African Helicobacter & Microbiota Study Group

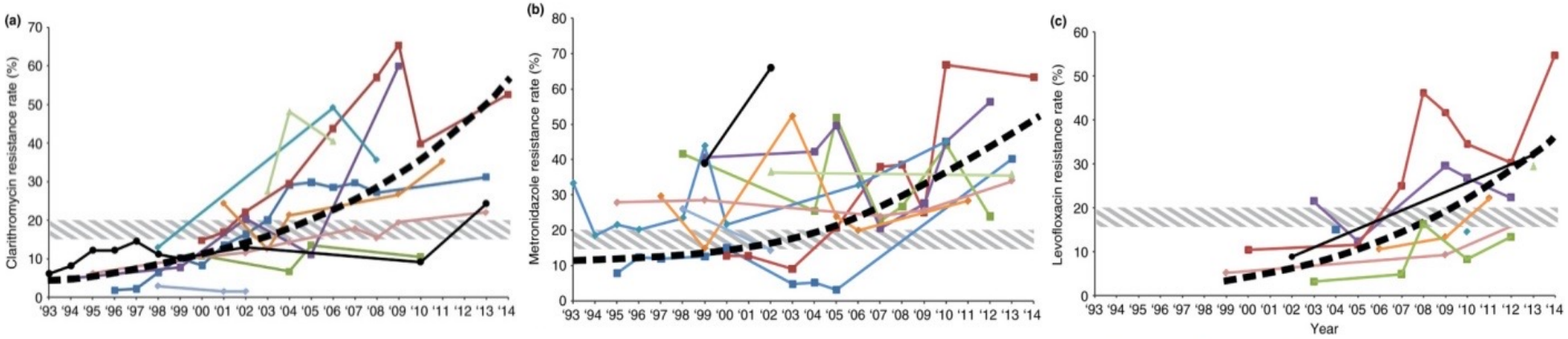
AHMSG 2024
16–17 AUGUST 2024
Cape Town, South Africa

Evidence-based medicine for *Helicobacter pylori* AST-based molecular methods

Evariste Tshibangu-Kabamba[1,2], Alain Cimuanga Mukanya[1,3], Yoshio Yamaoka[2,4], and the Congolese Observatory of *Helicobacter pylori* & Gastric cancer (OCIP)

[1] Dpt. of Internal Medicine, University of Mbujimayi, DR Congo;
[2] Research Center for Infectious Disease Science, Osaka Metropolitan University, Japan;
[3] Dpt. Environmental & Preventive Medicine, Oita University, Japan
[4] Dpt. of Gastroenterology, Baylor College of Medicine, Tx, USA

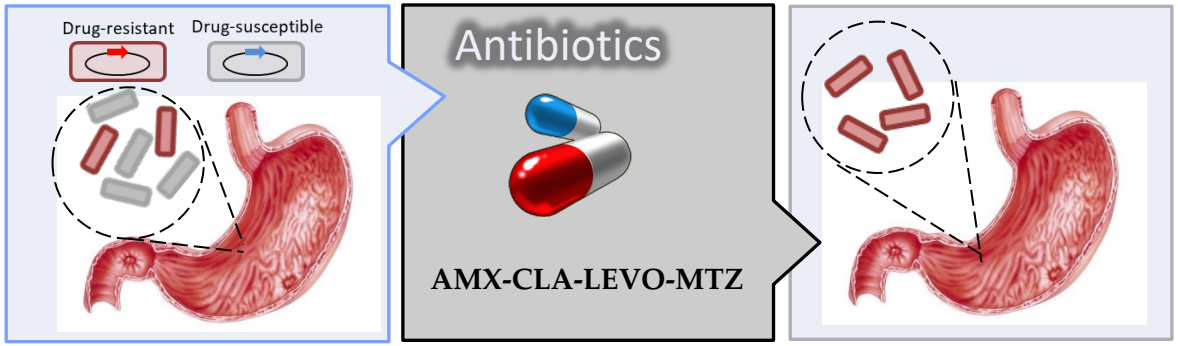
***H. pylori* drug-resistance is increasing worldwide** and poses a significant clinical challenge, leading to ramping treatment failures.



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

Aliment Pharmacol Ther 2016; **43**: 514–533

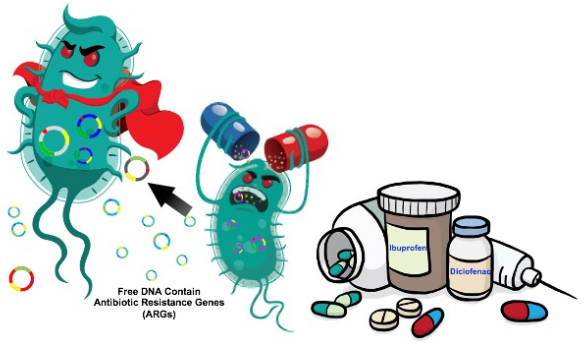
I. Thung*,†, H. Aramin*,†, V. Vavinskaya*, S. Gupta†, J. Y. Park‡, S. E. Crowe† & M. A. Valasek*



Exceptional adaptation abilities of *H. pyori*

Limited therapeutic choices and extensive misuse of drugs

Emergence of AMR



Clinical implications of antibiotic resistance in *H. pylori* species

↓↓↓ *H. pylori* treatment efficacy + (theoretically!) ↑↑ of clinical complications (e.g., gastric cancer or peptic ulcers)

Received: 15 April 2020 | Revised: 22 May 2020 | Accepted: 25 May 2020
DOI: 10.1111/hel.12714

ORIGINAL ARTICLE

Helicobacter WILEY

The effect of antibiotic resistance on *Helicobacter pylori* eradication efficacy: A systematic review and meta-analysis

Yunzhi Zou[1,2] | Xing Qian[1,3] | Xiaoqun Liu[1,4] | YanPing Song[1] | Conghua Song[1] | Shuang Wu[1] | Ying An[1] | Rui Yuan[1,5] | Youhua Wang[1] | Yong Xie[1]

Infection and Drug Resistance Dovepress
open access to scientific and medical research
Open Access Full Text Article REVIEW

Primary Resistance Pattern of *Helicobacter pylori* to Antibiotics in Adult Population: A Systematic Review

Infection and Drug Resistance 2020:13 1567–1573

Clin Gastroenterol Hepatol. 2014 February ; 12(2): 177–186.e3. doi:10.1016/j.cgh.2013.05.028.

Rational *Helicobacter pylori* therapy: evidence based medicine rather than medicine based evidence (revision 2)

David Y. Graham, M.D.[1], Yi-Chia Lee, M.D.[2], and Ming-Shiang Wu, M.D.[2]

CLA-R ↔ ↓ the efficacy of all CLA-containing regimens by up to ~66% (CLA-T3T) and ~13% (CLA-Q4T)

MTZ-R ↔ ↓ the efficacy of MTZ-containing regimens by ~38% (T3T) and ~14% (non-bi Q4T) while a very high therapeutic efficiency is still obtained with bi-Q4T regardless of the resistance of strains

LEVO-R ↔ ↓ the efficacy of LEVO-containing regimens by ~21% (five studies)

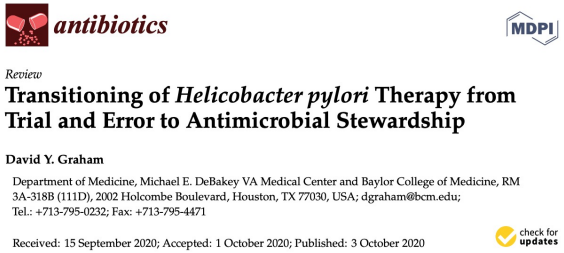
AMX-R, TET-R, and RIB-R have been lesser evaluated (therapy failures due to AMX-R and TET-R have been reported).

Consequently, the ↑ SDR pattern in *H. pylori* that has been noted worldwide may be suggestive of an overall decrease of the clinical efficacy of the eradication therapy

Critical antibiotics becoming clinically ineffective have resulted in strong pressure to utilize the principles of antimicrobial stewardship for selecting and managing therapy for infectious diseases

> "*The principles of antimicrobial stewardship codify and extend thinking and practices involved in the development and implementation of methods to simultaneously improve antimicrobial therapy, prevent antimicrobial misuse, and reliably achieve high cure rates, while minimizing the risk of developing resistance in order to prolong the useful life of antibiotics*" **Graham, 2020**

H. pylori therapies have to transit from "trial and error" to antimicrobial stewardship, from empirical to antimicrobial susceptibility testing – based therapies



antibiotics

MDPI

Review

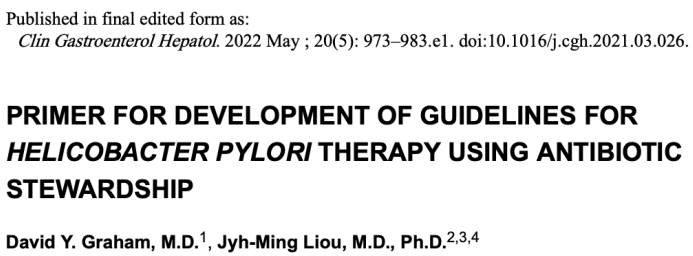
Transitioning of *Helicobacter pylori* Therapy from Trial and Error to Antimicrobial Stewardship

David Y. Graham

Department of Medicine, Michael E. DeBakey VA Medical Center and Baylor College of Medicine, RM 3A-318B (111D), 2002 Holcombe Boulevard, Houston, TX 77030, USA; dgraham@bcm.edu; Tel.: +713-795-0232; Fax: +713-795-4471

Received: 15 September 2020; Accepted: 1 October 2020; Published: 3 October 2020

check for updates



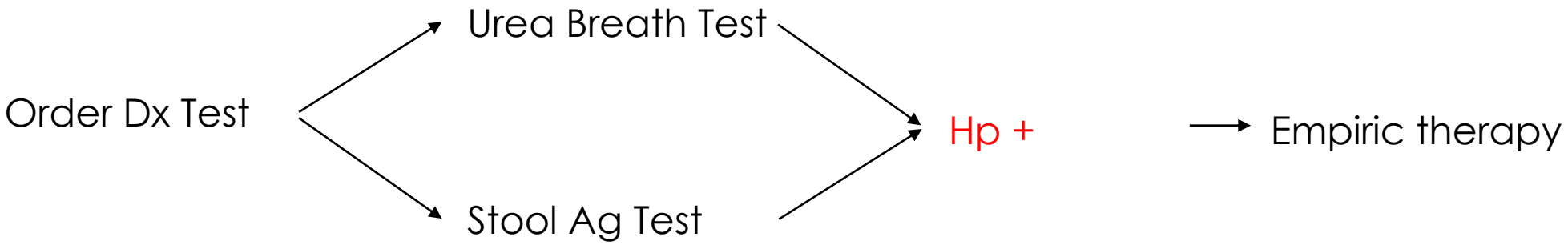
Published in final edited form as:
Clin Gastroenterol Hepatol. 2022 May ; 20(5): 973–983.e1. doi:10.1016/j.cgh.2021.03.026.

PRIMER FOR DEVELOPMENT OF GUIDELINES FOR *HELICOBACTER PYLORI* THERAPY USING ANTIBIOTIC STEWARDSHIP

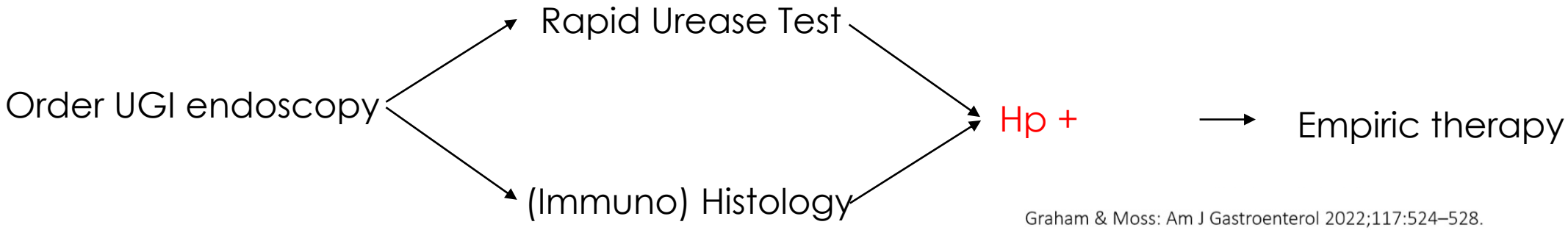
David Y. Graham, M.D.[1], Jyh-Ming Liou, M.D., Ph.D.[2,3,4]

