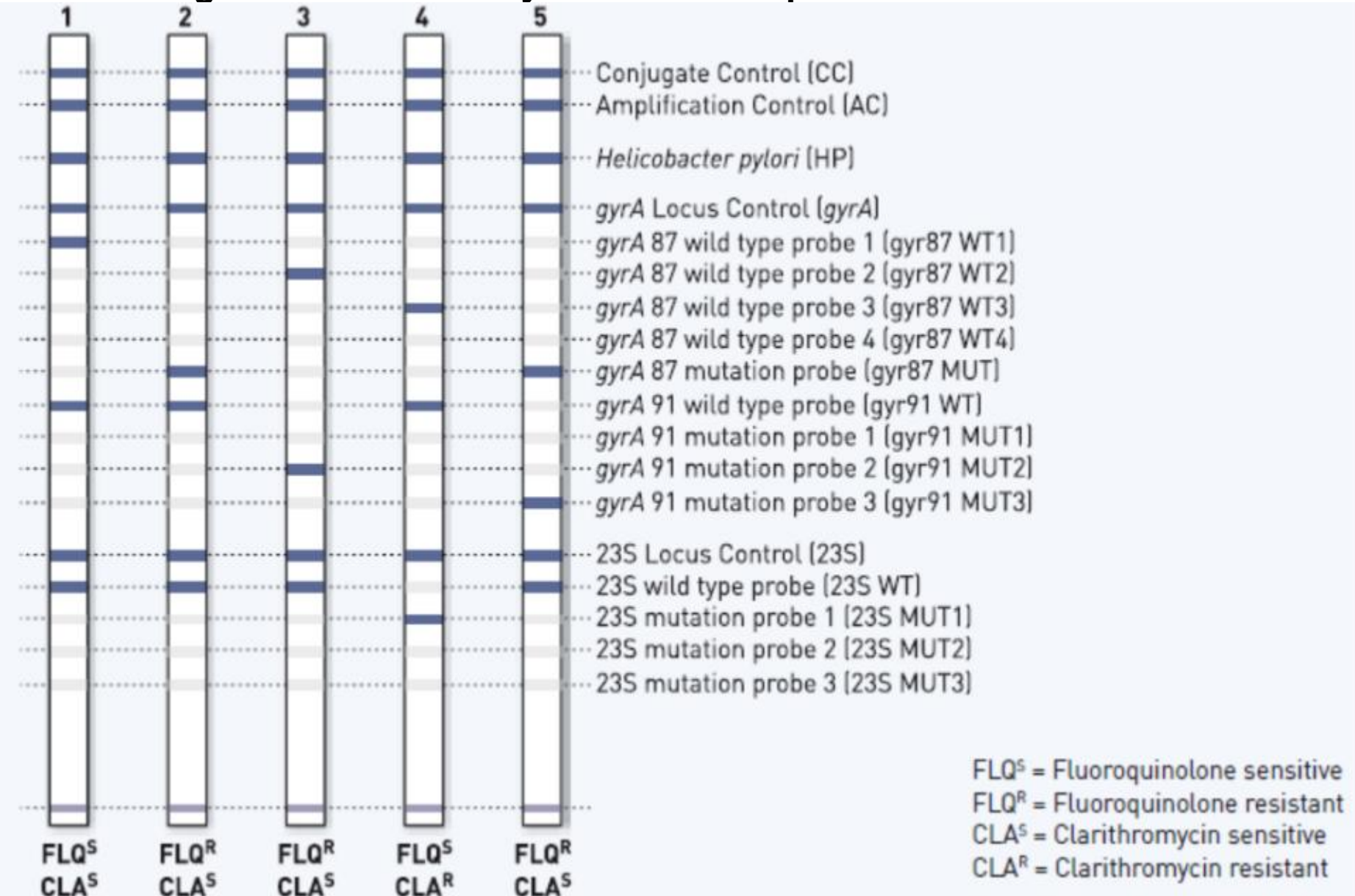
**PCR-RFLP**  
**Real time PCR**  
**FISH**  
**Pirosequencing**  
**Immunoassays**

