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In vitro efficacy of antibiotics used in clinical practice on clinical isolates of *Helicobacter pylori*

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Background

The treatment of *Helicobacter pylori* (Hp) consists of a combination of several antibiotics among amoxicillin (AMX), clarithromycin (CLA), levofloxacin (LEV), rifampicin (RIF), tetracycline (TET) and metronidazole (MTZ), with a proton pump inhibitor. However, the therapeutic arsenal has been built up empirically and the emergence of resistance prompts a reassessment of these combinations. **The aim** of this work was to study the *in vitro* efficacy of antibiotic combinations used in the current treatments of Hp and original ones including AMX, LEV, CLA, RIF, TET and MTZ.

Materials and methods

Clinical strains: 19 clinical isolates of Hp were obtained from gastric biopsies from the University Hospital of Poitiers (UHP). The reference strain was J99. The bacteria were identified as Hp by Gram straining and positive urease tests. E-Tests were routinely performed. The strains were held at -80°C in Brain Heart Infusion with 50% of glycerol, and sub-culturated after thawing to ensure reliable growth on Campylobacter agar under microaerophilic atmosphere at 37°C for 2 days. For each experiment, the inoculum was prepared from a 24 h culture on Campylobacter agar.

MICs determination: was carried out using the microdilution method (MD) in 96-well plates. A serial dilution of the antibiotics was performed in Brucella Broth supplemented with 10% fetal calf serum at neutral pH (BB10%). Each well was inoculated with Hp at a final inoculum of 10^6 CFU/mL. The plates were incubated for 2 days in microaerophilic conditions at 37°C with shaking (150 rotation per minutes (rpm)). MICs were interpretated according to EUCAST breakspoints.

Checkerboards assay: A two-dimensional, two-agent broth microdilution checkerboard titration method with 96-well microtitration plates was used to study the interaction between antibiotics in BB10%. The first antibiotic of the combination was serially diluted along the ordinate and the second one along the abscissa. Bacterial suspension of approximately 10^6 CFU/mL was then added into wells. The growing conditions were the same as for MICs.

The **FIC index** (Fractional Inhibitory Concentration) value was calculated to characterise interactions as :