Historical Dx approach

New patient or post-therapy
No alarm features



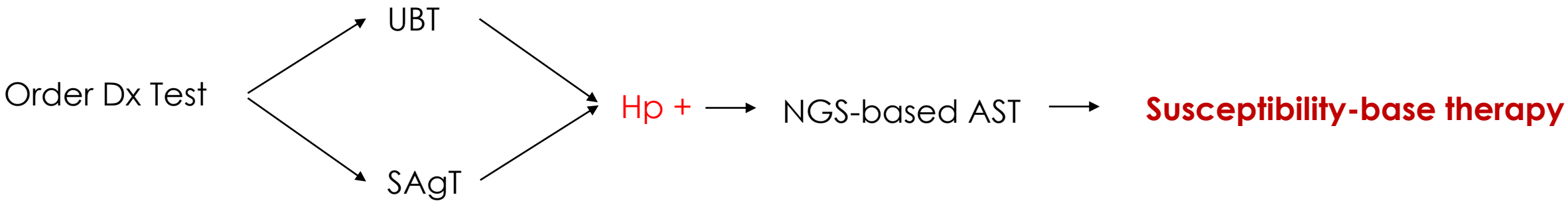
New patient or post-therapy
With alarm features



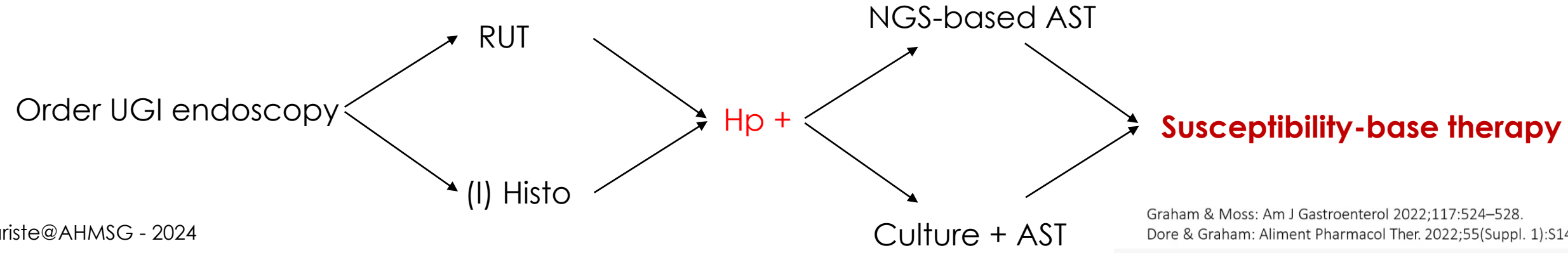
Graham & Moss: Am J Gastroenterol 2022;117:524–528.
Dore & Graham: Aliment Pharmacol Ther. 2022;55(Suppl. 1):S14–S21.

Shift to 'Diagnosis to Tailored Tx' in increasing AMR context

New patient or post-therapy
No alarm features

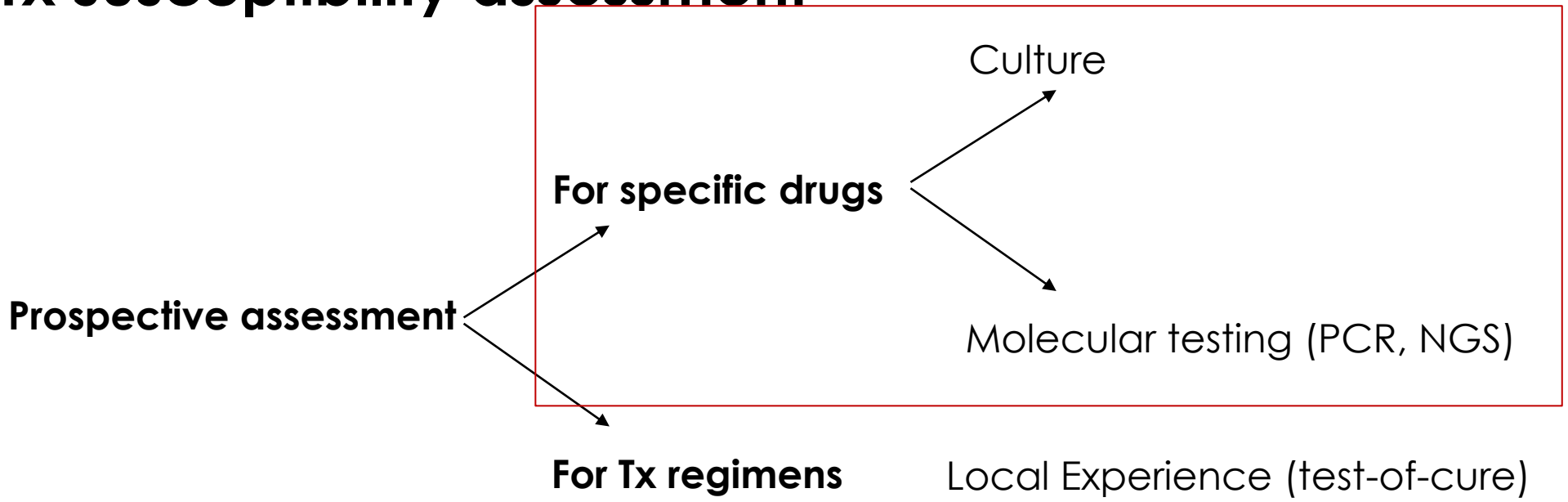


New patient or post-therapy
With alarm features

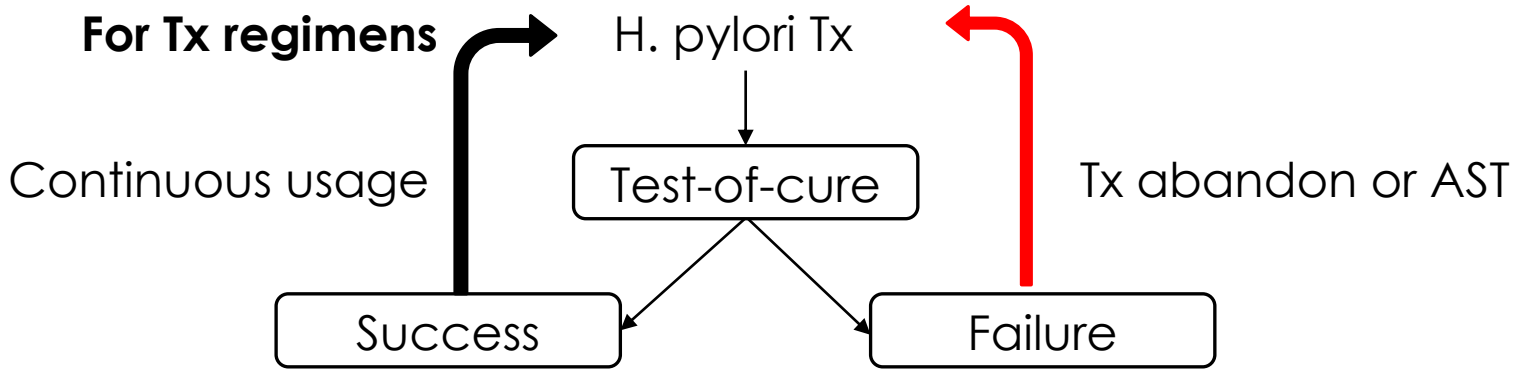


Graham & Moss: Am J Gastroenterol 2022;117:524–528.
Dore & Graham: Aliment Pharmacol Ther. 2022;55(Suppl. 1):S14–S

Tx susceptibility assessment

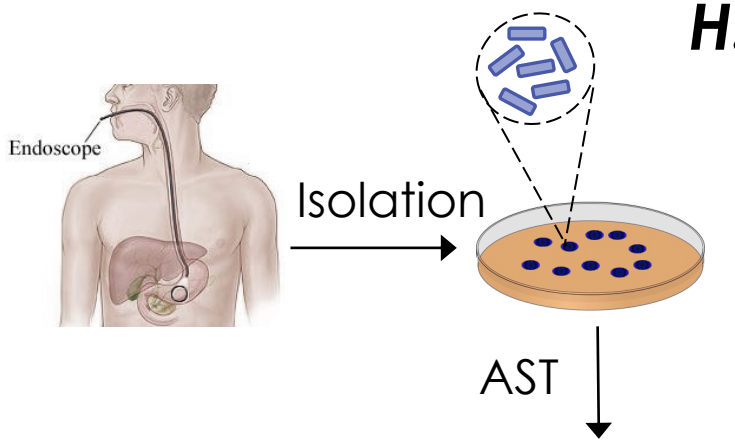


Retrospective assessment



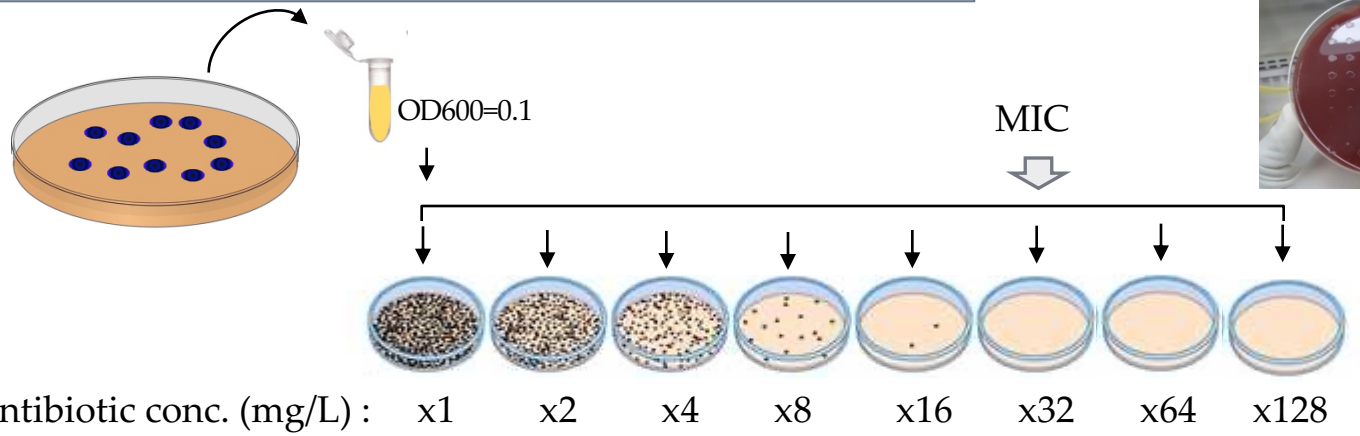
Graham & Moss: Am J Gastroenterol 2022;117:524–528.
Dore & Graham: Aliment Pharmacol Ther. 2022;55(Suppl. 1):S14–S21.

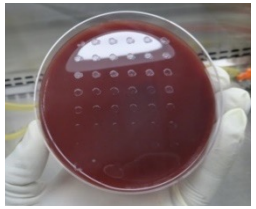
H. pylori antimicrobial susceptibility testing (AST)



Very slow, tedious, technically demanding, expensive, microaerophilic conditions, specific culture and conservative media ... unavailable in clinical settings of most African countries

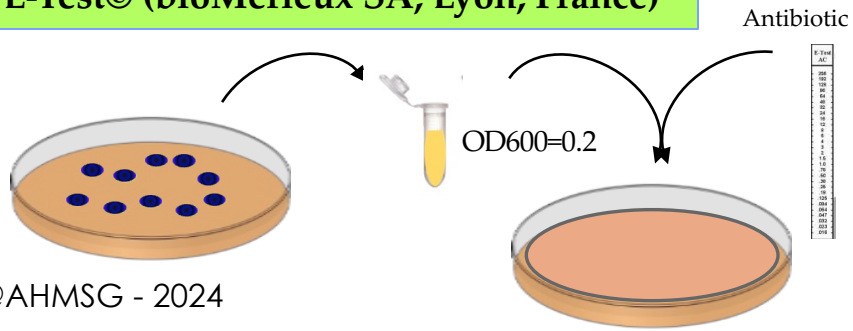
Agar dilution method (CLSI Protocols, Wayne, PA, USA)

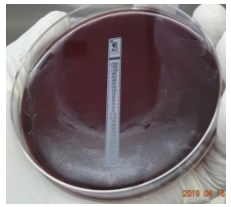


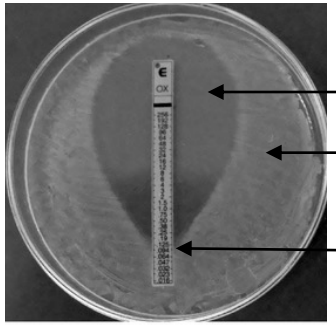


Clinical breakpoints (EUCAST) :
- AMX : 0.125 mg/L
- CLA : 0.25 mg/L
- LEVO : 1 mg/L
- MTZ : 8 mg/L

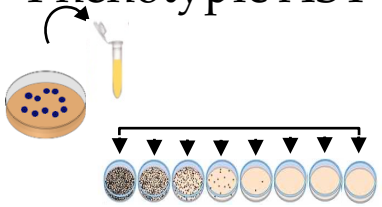
E-Test© (bioMérieux SA, Lyon, France)







Emerging techniques for molecular AST (mAST) of *H. pylori*

AST method	Application	Performance	Operability
WGS by NGS/TGS 	No limitation for structural mechanisms, All possible known and novel variants, All drug-R with gene structure alteration	Fast, accurate (less if read-base), reproducible	Computationally demanding (less if reads-based), cost-effective
Phenotypic AST 	All drug-R (drugs stable and effective in culture conditions), Limited to cultivable strains	Gold standard, less reproducible, very slow	Very fastidious, interpretation problem, expensive