Rapid result  
Good correlation with phenotypic tests  
Detect mix infections (S-R) and several mutations

## Commercially available molecular methods for *H. pylori* antibiotic resistance

Product name	Seeplex	ClariRes	GENECUBE	HelicoDR
Manufacturer (Country)	Seegene (Korea)	Ingenetix (Austria)	TOYOBO (Japan)	Hain Life Science (Germany)
Assay technique	Dual priming oligonucleotide PCR	Real-time PCR using biprobe	Real-time PCR using quenching probe	DNA strip genotyping test combining PCR and hybridization
Time-to-result	4 h		30 min	6 h
Target gene Detectable mutation	23S rRNA A2142G, A2143G	23S rRNA A2142G, A2143G A2142C	23S rRNA A2142G, A2143G	23S rRNA gyrA A2142G, A2143G 87 (Asn to Lys) A2142C 91 (Asp to Gly, Asn, or Tyr)
<i>H. pylori</i> detection	Sensitivity 97.7% Specificity 83.1% (Reference test: culture)	Sensitivity 100% Specificity 98–100% (Reference test: culture, RUT, histology)	Sensitivity 100% Specificity 100% (Reference test: Hp -IgG, RUT, UBT)	
Comparison with direct sequencing			Sensitivity 100% Specificity 100%	Sensitivity 94.9% Sensitivity 98.2% Specificity 87.1% Specificity 80.0%
Comparison with susceptibility test	Concordant rate 95.3% (41/43)	Sensitivity 82-100% Specificity 100%		Sensitivity 94% Sensitivity 87% Specificity 99% Specificity 98.5%

# GenoType HelicoDR (Hain, Alemania) for diagnosis and detection of resistance genes to clarithromycin and fluoroquinolones



## DIAGNOSIS OF *Helicobacter pylori*

Invasive tests	Non-invasive tests
Histology	Urea breath testing (urea-C13,C14)
Rapid urease tests	Detection of fecal antigen
Culture	Serology (ELISA, immunochromatography, Western blot)
PCR	PCR (faeces, saliva)

### Post treatment testing for *Helicobacter pylori*

- The urea breath testing (UBT) is considered the most reliable non-endoscopic test to confirm *H. pylori* eradication
- The faecal antigen test may be an alternative to the UBT, but this assay has not been as validated in the post-treatment setting
- Serology should be avoided post-treatment owing to persistence of *H. pylori*-specific antibodies after the infection has been cleared

# *H. pylori*. Genetic basis of antibiotic resistance

Drug class	Genes involved
Macrolides	<i>23SrRNA</i>
Metronidazole	<i>rdxA, frxA</i>
Fluoroquinolones	<i>gyrA, gyrB</i>
Amoxicillin	<i>pbp1</i>
Tetracycline	<i>16SrRNA</i>
Rifamycins	<i>rpoB</i>

## Clarithromycin resistance

There are essentially three point mutations, which can occur at the **position 2142 (A2142C, A2142G) and 2143 (A2143G)**, which resulted in:

- Decrease in binding of the drug
- No impact on bacterial viability
- Decreased affinity of triple therapy by 66,2 %
- **Problem can be induced by any type of macrolid**