Synergistic ≤0,5 Additive >0,5 to 1
Indifference >1 to 4 Antagonistic >4

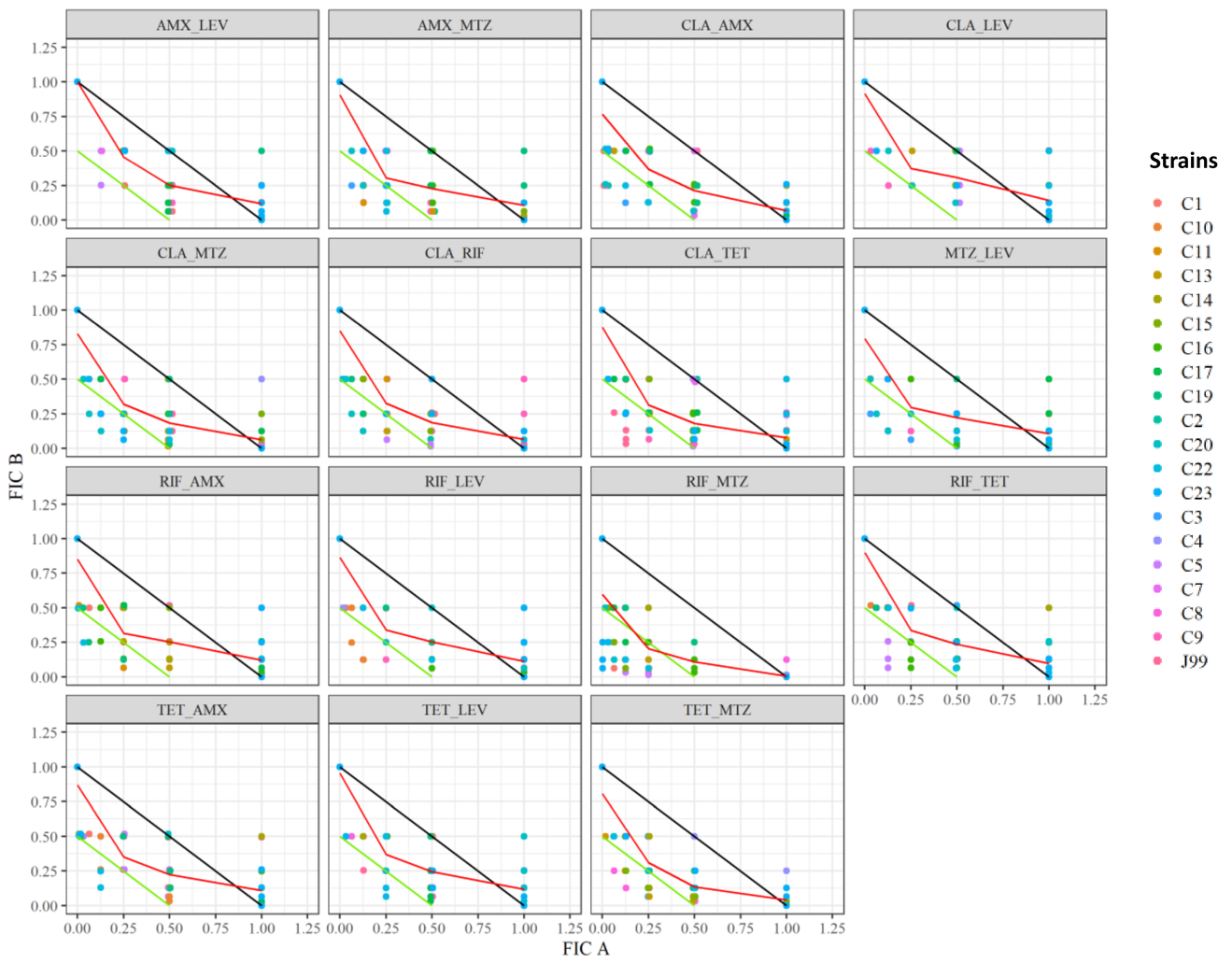
$$\textbf{FIC Index} = \text{FIC A} + \text{FIC B} = \frac{MICA_{\text{combination A and B}}}{MIC\ A} + \frac{MICB_{\text{combination A and B}}}{MIC\ B}$$

Results

Table 1. Antibiotic phenotypes of the 20 strains determined by the MD method for the 20 strains (S: Susceptible; R: Resistant)

Strains	Phenotypic antimicrobial succeptibility testing					
	AMX	RIF	LEV	CLA	TET	MTZ
C1	S	S	S	R	S	S
C2	S	S	S	S	S	S
C3	S	S	S	S	S	S
C4	R	S	S	S	S	S
C5	S	S	S	S	S	S
C7	S	S	S	S	S	R
C8	S	S	R	R	S	S
C9	S	S	S	S	S	S
C10	S	S	R	S	S	R
C11	S	S	S	S	S	S
C13	S	S	S	S	S	R
C14	S	S	R	S	S	S
C15	S	S	S	S	S	S
C16	S	S	S	S	S	S
C17	S	S	S	S	S	R
C19	S	S	R	S	S	S
C20	S	S	S	S	S	R
C 22	R	S	R	R	R	R
C23	S	S	S	R	S	R
(J99)	S	S	S	S	S	S

Figure 1. FIC B versus FIC A of all two-antibiotic combinations for the 20 strains



The colored points indicate the values observed for the different strains. Green line corresponds to the upper limit of the synergy zone. Black line shows an additivity of effects. Red curve represents the average of all FIC B versus FIC A for the 20 strains.

Our MICs determined with the MD method results were in agreement with those obtained with the routine E-test method **(Table 1)**. No antagonism was observed when crossing all combinations. The effects of all antibiotics used in combination were predominantly additive **(Figure1)**. Synergy was more evident when MTZ was used in combination. We also observed that there is no relationship between the resistance profile of the strain and the results of the checkerboards found, i.e. we have no more synergy with resistant strains than with susceptible strains to a given antibiotic.

Conclusion

The pharmacodynamic interactions were mainly additive. This justifies a choice of antibiotics to be used in guided therapy based on their respective MICs according to European recommendations (Maastricht VI, published in 2022). Carrying out bactericidal experiments would allow the evaluation of pharmacodynamic interactions over time.

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