As the demand for antimicrobial stewardship grows, molecular AST-based methods have emerged as crucial tools for detecting mAMR to predicting, respond to, and monitoring drug resistance.

mAMI

MICs

Article

Next-Generation Sequencing of the Whole Bacterial Genome for Tracking Molecular Insight into the Broad-Spectrum Antimicrobial Resistance of *Helicobacter pylori* Clinical Isolates from the Democratic Republic of Congo

(Evariste Tshibangu-Kabamba et al., 2020)

Helicobacter pylori infection and antibiotic resistance — from biology to clinical implications

Evariste Tshibangu-Kabamba[1] and Yoshio Yamaoka[1,2]✉

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Methods for the detection of antimicrobial resistance in *H. pylori* species

Assay	Existing applications for AST
Culture-based techniques	
Agar dilution method (ADM) [214, 215]	CLA, AMX, TET, MTZ, LEVO, RIB, and other drugs
Epsilometer Test method (E-test) [214, 215]	CLA, AMX, TET, MTZ, LEVO, RIB, and other drugs
PCR-based assays	
PCR-DNA sequencing by conventional and pyrosequencing techniques[216]	CLA, AMX, TET, MTZ, LEVO, RIB
Allele-specific PCR alone [181, 217]	LEVO, CLA
PCR- restriction fragment length polymorphism (RFLP) [218-222]	CLA, TET
PCR- oligonucleotide ligation assay (OLA) [223, 224]	CLA
Dual priming oligonucleotide (DPO)-PCR[225]	CLA
PCR- DNA enzyme immunoassay (DEIA) [226]	CLA
PCR- line probe assay (LipA) [227]	CLA
PCR- preferential homoduplex formation (PHFA) [228]	CLA
3'-mismatch reverse primer PCR method (3M)-PCR [229]	CLA
Real-Time (RT)-PCR based on hybridization assay [230-235]	CLA, TET
Real-Time (RT)-PCR based on fluorescence resonance energy transfer (FRET)[216, 225, 235]	CLA, LEVO, TET
Real-Time (RT)-PCR based on peptide nucleic acid probe (PNA)[224, 236]	CLA
Digital droplet PCR (ddPCR) [130, 204]	CLA
***In situ* probe hybridization-based assay**	
Fluorescent in situ hybridization (FISH) [149, 179, 237, 238]	CLA
Peptide nucleic acid probe (PNA)- Fluorescent in situ hybridization (FISH)[239]	CLA
Whole genome-sequencing based assays	
Mapping High-throughput short reads to reference genome sequences[21, 210]	CLA, MTZ
Mapping High-throughput short reads to targeted reference gene sequences[16, 17]	AMX, TET, MTZ, CLA, LEVO, RIB
Targeting genes in *de novo* assembly genomes from High-throughput short reads and long reads [20]	AMX, MTZ, CLA, LEVO
Protein-based molecular assay	
Immunoblotting with specific antibodies [208, 240]	MTZ

(*) Abbreviations: AMX, amoxicillin; LEVO, levofloxacin; MTZ, metronidazole; TET, tetracycline ; CLA, clarithromycin ; RIB, rifabutin;

Availability of AST methods

Culture-based AST

Clarithromycin
Levofloxacin
Metronidazole
Amoxicillin
Tetracycline
Rifabutin

Available for
Well conserved gastric biopsies

mAST

Molecular (NGS and PCR)

Clarithromycin* PCR
Levofloxacin
Metronidazole
Amoxicillin
Tetracycline
Rifabutin

Next Generation Sequencing

Available for
Gastric biopsies
Fresh or Formalin-fixed
Stools
Cultures

Other drugs? NTZ, MIN, RIF and FUZ

NGS-based AST: powerful, reliable, reproducible tool

Prof Yamaoka's Lab

Article

Next-Generation Sequencing of the Whole Bacterial Genome for Tracking Molecular Insight into the Broad-Spectrum Antimicrobial Resistance of *Helicobacter pylori* Clinical Isolates from the Democratic Republic of Congo

Home › BMC Microbiology › Article

Genetic determinants of Biofilm formation of *Helicobacter pylori* using whole-genome sequencing

Open Access Article

A Next-Generation Sequencing-Based Approach to Identify Genetic Determinants of Antibiotic Resistance in Cambodian *Helicobacter pylori* Clinical Isolates

Research Article

Genetic Determinants of Biofilm Formation and Antibiotic Resistance of Helicobacter Pylori using Whole Genome Sequencing

Mutations Related to Antibiotics Resistance in *Helicobacter pylori* Clinical Isolates from Bangladesh

Synergistic effects of novel penicillin-binding protein 1A amino acid substitutions contribute to high-level amoxicillin resistance of *Helicobacter pylori*

… How to interpret their outcomes in clinical practice? How to identify the most relevant mAMR?

Helicobacter pylori infection and antibiotic resistance — from biology to clinical implications

Evariste Tshibangu-Kabamba[1] *and Yoshio Yamaoka*[1,2]✉

How does *H. pylori* develop drug resistance? What are the clinical implications?

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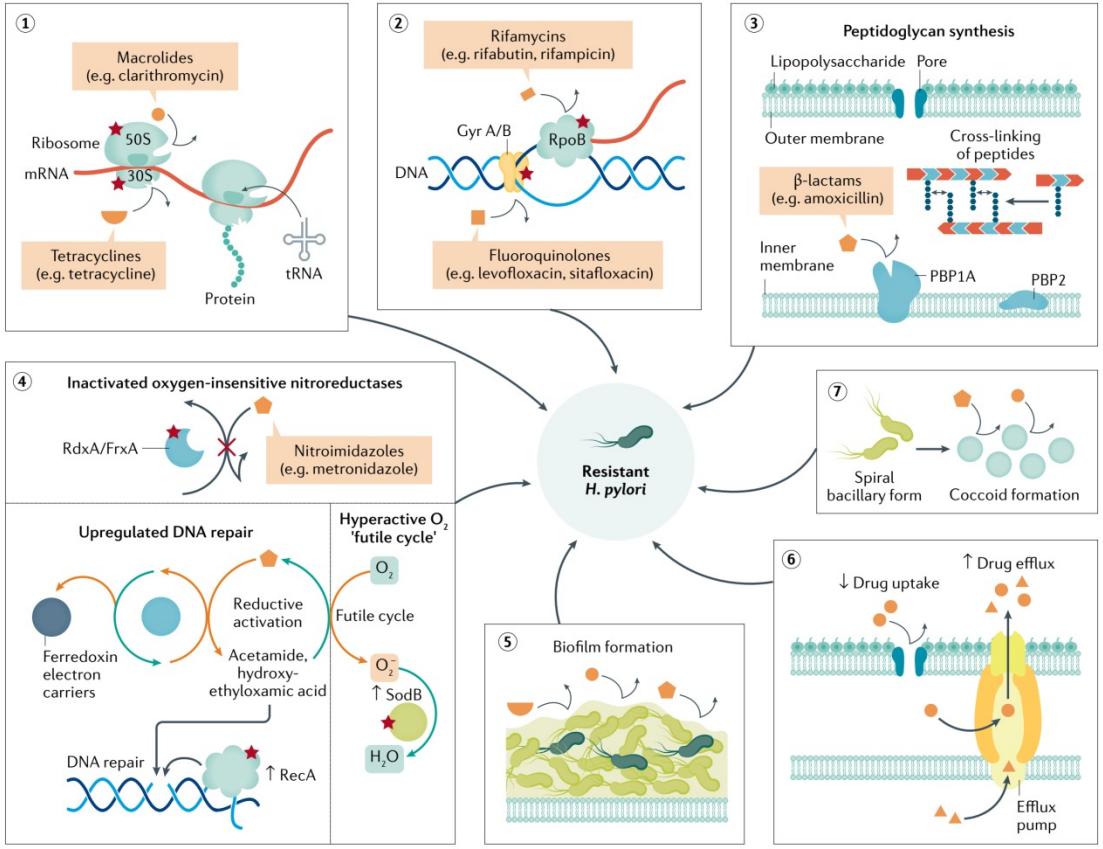
Key points

- Antibiotic resistance in *Helicobacter pylori* is a global threat to human health.
- Attributes driving this resistance include mainly mutations encoded chromosomally but also physiological changes such as impaired regulation of drug uptake and/or efflux, and biofilm and coccoid formation.
- *H. pylori* frequently displays three different profiles of resistance including single drug resistance, multidrug resistance and heteroresistance, probably with nested fundamental mechanisms and clinical implications.
- In individual patients, mechanisms of resistance deployed by *H. pylori* cause treatment failures, diagnostic difficulties and ambiguity in clinical interpretation of therapeutic outcomes.
- At the population scale, increasing antibiotic resistance has globally led to a substantial decrease in *H. pylori* treatment efficacy and probably an increased risk of complications such as peptic ulcers and gastric cancer.
- To fight this resistance, efforts needed include development of efficient vaccines, setting new treatment strategies, improving diagnostic tools for optimizing clinical decisions, and a better understanding of driving mechanisms.



| Biological attributes of resistance in *Helicobacter pylori* species.

Using Next Generation Sequencer

Illumina NGS sequencing platforms

Ion Torrent platforms (Thermo Fisher Sci)

Ion GeneStudio

Ion Proton

Ion PGM

Illumina Miseq
US$120,000
15Gb, read length <600 bases
Running cost: US$3000

Whole genomes of 16 subjects
Within 3 days
1,000 USD/ subject

Max. Throughput	7.5 Gb	15 Gb	120 Gb	1.5 Tb	6 Tb*	1.8 Tb
Max. read length	150bp x 2	300bp x 2	150bp x 2	150bp x 2	150bp x 2	150bp x 2
DNA cluster density	25 M	25 M	400 M	5000 M	5000M	6000 M

Throughput (bp) ≒ Rread length x DNA cluster density

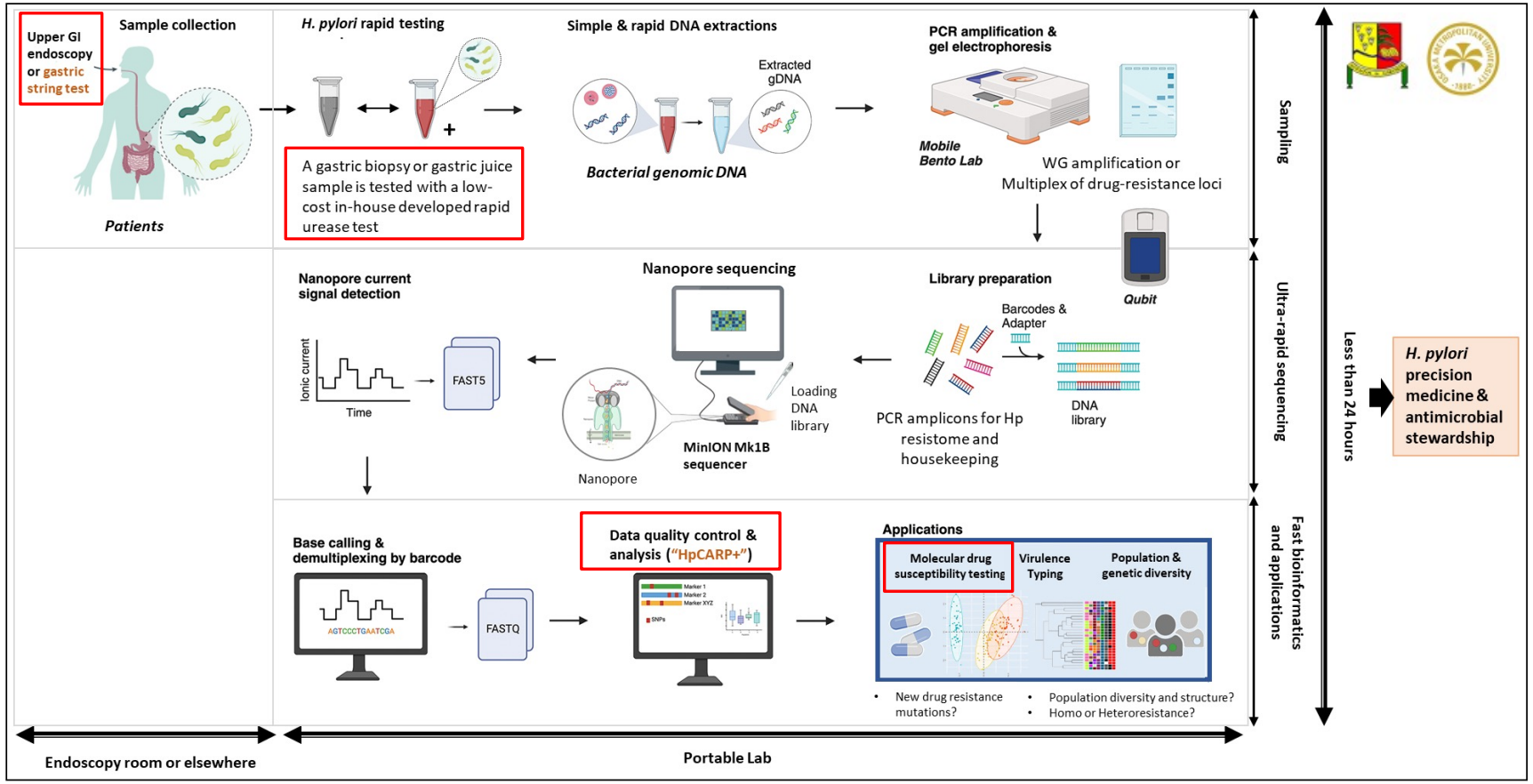
Using Third Generation Sequencers: the future is now

PacBio

Oxford NANOPORE Technologies

Low starting cost
Label-free
+/- PCR-free
+/- multiplex
Flexibility
Ultra-rapid
Ultra-long reads
Low material requirement
High Accuracy with newer chemistry and post sequencing tools

Time for precision medicine for best *H. pylori* management in Africa…



To bypass *H. pylori* culture … and possibly GI endoscopy … to accurately detect *H. pylori* anywhere in Africa…

To uncover genomic *H. pylori* genomic features with ultra-rapid ONT sequencing…

And quickly deliver good *H. pylori* precision medicine and antimicrobial stewardship through an innovative point-of-care system

… Using the right treatments for the right patients at the right time!