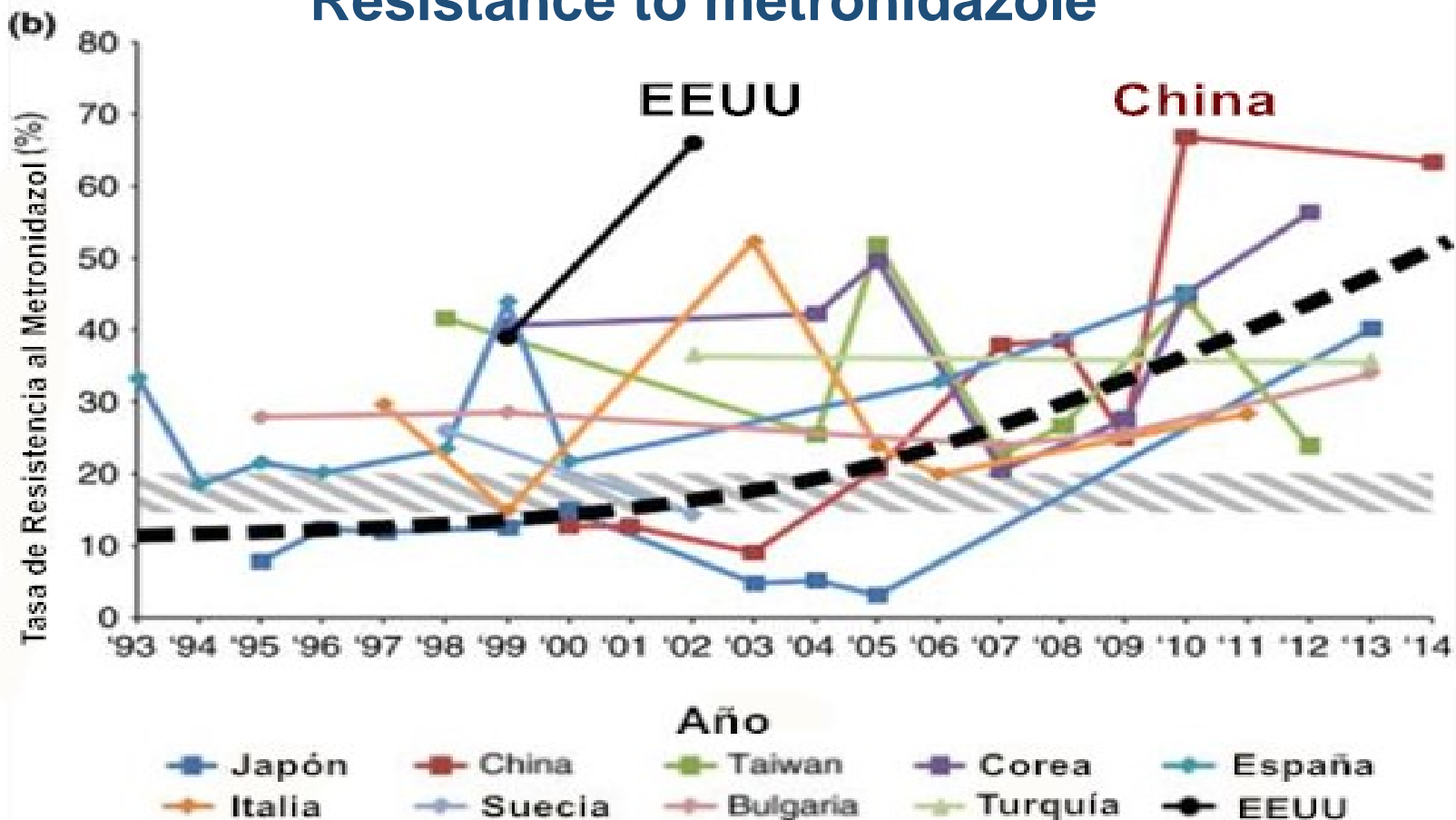
# *Helicobacter pylori* & antibiotic resistance

- Cross resistance in each family of antibiotics  
Resistance to clarithromycin → resistance to all macrolides  
Resistance to levofloxacin → resistance to all fluoroquinolones
- No cross resistance between different families of antibiotics
- Important to use compound indicated to get good results  
Clarithromycin for macrolides  
Tetracycline HCL and not doxycycline  
Levofloxacin but not ciprofloxacin for fluoroquinolones