OITA UNIVERSITY
RCGLID grant
DS-I Africa Data Science for Health Discovery and Innovation in Africa
NIH Fogarty International Center
ACEGID REDEEMER'S UNIVERSITY, NIGERIA
Combatting AntiMicrobial Resistance in Africa Using Data Science (CAMRA)

- Several molecular markers for *H. pylori* antimicrobial resistance (Hp-AMR) have been reported.

- However, the biological and clinical relevance of these markers remains to be fully elucidated to guide the interoperability of molecular testing.

Aim

- This study aims to evaluate the evidence basis for individual mAMR likely to enable interpretation and implementation of molecular AST-based approaches in *H. pylori* management.

Method

Approach

- A comprehensive search is conducted across 8 public databases, adhering to PRISMA guidelines, to identify relevant studies reported from inception to 2024.

- A critical and systematic review is performed to assess the biological significance of each reported molecular marker.

- **A scale with 4 strenght of evidence levels is used to assess the relevence of each mAMR reported**

Inclusion criteria :

Hp-AMR molecular marker discovery articles

Main data collected and interpreted:

1) Methods used to link molecular markers to Hp-AMR

2) Genes linked to Hp-AMR

3) Mutations linked to Hp-AMR

4) Putative biological function

We published a similar work on malaria artemisinin resistance

THE LANCET
Infectious Diseases

REVIEW | VOLUME 21, ISSUE 4, E82-E92, APRIL 2021 Download Full Issue

Spatial and molecular mapping of *Pfkelch13* gene polymorphism in Africa in the era of emerging *Plasmodium falciparum* resistance to artemisinin: a systematic review

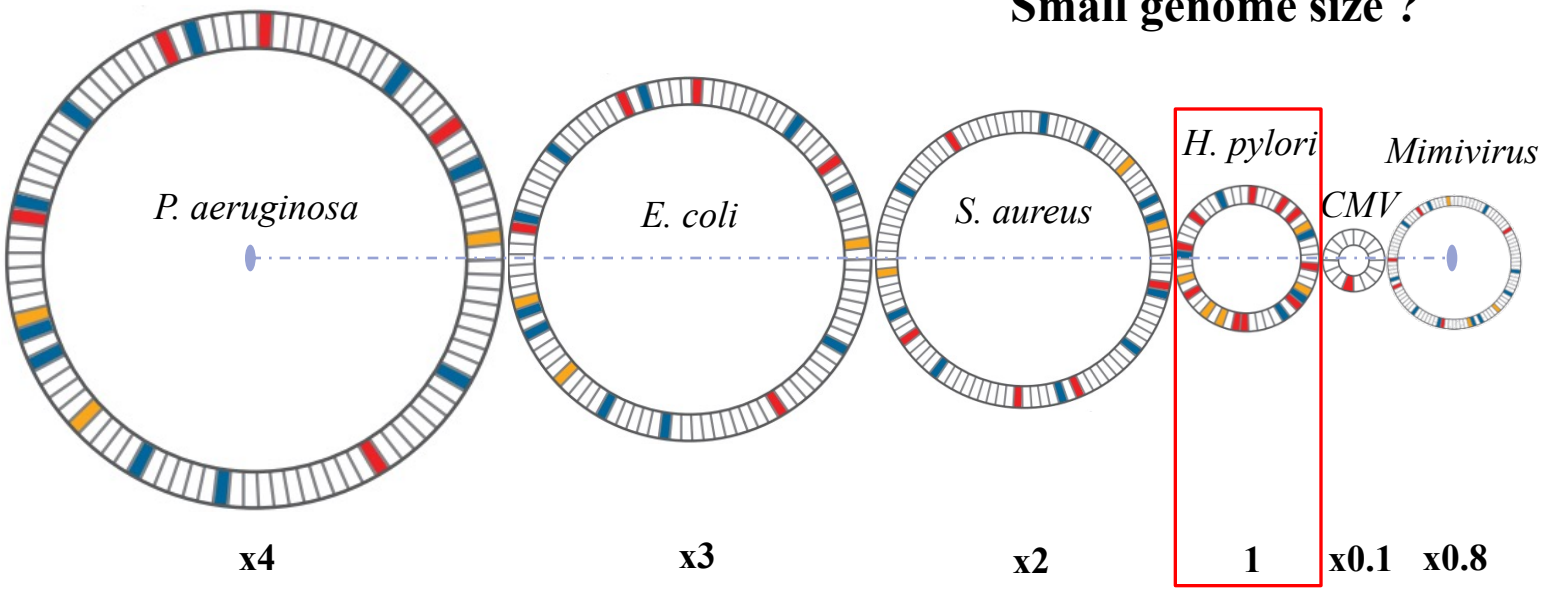
Nadine K Kayiba, MPH • Doudou M Yobi, MSc • Evariste Tshibangu-Kabamba, PhD • Vo P Tuan, PhD • Prof Yoshio Yamaoka, PhD • Brecht Devleesschauwer, PhD • Dieudonné M Mvumbi, PhD • Prof Emile Okitolonda Wemakoy, PhD • Prof Patrick De Mol, PhD • Prof Georges L Mvumbi, PhD • Prof Marie-Pierre Hayette, PhD • Angel Rosas-Aguirre, PhD • Prof Niko Speybroeck, PhD • Show less

Published: October 27, 2020 • DOI: https://doi.org/10.1016/S1473-3099(20)30493-X • Check for updates

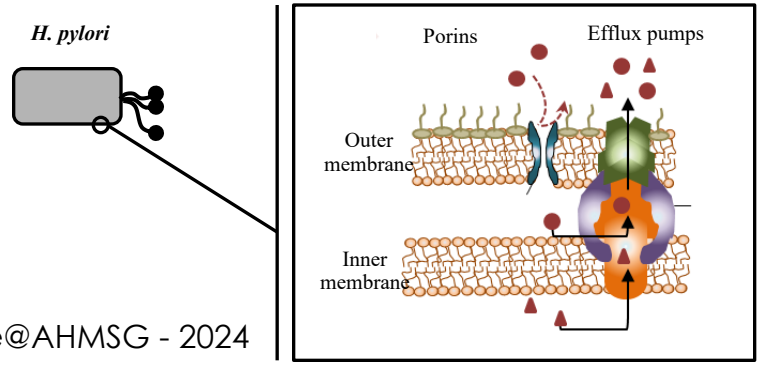
Outcomes

Intrinsic resistance

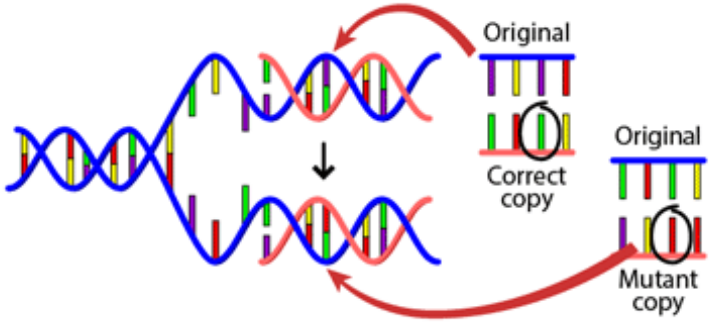
Small genome size ?



The permeability of the bacterial cell wall

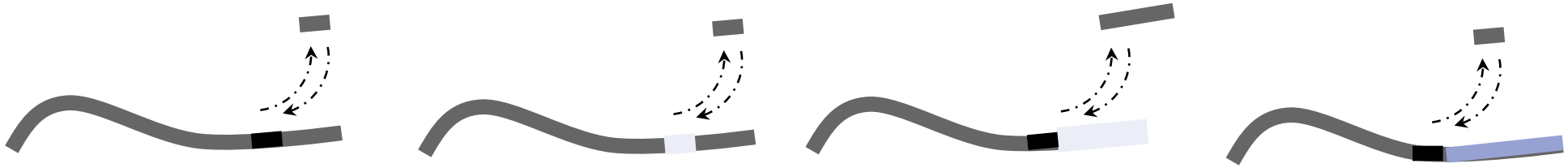


Acquired resistance: Antibiotic resistance-encoding genetic changes are mostly gene mutations

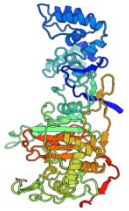


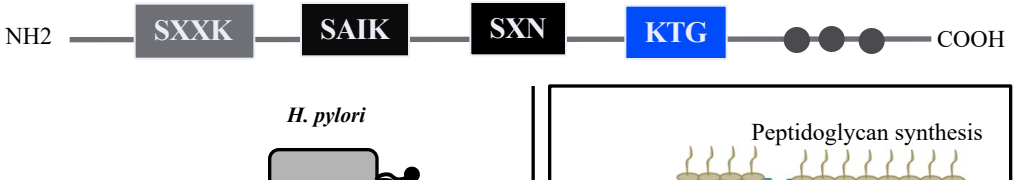
	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr

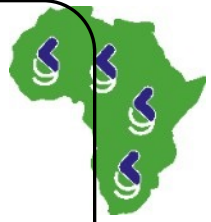
basic
polar



β-lactams : e.g., amoxicillin (AMX)



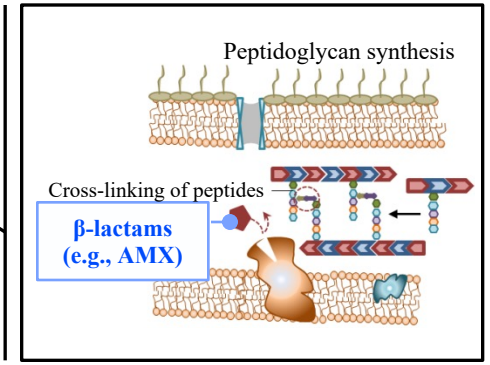




Resistance

Drug uptake limitation	?	Mutation in porins encoded by the ***hofH***, ***hefC***, or ***hopC*** genes
Drug inactivation	?	β-lactamase activity
Drug target modification	✓	Mutations on Penicillin-binding proteins (PBPs) encoded by ***pbp1A*** rather than ***pbp2***, ***pbp3***, and ***pbp4*** genes

H. pylori



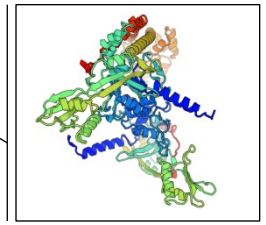
Fluoroquinolones : e.g., levofloxacin (LEVO), moxifloxacin (MOFX), sitafloxacin (STFX), gatifloxacin (GAT), garenoxacin (GRNX), and delafloxacin (DLFX)

Resistance

Drug target modification ✓

Mutations in the quinolone resistance-determining region (QRDR) of genes encoding DNA gyrase subunit A and B (*gyrA* and *gyrB*)

H. pylori

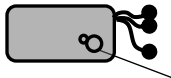


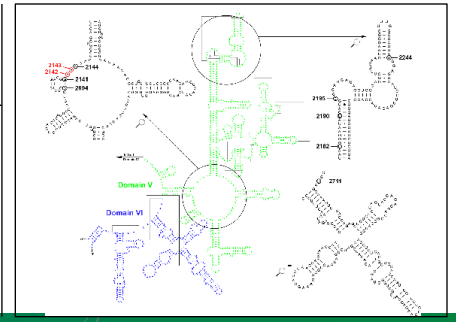
Macrolides: e.g., clarithromycin (CLA)

Resistance

Drug target modification ✓

- Adenine to Guanine substitution at nucleotide-positions A2142 and A2143 in domain V of the gene encoding 23S rRNA;
- ? Absence of mutations on the *rpl22* gene (encoding a ribosomal protein that interacts with the 23S rRNA domains) ;
- ? G160A on *infB* (encoding a translation initiation factor, IF-2) in only one susceptible isolate

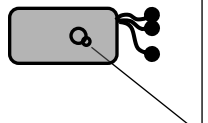


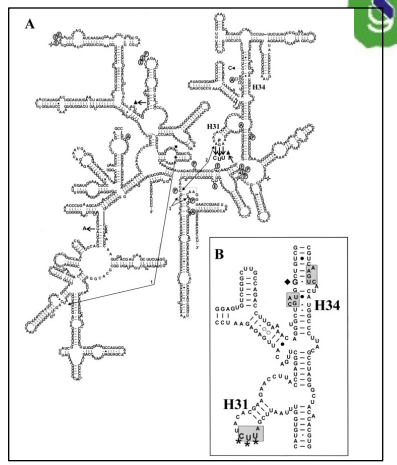


Tetracyclines : e.g., tetracycline (TET) and minocycline (MIN)

Resistance

Drug target modification	✓	- Mutations inside the TET-binding site of 16S rRNA (e.g., AGA926_928(TTC/GGC), AG926_927GT, GA927_928TC, A926(C/G/T) , A928C, A939C) ;
Other hypothetical	?	- (i) efflux of TET-cation complexes by proteins such as TetA; (ii) cytoplasmic overexpression of proteins for ribosomal protection such TetO; and (iii) inactivation of TET by enzymes such as TetX.