# *Helicobacter pylori* & antibiotic resistance

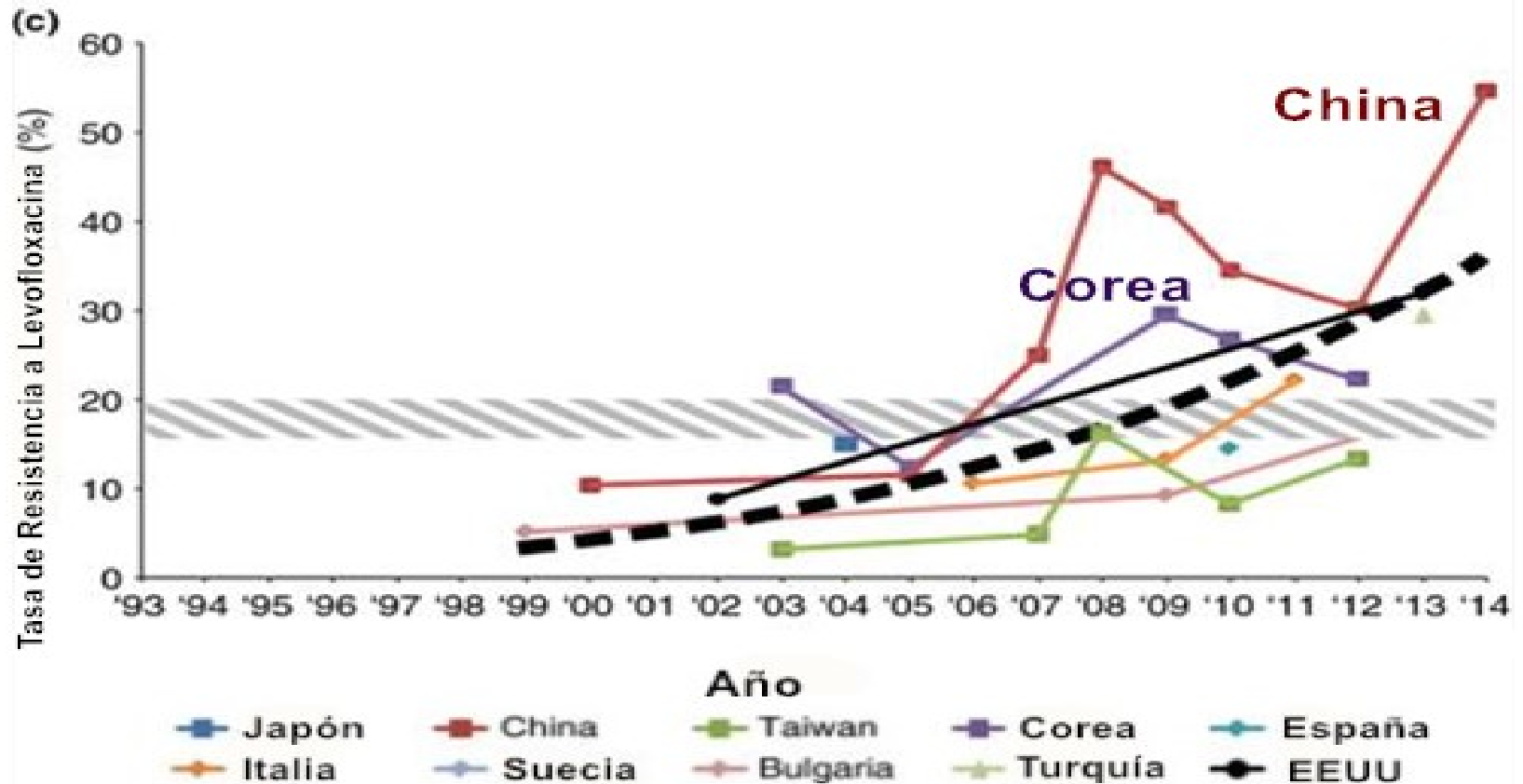
Antibiotic	Description
Clarithromycin	Not overcome by increasing dose & duration Should not be used if prevalence > 15 %
Levofloxacin	Not overcome by increasing dose & duration Rapidly increasing worldwide
Metronidazol	Overcome by increasing dose and duration Should not be used if prevalence > 40 %
Amoxicillin	Rare in most regions
Tetracycline	Rare in most regions
Bismuth	Does not occur