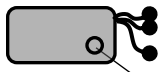


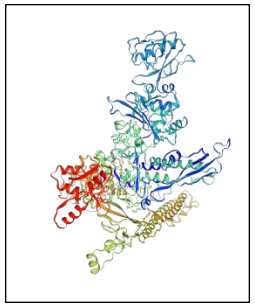
Rifamycins : e.g., rifampicin (RIF) and rifabutin (RIB)

Resistance

Drug target modification	✓	- Mutations inside/outside the RDR_{511_612}[89, 90, 211] – e.g., V149F, Q524P, L525P, Q527K, Q527R, D530(V/E), D530N, V538I, H540 (N/Y), H540N, S545L, A603T, I586N, I586L;
Other hypothetical	?	- ?

H. pylori





RpoB

- **HpResGene db (a Resistance Gene db newly established for this study)**

Gene	ATB
pbp4	AMX
hcpA	AMX
lytB	AMX
pbp1A	AMX
RodA1	AMX
mreC	AMX
mreB	AMX
mreB	
mreB	
ftsI	
pbp2	
llm	

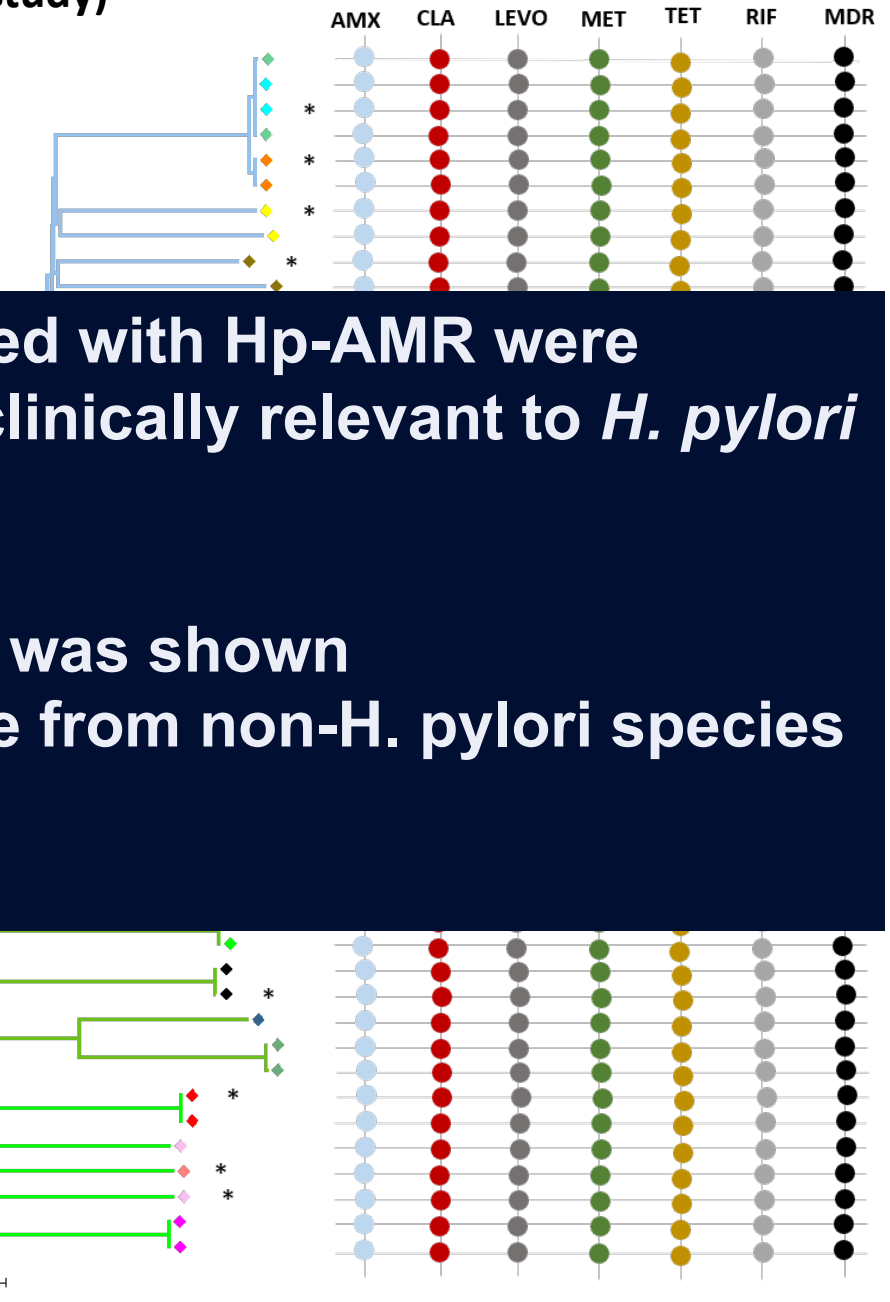
Gene
kefB
kefB
SrRNA2
SrRNA2
infB
rpl22

Gene
rpoB

Gene	
lepA	
yihK	TET
ketoGP	TET
fusA	TET
tufB	TET
tetA	TET
SecD	TET
SrRNA16	TET
SrRNA16	TET
infB	TET

id	ATB
dapF	MET
fdxA	MET
ddpB	MET
MSF	MET
sodB	MET
PorCDBA	MET
PorCDBA	MET
recA	MET
rnc	MET
ddpA	MET
rps4	MET
rpsU	MET

Gene	ATB
hefA	MDR
hefB	MDR
hefC	MDR
norM1	MDR
codA	MDR
hefI	MDR
hypothP	MDR
RND_TP	MDR
RND_MFP	MDR
rnd_OMP	MDR
copA2	MDR
abc_NikA	MDR



A total of 86 genes potentially associated with Hp-AMR were reported, covering various antibiotics clinically relevant to *H. pylori* eradication therapies.

So far, no gene acquisition mechanism was shown
1) no horizontally transferred AMR gene from non-H. pylori species was established
2) no plasmid-encoded AMR gene

Updated list of mutations and genes linked to Hp-AMR as of July 2024

Antibiotics	Gene/sequence	Putative resistance mutations[±,*]	
β-Lactams (e.g., AMX)	*pbp-1A*	p.	Mutations inside/outside PBP-motifs and in the c-terminus[20, 37, 38] – e.g., V45I, T337_S338insN, S338R, F366L, V374L, Y401_S402insY, S402G, N404S, S405N, S414R, L423F, S455N, K464insE, V469 (M/A), A474T, N504D, D535N, S543 (H/R), T556S, T558S, N562 (D/H/Y), N562D, N562H, N562Y, T593 (A/G/K/P/S), G595 (del/A/S), A599 (T/P/V), Y637Ter
	pbp2	p.	V312M, V313A, G353R
	pbp3	p.	F233L
	pbp4	p.	Y266H, Y267H
	hofH	p.	G22W
	hefC	p.	D131E, L378F
	hopC	p.	R302H
Fluoroquinolones (e.g., LEVO)	*gyrA*	p.	Mutations inside/outside the $QRDR_{A71\_Q110}$ (e.g., H57Y, S63P, V65I, V77A, S83A, D86N, N87A/K/I/Y/T, A88N/P/V, D91G/N/A/H/Y, A92T, D99V, R103H, A129T, R130K, D155N, D161N, V172I, P188S, D192N, A199V/I)
	gyrB	p.	Mutations inside/outside the $QRDR_{E415\_S454}$ (e.g., D435N , V437L, F438S, S429T, E463K, D481E, R484K, R579C)
Macrolides (e.g., CLA)	*23S rrna*	c.	Mutations inside/outside the V domain (e.g., T1942C, G1939A, C2147G, G2172T, T2182C, A2116G, A2142(C/G) , A2143G, A2144G/T, A2115G, G2111A, T2717C, T2289C, G2224A, C2245T)
	rpl22	c.	226_228delGTG, T265_T266insTTCCATGTA
	infB	c.	G160A
Nitroimidazoles (e.g., MTZ)	*rdxA* and *rdxA* -related promoter region	p.	Missense mutations at well-defined functional codons based on experimental studies[65] (e.g., R16, H17, S18, C19, K20, R41, L42, S43, Y47, Q50, V55, M56, N73, I142, A143, G145, G149, C159, G162, G163, V192, K198, K200, K202, L209), frameshift/nonsense mutations at any codon-position (all TfsTer, and Ter), large sequence deletions (e.g., K2_M21del, R131_K166del, K168_V172del, L137_I142del, N178_L185del; G189_R200del, S92_Q146del), large sequence insertions, missense mutations observed in clinical isolates (e.g., A22S, E27Q/V, T31E, D59N, R90K, H97T/Y, P106S, S108A, A118S/T, R131K, and G189C) [37, 38], and hypothetical mutations in the promoter region [208, 209].
	frxA	p.	Mutations at well-defined functional codons following experimental studies[63] (e.g., K17, R13, A15, K20, Q164, G165, R206), and null mutations at any codon-position (all TfsTer, and Ter).
	fur	p.	Mutations at well-defined functional codons following experimental studies[71, 73] – e.g., R3, M42, Y65, C78, E90, H99, E110, P114, and in the $HHDHxxCxxC_{96\_105}$-motif
	sodB-related promoter region	c.	A-5C[75].
	recA	p.	Y103H and S121D[72]
	mdaB		R99I and G98D[69]
	ribF	p.	T222M and A227T[69]
	omp11	p.	A1290D[69]
	rpsU	p.	D13T[210]
Tetracyclines (e.g., TET)	*16S rrna*	c.	Mutations inside the TET-binding site (e.g., AGA926_928(TTC/GGC), AG926_927GT, GA927_928TC, A926(C/G/T) , A928C, A939C)
Rifamycins (e.g., RIB)	*rpoB*	p.	Mutations inside/outside the RDR_{511_612}[89, 90, 211] – e.g., V149F, Q524P, L525P, Q527K, Q527R, D530(V/E), D530N, V538I, H540 (N/Y), H540N, S545L, A603T, I586N, I586L
Nitrofurans (e.g., FUR)	*porD*	p.	G353A, A356G, and C357T[93]
	oorD	p.	A041G, A122G, C349A/G[93]

(±) p., mutations reported by amino acid residues; c., mutations reported by nucleotide residues; (*) Nucleotide residues in DNA sequences are represented by heterocyclic bases as follows: A, Adenine; C, Cytosine; G, Guanine; and T, Thymine. Single-letter abbreviations for the amino acid residues in protein sequences are as follows: A, Alanine; C, Cysteine; D, Aspartic acid; E, Glutamic acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; and Y, Tyrosine. Other abbreviations are as follows : RDR, *rpoB* resistance-determining region; QRDR, Quinolone resistance-determining region; (#)

~ 136 mutations potentially associated with Hp-AMR were reported, covering 7 families of antibiotics clinically relevant to *H. pylori* eradication therapies.

Biological mechanisms of drug resistance described in *H. pylori* species*

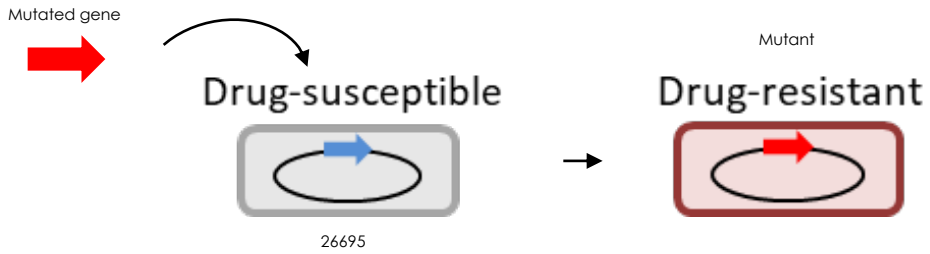
Antibiotics	Mode of resistance	Molecular target or change	Resistance mechanisms
β-Lactams (e.g., AMX)	Drug target-mediated resistance	PBPs (i.e., PBP-1A, PBP2, PBP3) alteration by missense, indel, or nonsense mutations in/around PBP-motifs (SxN, KTG, and SxxK) and PBPs c-terminus sequences	Protection of peptidoglycan synthesis during cell wall synthesis
	Drug uptake limitation	HopC and HofH porins alteration by missense mutations	†Putative decrease of membrane permeability to the drug
Fluoroquinolones (e.g., LEVO)	Drug target-mediated resistance	GyrA and GyrB with sequence alterations inside/outside QRDR by missense mutations	Protection of chromosomal supercoiling during DNA synthesis, transcription and cell division
Macrolides (e.g., CLA)	Drug target-mediated resistance	23S rRNA with a V domain altered by due single or double, or triple base-pair substitutions	Protection of the mRNA-tRNA translocation step during protein synthesis
		Rpl22 or InfB with alterations by missense mutations, or indels	†Putative protection of ribosomal domains
Nitroimidazoles (e.g., MTZ)	Drug detoxication	FrxA and/or RdxA with altered molecule stability, dimerization or flavin mononucleotide binding by frameshift, nonsense, indel, or missense mutations	Reduced or suppressed drug reductive activation by altered oxyger insensitive nitroreductases
		Down-regulated expression of RdxA likely by mutations in related promoter region	† Reduced or suppressed drug reductive activation by down-regula oxygen-insensitive nitroreductases
		Hyperactivity of oxygen "futile cyle"; upregulation of SodB by an inactivation of Fur activity due to Fur missense or nonsense mutation, and single, base-pair substitution in sodB promoter region	Regeneration of inactive drug compounds by increased futile cycli of oxygen and drug; protection against oxidative reactions
	Drug target-mediated resistance	Upregulation of RecA DNA repair effector due to missense mutations	Protection of DNA from damage by SOS and drug toxic derivative
Tetracyclines (e.g., TET)	Drug target-mediated resistance	16S rRNA with a TET-binding pocket altered by single, double, or triple base-pair substitutions	Protection of the peptide-chain elongation step during protein synthesis
Rifamycins (e.g., RIB)	Drug target-mediated resistance	RpoB with altered Rifamycins binding sites by missense mutations	Protection of the extension of RNA chain during DNA transcriptio
Nitrofurans (e.g., FUR)	Drug detoxication	PorD and/or OorD with missense mutations	Probably reduced or suppressed drug reductive activation by altere ferredoxin-like subunits
Multiple drugs (e.g., AMX, TET, MTZ, CLA)	Cumulative MDR profile	Alterations of multiple drug targets	Accumulation of resistance mutations for separated drug families
	Drug efflux	Upregulated or overexpressed RDN efflux systems Hef ABC, DEF, and GHI (substrates e.g., TET, MTZ, CLA, and AMX)	Reduction of intracellular drug concentration below lethal doses
		Overexpressed MFS efflux system GluP (substrates e.g., AMX, CLA, TET, MTZ, FUR)	Reduction of intracellular drug concentration below lethal doses
		Upregulated or overexpressed ABC transporters MsbA and Imp/OstA (substrates e.g., erythromycin, novobiocin, rifampicin)	Reduction of intracellular drug concentration below lethal doses
	Biofilm formation	Biofilm matrix; efflux pump overexpression; genetic mutations	†Multifactorial barriers (mechanical, physiological, or genetic) to d penetration
	Coccoid formation	Shape/surface modifcations accompanied with ultrastructural and metabolic changes	†Multifactorial barriers (mechanical, physiological, or genetic) to d penetration and activity

(*) Abbreviations: AMX, Amoxicillin; TET, Tetracycline; CLA, Clarithromycin; LEVO, Levofloxacin; MTZ, Metronidazole; RIB, Rifabutin; PBPs, Penicillin-binding proteins; HopC, *Helicobacter pylori* outer membrane pro C; HofH, *Helicobacter* outer membrane protein family; rRNA, ribosomal RNA; mRNA, messenger RNA; RpoB, b-subunit of DNA-dependent RNA polymerase; GyrA, DNA gyrase subunit A; GyrB, DNA gyrase subuni QRDR, Quinolone resistance-determining region; RdxA, oxygen-insensitive NAD(P)H nitroreductase; FrxA, NAD(P)H flavin nitroreductase; Fur, ferric uptake regulator; SodB, superoxide dismutase; RecA, DNA recombinas PorD, pyruvate:flavodoxin oxidoreductase subunit D; OorD, 2-oxoglutarate:acceptor oxidoreductase subunit D, InfB, translation initiation factor 2; Rpl22, ribosomal protein L22p; RDN, restriction-nodulation-division; O organic solvent tolerance; Imp, increased membrane permeability; MsbA, multicopy suppressor of null mutations in the htrB gene; GluP, glucose/galactose transporter.
(†) Hypothetical mechanism

Methods for assessing molecular markers of drug resistance in *H. pylori*

- Phenotypic screening
- Amplicon sequencing (AmpSeq)
- Whole Genome Sequencing (WGS)
- Genome-Wide Association Studies (GWAS)
- Functional analysis (functional genomics, transcriptomics, proteomics, metabolomics, Bioinformatics and In Silico Modeling)

Site-directed mutagenesis by natural transformation



Validation methods for Hp-AMR molecular markers primarily included site-directed mutagenesis approaches and statistical associations with in vitro resistance phenotypes.

Type of Evidence

Validation

Functional studies, e.g. Site-directed mutagenesis

Association

Statistical association between an observed marker and a resistance phenotype (association # causality)

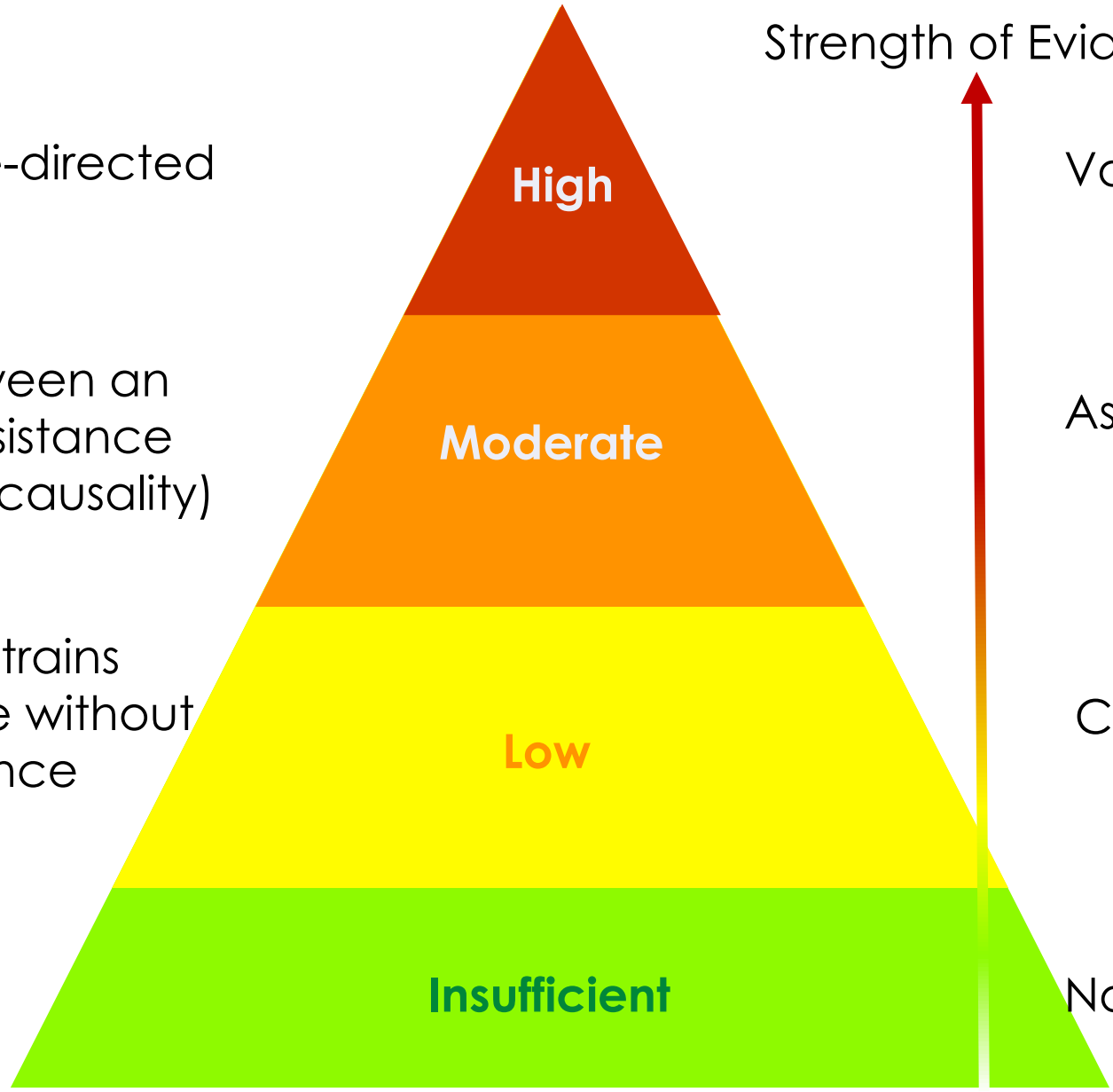
Suspicion

Observation of a marker in strains with a resistance phenotype without reaching statistical significance

No suspicion

Simple observation of a marker in both resistant and susceptible strains

Four Strenght of Evidence Levels



We thus propose a practical categorization of Hp-AMR molecular markers into three groups:

1) **"validated" markers**, which have functionally demonstrated biological relevance (<10%);

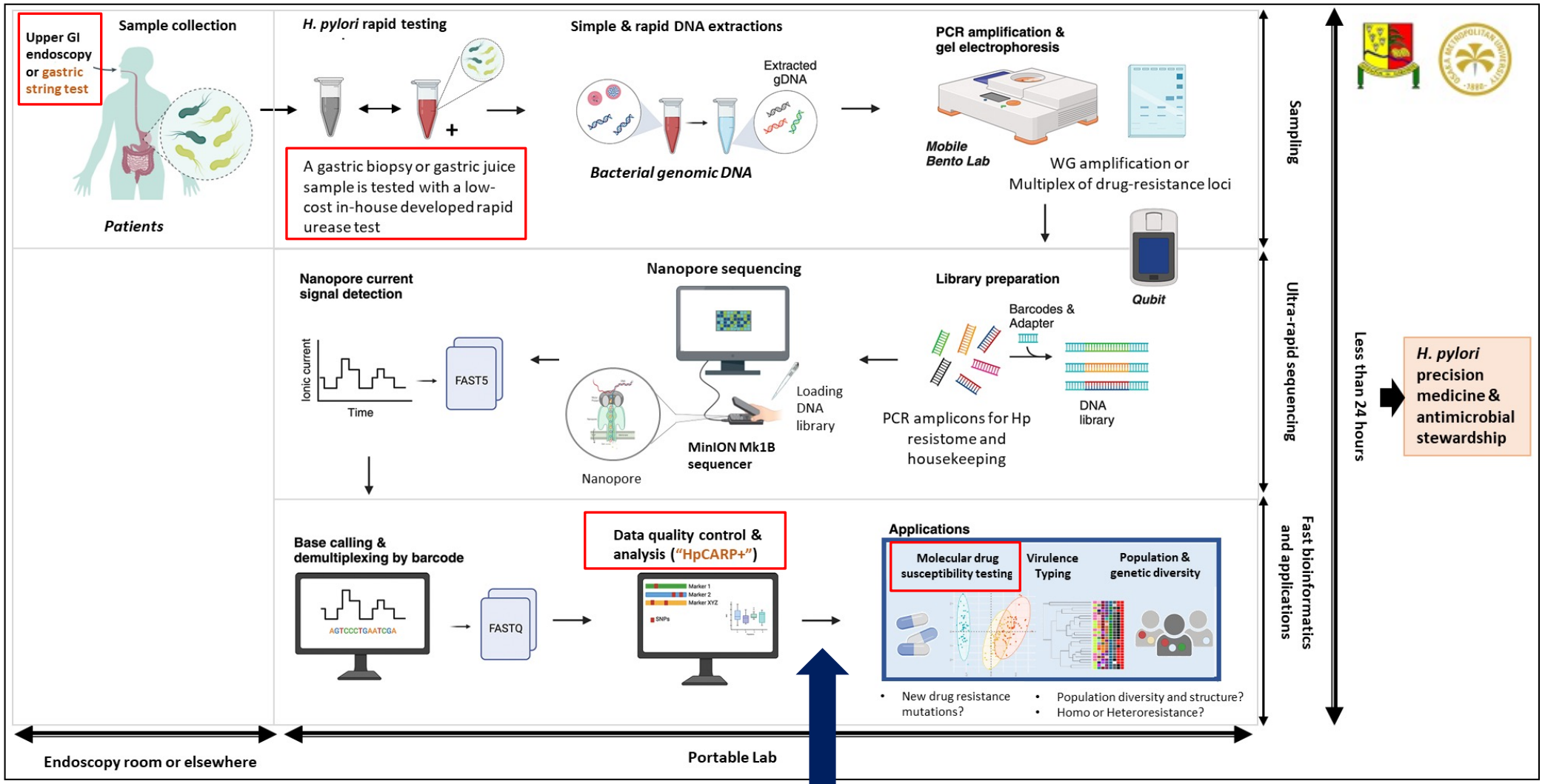
2) **"candidate" Hp-AMR markers**, statistically associated with in vitro resistance (~15%);

3) **"suspect" Hp-AMR markers**, which are mutations suspected of in vivo resistance without reaching statistical significance due to the low number of observed mutants (~75%).