## Resistance to metronidazole



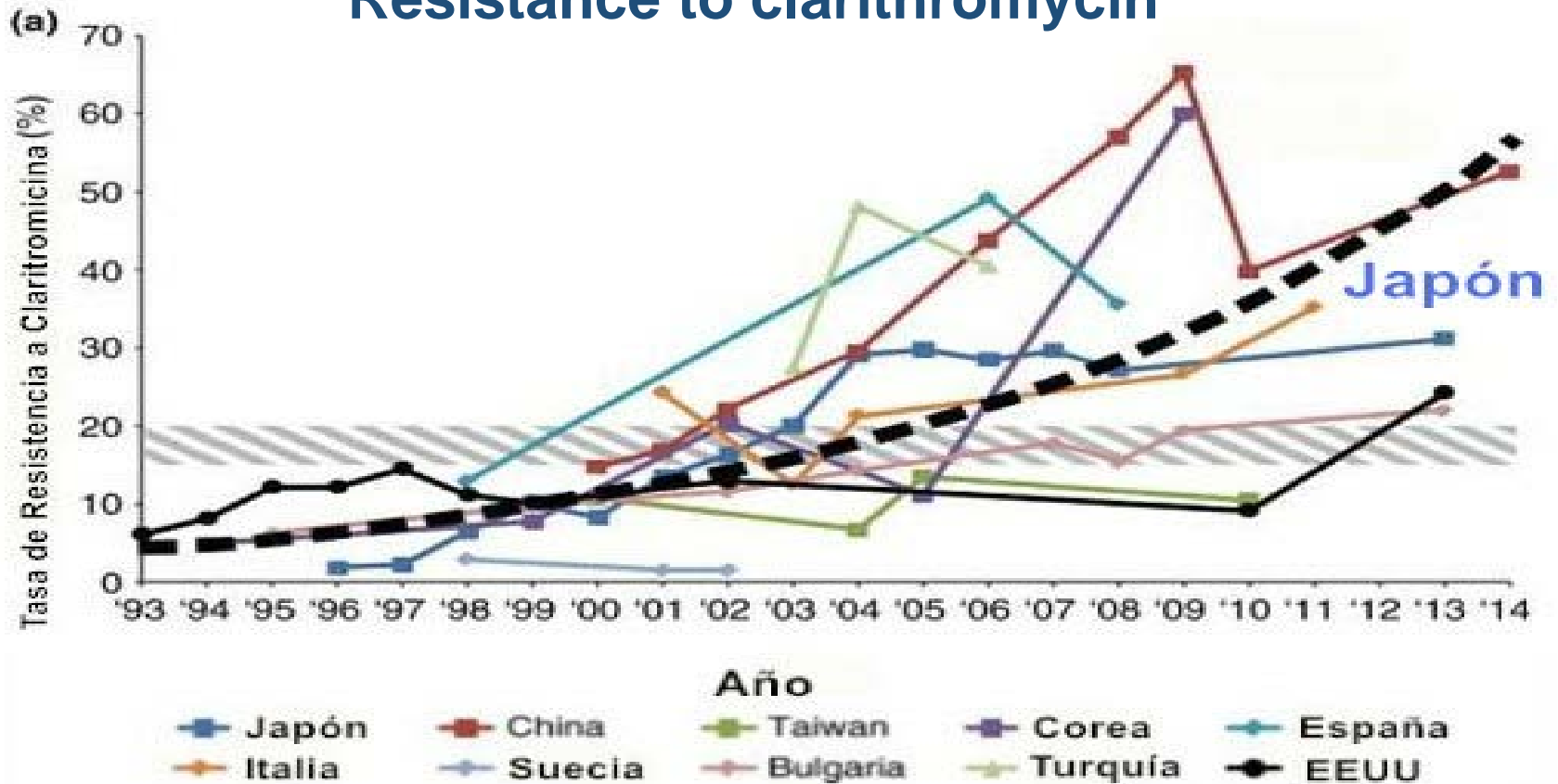
The rate of resistance has increased due to its use for treatment of HP infections and some others: parasites, vaginal and dental infections

## Resistance to levofloxacin



The rate of resistance has rise due to its increasing use for treatment of HP infections and/crossing resistance to other fluoroquinolones (ciprofloxacin) used for the therapy of digestive, respiratory and urinary tract infections.

## Resistance to clarithromycin



The rate of resistance has increased as consequence of its use for treatment of HP infections and other infections and/or crossing resistance to other macrolides (azithromycin) used for treatment of respiratory, infections and STIs.

## Systematic review/metanalysis Savoldi *et al*, *Gastroenterology* 2018

Records screened: 1605; Full text articles assessed: 572 (Jan 2007-Jun 2017)

Studies included in quantitative analysis: 178

### Geographic distribution of the studies, number of tested isolated, and participants according to the WHO region.

WHO Region number of studies	Countries providing data	No.isolates	No. patients
Africa (3)	3	296	248
America (13)	6	1646	1506
East Mediterranean (24)	5	2668	3443
Europe (58)	17	18201	17610
South-East Asia (14)	5	1397	1830
Western Pacific (66)	10	41933	28946
Total (178)	45 countries	66142	53583
Six studies does not report number of patients			

## Pooled prevalence of overall antibiotic resistance, stratified by WHO region.

WHO regions		Pooled prevalence of antibiotic resistance, % (95% CI)			
Region	CLA	MET	LEV	CLA-MET	AMO
Africa	15	91	14	-	38
America	14	27	14	3	8
East Mediterraneo	29	61	23	14	14
Europe	32	38	14	15	0
South East Asia	17	59	25	6	12
Western Pacific	34	55	24	11	1

**Prevalence of antibiotic resistance by age group, stratified by WHO region. Data for AMO and TET were not pooled by age-group due to the lack of studies.**

WHO región patient population		Pooled prevalence of antibiotic resistance, % (95% CI)	
<b>America</b>	Clarithromycin	Metronidazole	Levofloxacin
Adults	13	22	18
Children	19	40	-
<b>East Mediterraneo</b>			
Adults	29	61	18
Children	10	81	29
<b>Europe</b>			
Adults	28	40	11
Children	10	81	29
<b>South East Asia</b>			
Adults	16	25	11
Children	29	-	-
<b>Western Pacific</b>			
Adults	32	53	27
Children	85	43	27



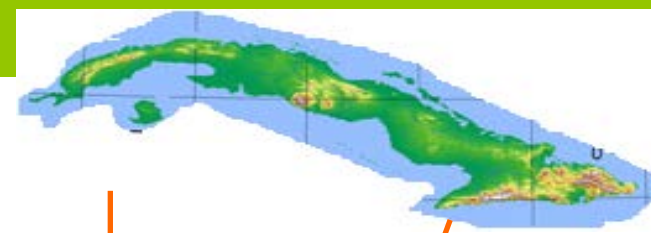
Treatment		Regimen								Duration of therapy		
		Amo	Cl	Met	Tet	Lev	Rif	Fur	Bis		PPI	
First Line therapy	Standard triple therapy		1g	0.5g							SD	7–14 d
	Concomitant therapy		1g	0.5g	0.5g						SD	7–10 d
	Bismuth-containing quadruple therapy				0.25g	0.5g				SD	SD	10–14 d
	Sequential therapy	First phase	1g								SD	5 d
		Second phase		0.5g	0.5g						SD	5 d
	Hybrid therapy	First phase	1g								SD	7d
		Second phase	1g	0.5g	0.5g						SD	7d
Second Line therapy	Bismuth-containing quadruple therapy				0.5g	0.5g				SD	SD	10-14d
	Levofloxacin-based triple therapy		0.5g				0.5g				SD	10d
Third Line therapy	Culture guided therapy		Two antibiotics selected by sensitivity tests						SD	SD	NA	
	Levofloxacin-based quadruple therapy		0.5g				0.5g			SD	SD	10d
	Rifabutin-based triple therapy		1g					0.15g			SD	14d
	Furazolidone-based quadruple Therapy					1g			0.2g	0.24g	SD	NA