Other mutations are not Hp-AMR markers.

Using Hp 26695 as wild-type reference strain / sequence

Time for precision medicine for best *H. pylori* management in Africa…



GPT-based tool to interpret mAST outcomes

RCGLID grant

Combatting AntiMicrobial Resistance in Africa Using Data Science (CAMRA)

Provide a practical guidance for *H. pylori* mAST

1) **"validated" markers** (<10%) ⟶ Confidently treat the strain as resistant

2) **"candidate" Hp-AMR markers** (~15%) ⟶ Treat the strain as potentially resistant with some confidence, but expect further confirmation

3) **"suspect" Hp-AMR markers** (~75%) ⟶ Don’t treat the strain as resistant, request phenotypic assessment

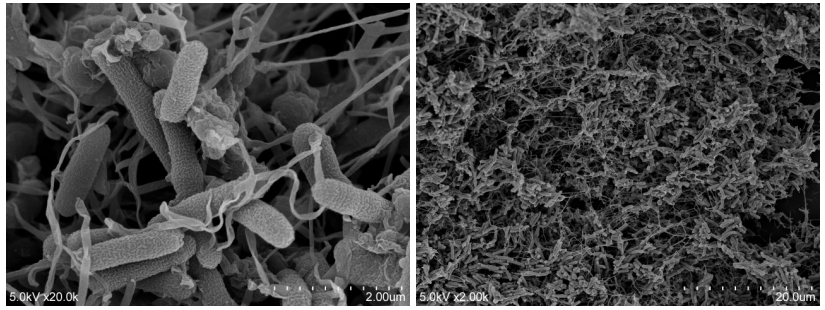
We believe this stratification can support antimicrobial stewardship in H. pylori management by avoiding unnecessarily ATBs usage

Some complex cases…

1) ‘Structurally mimicking Hp-AMR markers’
e.g. QRDR GyrA **D86M** instead **D86N**
> Substituting AA is from a family that differ from the wild-type AA : considerer as a candidate Hp-AMR marker.

2) ‘Synergic mutations’
e.g. PBP1A

3) ‘Metabolic resistance’ in ‘Complex resistance phenotypes’ e.g. MTZ and AMX
e.g. Biofilm forming strains, stress-related changes, environment-related change, efflux pumps



mAST cannot definitively replace pAST; they need to complement each other

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| Antimicrobial Chemotherapy | Research Article

Synergistic effects of novel penicillin-binding protein 1A amino acid substitutions contribute to high-level amoxicillin resistance of *Helicobacter pylori*

Alain Cimuanga-Mukanya,[1,2] Evariste Tshibangu-Kabamba,[2,3] Patrick de Jesus Ngoma Kisoko,[4] Kartika Afrida Fauzia,[5] Fabien Mbaya Tshibangu,[1,2] Antoine Tshimpi Wola,[4] Pascal Tshiamala Kashala,[6] Dieudonné Mumba Ngoyi,[7] Steve Ahuka-Mundeke,[8] Gunturu Revathi,[9] Ghislain Disashi-Tumba,[2] Yasutoshi Kido,[3] Takashi Matsumoto,[1] Junko Akada,[1] Yoshio Yamaoka[1,10,11,12]

‘Synergic mutations’

Given mutation A, B, and C

Mutants with A, B, or C alone ⟶ ~ no change in MICs compared to WT strain

Certain combinations of A, B, or C ⟶ ~ gradual increase of MICs compared to WT strain

Are these Hp-AMR marker or not?

Prof Yamaoka's Lab

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Antimicrobial Chemotherapy | Research Article

Synergistic effects of novel penicillin-binding protein 1A amino acid substitutions contribute to high-level amoxicillin resistance of *Helicobacter pylori*

'Synergic mutations'

Strain	Genotype or description	Reference (no.)
Wild-types:		
KIN76	AMX^R, carrying mutations T558S, N562H, T593A, and G595S in PBP1A, and used as DNA donor	(16) (OR855
26695	AMX^S, used as DNA recipient	(31)(NC_00
Transformants:		
Originating from 26695, transformed with the *pbp1a* fragment of KIN76 and selected as AMX^I		
26695_Fr3_1	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_2	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_3	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_4	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_5	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_6	Carrying N562H in PBP1A^{26695}	This study
26695_Fr3_7	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr3_8	Carrying T558S and N562H in PBP1A^{26695}	This study (JAXCGF00
26695_Fr4_1	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr4_2	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr4_3	Carrying T558S and N562H in PBP1A^{26695}	This study
26695_Fr4_4	Carrying T558S and N562H in PBP1A^{26695}	This study
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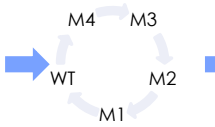
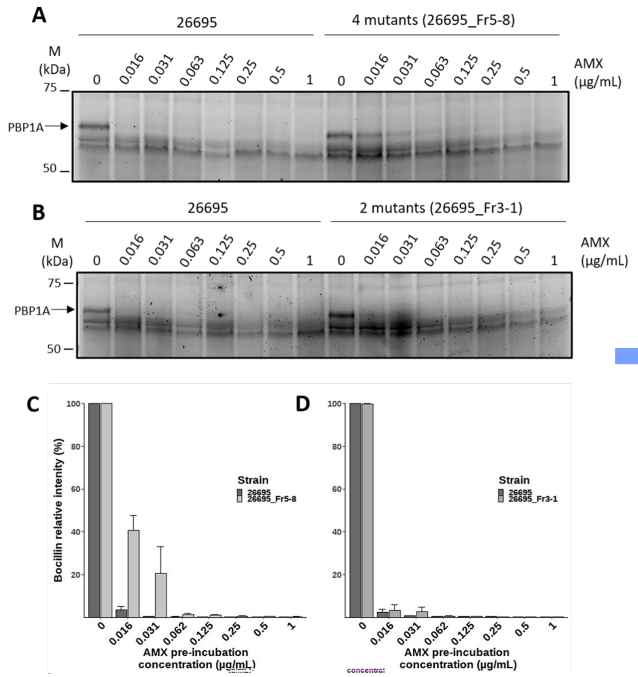
26695_Fr5_1	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_2	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_3	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_4	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_5	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_6	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study
26695_Fr5_7	Carrying T593A, G595S, and T593A in PBP1A^{26695}	This study
26695_Fr5_8	Carrying T593A, G595S, T593A, and G595S in PBP1A^{26695}	This study

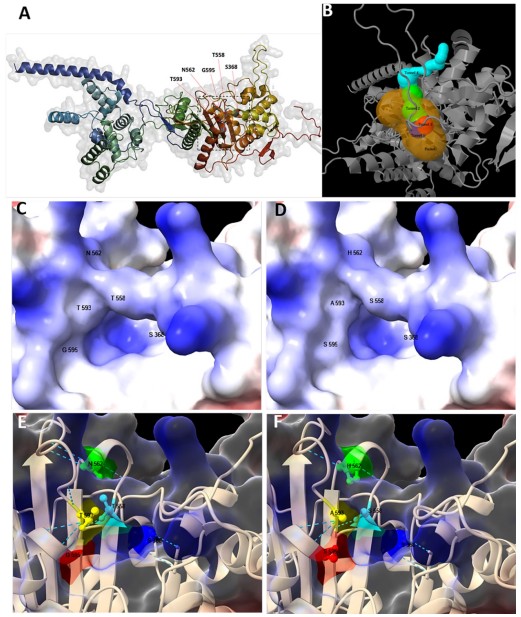
***H. pylori* strains used**



Comparative genomics

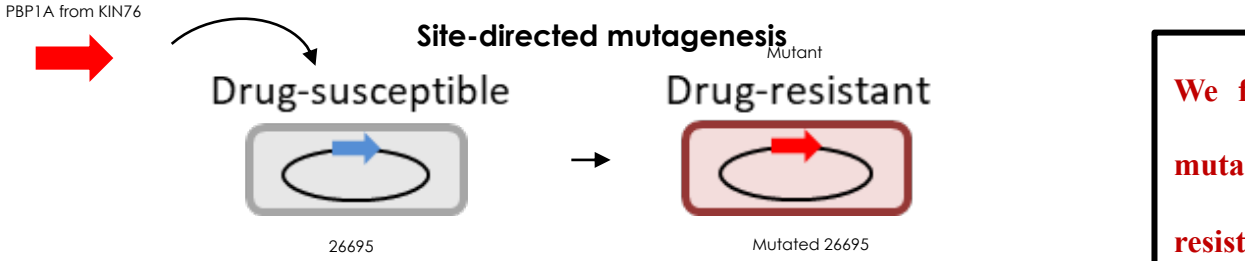


AMX-Bocillin competitive binding assay



***H. pylori* PBP1A tertiary structure models**

Dr. Alain Cimuanga



Genetically modified strains with various combinations of **T558S, N562H**, G595S, T593A

We found that *H. pylori* strains from the RDC use newly discovered mutations with synergistic effects on MICs to establish high-level AMX resistance

AMR-Related Genetic Determinants and Prediction of Phenotypic Resistance : Metronidazole (MTZ)

(p.8&9; Suppl. p.9,10&11)

Possible mechanisms	Outcomes
Drug detoxication	Mutations related to MTZ could be identified mainly in genes encoding the **oxygen-insensitive NAD(P)H nitroreductase (*rdxA*)** and the NAD(P)H flavin nitroreductase (*frxA*) and secondarily in genes encoding the ferric uptake regulator (*fur*) and in the promoter region of superoxide dismutase (*sodB*)
Drug target modification	No relevant mutation of RecA DNA repair effector

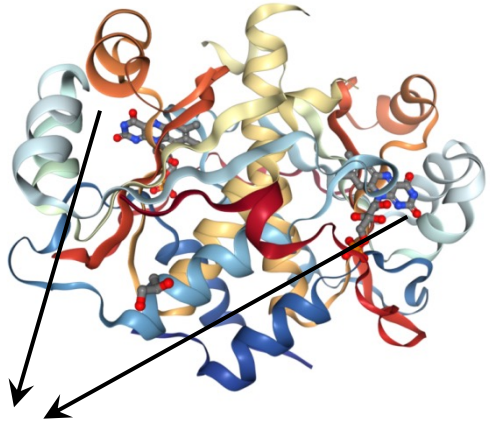
Table. Potential genotype encoding MTZ-R in Hp clinical isolates from DRC

Genotype	MTZ-S				MTZ-R				p-value
	n_0	%	n_1	%	n_0	%	n_1	%	
***rdxA* gene**									
Wild type sequence	4	40.0	6	60.0	79	85.9	13	14.1	0.003
Mutations at a known functional loci	9	90.0	1	10.0	25	27.2	67	72.8	0.000
Null mutations	10	100.0	0	0.0	44	47.8	48	52.2	0.001
Frameshift mutations (e.g., Q65TfsTer10)	10	100.0	0	0.0	67	72.8	25	27.2	0.114
Premature stop codons (e.g., Q50Ter)	10	100.0	0	0.0	80	87.0	12	13.0	0.602
Large sequence deletions (e.g., K2_M21del§)	10	100.0	0	0.0	82	89.1	10	10.9	0.592
Large sequence insertions ending with a stop	10	100.0	0	0.0	91	98.9	1	1.1	1.000
Point-mutations at functional codons (e.g., R16C/H; H97T/Y)	9	90.0	1	10.0	64	69.6	28	30.4	0.274

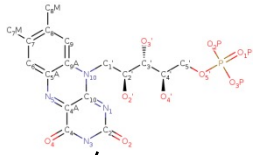
(Modified from Table S5 (A))

Mechanism of resistance to Metronidazole (MTZ)

RdxA : a flavoprotein with nitroreductase activity



FMN cofactors



The Flavin Mononucleotide (FMN) coenzyme helps the RdxA to reduce MTZ molecules into active compounds

MTZ (prodrug that needs intracellular activation by nitroreductases)

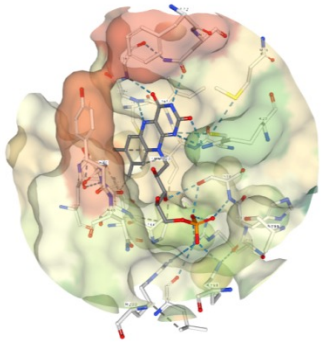
↓ Reductive activation

Active MTZ compounds
(Acetamide, HO-ethyl oxamic acid, etc.)

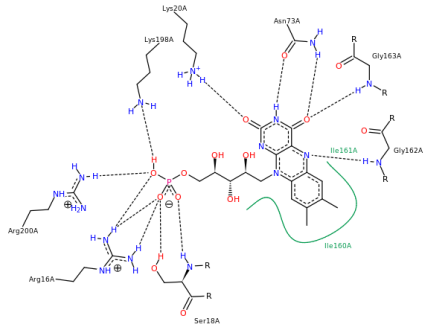
↓

Bactericidal effect

Crystal structure of RdxA of H. pylori



The FMN ligand pocket.