**Figure 1. Current recommended regimens for *H. pylori* eradication. Wu et al, 2012**

The figure in the ball stands for dose. Green ball: b.i.d, Blue ball: t.i.d, Dark Green ball: q.i.d. A: amoxicillin, C: clarithromycin, tetracycline, L: levofloxacin, R: rifabutin, F: furazolidone, SD: standard dose, BIS: bismuth, PPI: proton pump inhibitor

# What about Hp resistance in Cuba?





- High incidence of gastric cancer in patients over 60 years of age
- Gastric cancer produces 890 deaths in 2016 (rate 7,9/100 000 inhab)
- Some studies reveal a prevalence of HP in symptomatic patients of 50-70% in adults and 30% in children

Scarse institutions developing laboratory diagnosis of HP

# Antibiotic susceptibility in *H. pylori*, Cuba, 2005-2007



High resistance MET: 85,7 %

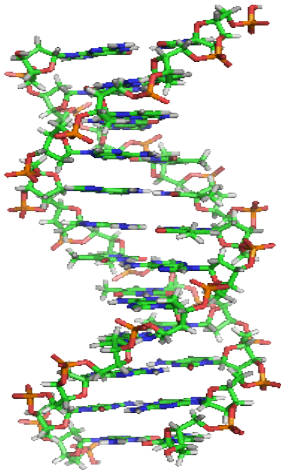
High resistance CIP: 22,5 %

Moderate resistance CLA: 11,4 %

Low resistance AMO: 1,6%

No resistance TET

## Genetic mechanisms resistance



Clarithromycin 23 S rRNA

A (2142) → G    A (2143) → G

Metronidazole: several mutation in *rdxA* gene  
including new mutations

# Conclusions

- **Resistance of *H. pylori* to antibiotics has reached alarming levels worldwide, which has a great effect on efficacy of treatment**
- **In most WHO regions pooled prevalence of both primary and secondary resistance of *H. pylori* to CLA, MET and LEV is higher than 15%, the common threshold for choosing alternative empiric regimens. Tetracycline and amoxicillin resistance were both <10% worldwide**
- **Although fluoroquinolones are contraindicated for the treatment of any infection in children, the resistance rate to LEV was remarkable in EMR (29%) and WPR (17%), possibly reflecting resistance in the infecting strain and intrafamilial spread of quinolone-resistant isolates from adults**
- **The high level of global HP resistance in treatment-naive patients can be correlated with the increasing and uncontrolled consumption of antibiotics that are commonly used in HP empirical therapy and also used to treat other common infections in the general population (e.g., respiratory, genital, urinary infections, parasite infestation)**
- **Local surveillance networks are required to select appropriate eradication regimens for each region**

The image features a dark blue, gradient background. Scattered across the scene are several green, teardrop-shaped objects with a dense, fuzzy, or fibrous texture. From the base of each object, several thin, white, hair-like filaments extend outwards. The objects are positioned at various angles and depths, creating a sense of three-dimensional space. The word "Gracias" is centered in the lower half of the image in a clean, white, sans-serif font.

Gracias