FMN binding sites

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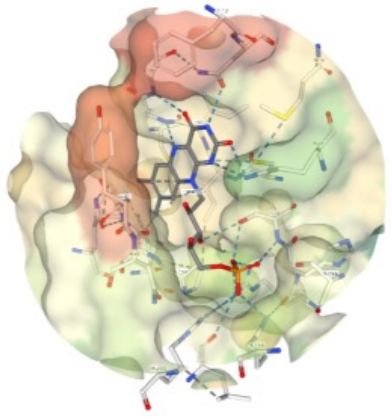
Structure of RdxA: an oxygen insensitive nitroreductase essential for metronidazole activation in *Helicobacter pylori*

Marta Martínez-Júlvez[1,2,#], Adriana L. Rojas[3,#], Igor Olekhnovich[4], Vladimir Espinosa Angarica[1,2], Paul S. Hoffman[4,*], and Javier Sancho[1,2,*]

Specific drug resistance mechanisms

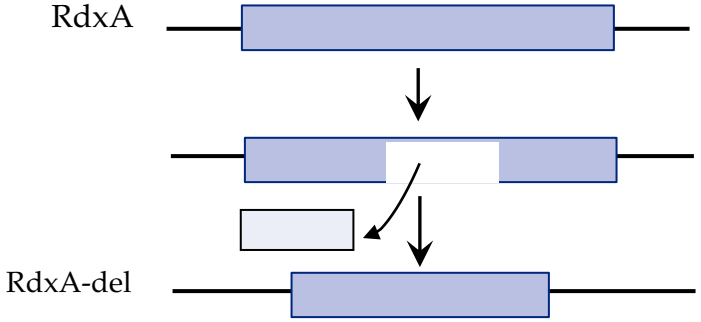
RdxA-del : A new and emerging mechanism of resistance to Metronidazole (MTZ) in the DRC

Flavin mononucleotide (**FMN**)

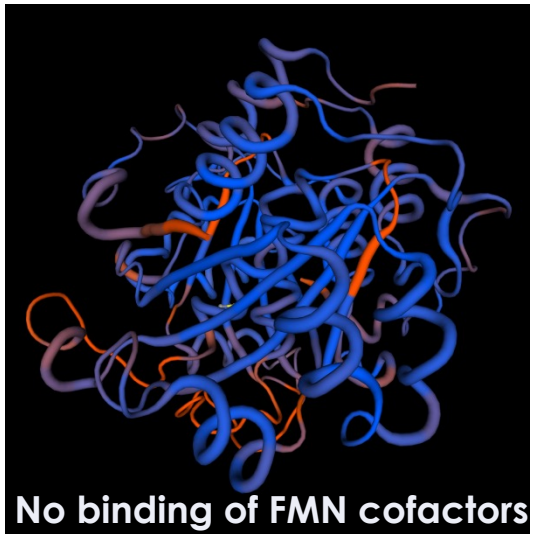


The FMN ligand pocket

RdxA protein

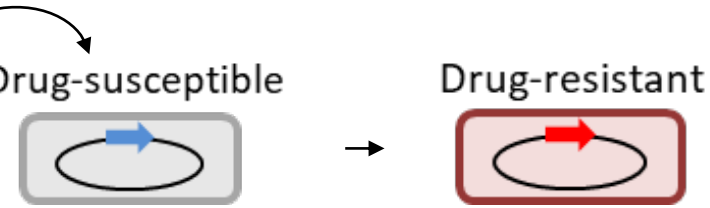


RdxA (K2_M21del**)**



RdxA-del =
1) ↔ non optimal ability of binding and catalysis of substrates
2) ↔ loss of ability to reduce the lower redox MTZ
2) ↔ loss of ability to bind the FMN cofactor

RdxA-del



Genetically modified strains with RdxA-dels

We found that *H. pylori* strains from the RDC use gene deletions (not only SNPs!) to irreversibly establish high-level MTZ resistance

RdxA-del as a new mechanism of resistance to Metronidazole (MTZ)

- 10% of MTZ-R were due to RdxA-del : $RdxA_{(R131\_P166del)}$, $RdxA_{(N178\_L185del;\ G189\_R200del)}$, $RdxA_{(L137\_I142del)}$, and $RdxA_{(K168\_V172del)}$, and $RdxA_{(K2\_M21del)}$.

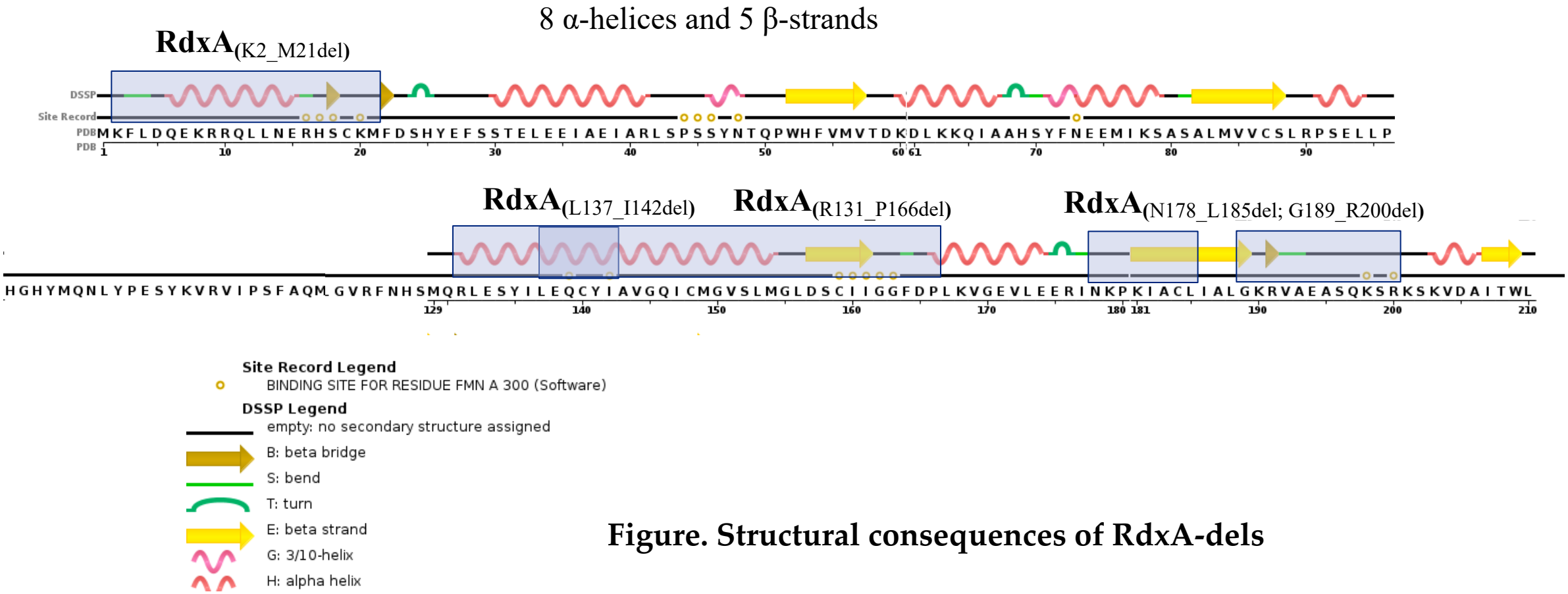
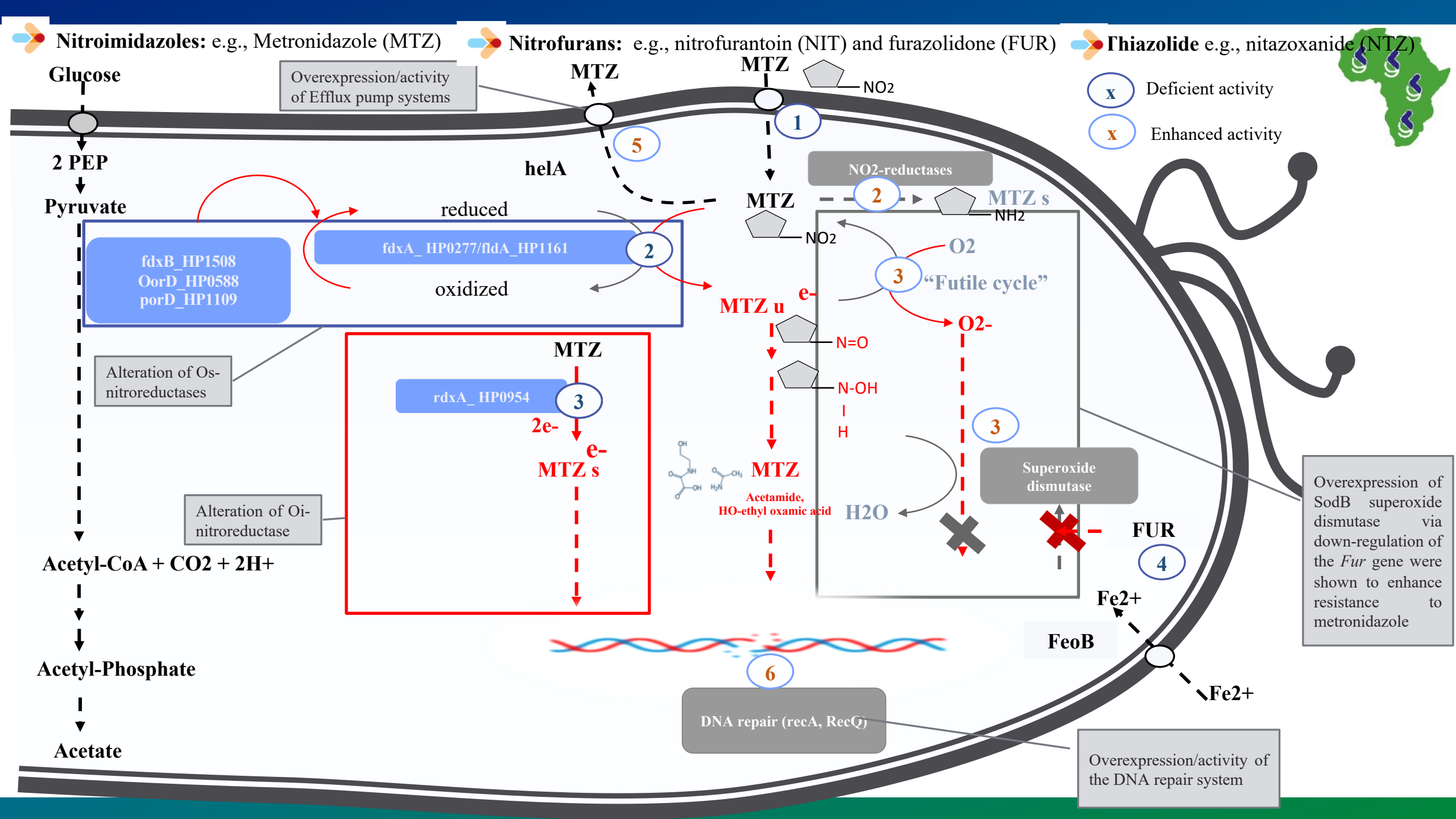


Figure. Structural consequences of RdxA-dels


Nitroimidazoles: e.g., Metronidazole (MTZ)
Nitrofurans: e.g., nitrofurantoin (NIT) and furazolidone (FUR)
Thiazolide e.g., nitazoxanide (NTZ)
x Deficient activity
x Enhanced activity
Glucose
2 PEP
Pyruvate
Acetyl-CoA + CO2 + 2H+
Acetyl-Phosphate
Acetate
Overexpression/activity of Efflux pump systems
MTZ
NO2
1
5
helA
NO2-reductases
2
MTZ s
NH2
reduced
oxidized
fdxB_HP1508
OorD_HP0588
porD_HP1109
fdxA_ HP0277/fldA_HP1161
2
MTZ u
e-
O2
3
"Futile cycle"
O2-
N=O
N-OH
H
Alteration of Os-nitroreductases
rdxA_ HP0954
3
2e-
e-
MTZ s
Alteration of Oi-nitroreductase
Acetamide,
HO-ethyl oxamic acid
H2O
Superoxide dismutase
FUR
4
Fe2+
FeoB
Fe2+
6
DNA repair (recA, RecQ)
Overexpression of SodB superoxide dismutase via down-regulation of the Fur gene were shown to enhance resistance to metronidazole
Overexpression/activity of the DNA repair system

Conclusion

- We propose a categorization of Hp-AMR molecular markers and a practical framework to potentially support antimicrobial stewardship and feasibility of mAST in *H. pylori* management.

- But significant gaps in the evidence regarding the biological relevance of reported molecular markers for Hp-AMR. Need for more validations (e.g., using CRISPR-Cas techniques).

Stop AMR

Antimicrobial Resistance

Stop the spread of drug-resistant bacteria

Japan Pharmaceutical Manufacturers Association

African Helicobacter & Microbiota Study Group

AHMSG 2024
16-17 AUGUST 2024
Cape Town, South Africa

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