P00462

In vitro bactericidal activity of amoxicillin combined to clarithromycin or levofloxacin on clinical strains of *Helicobacter pylori*

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Background

Helicobacter pylori (Hp) is the major agent in the development of peptic ulcers and gastric cancers. The guided empirical therapy consists in combination of amoxicillin (AMOX) either with clarithromycin (CLA) to treat patients infected by CLA susceptible isolates, or with levofloxacin (LEV) to treat patients infected by CLA resistant isolates **(Maastricht VI/Florence European consensus report)**. These regimens have been reported more successful than the probabilistic regimens. Despite guided therapies, there are still failures to eradicate Hp in some patients. **The aim** of this study was to characterise the *in vitro* pharmacodynamic interactions between AMX and LEV or AMX and CLA against clinical isolates of Hp.

Materials and methods

Clinical strains: 4 clinical isolates (C1, C4, C10 and C23) of Hp were obtained from gastric biopsies from the University Hospital of Poitiers (UHP). The reference strain was J99. Isolates 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.

MICs determination: was carried out using the microdilution method 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 bacteria at a final inoculum of 10^6 CFU/mL. The plates were incubated for 2 days under microaerophilic conditions at 37°C and shaking (150 rotation per minutes (rpm)). MICs are the smallest concentration of antibiotic needed to inhibit the visible growth of Hp, results were interpretated according to EUCAST breakpoints.

Time killing Assay (TKC) of AMOX combined with LEV or CLA: Kinetic studies were performed in BB10% at neutral pH using 6-well plates. Antibiotic were combined and tested at concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 x MIC. Bacteria from 24 h culture, were inoculated to a final inoculum of 10^6 CFU/mL. The plates were incubated for 30 h under microaerophilic conditions at 37°C with shaking 150 rpm. Samples (50 µL) were removed after 0, 4, 8, 24 and 30 h of incubation, and serially diluted. 20 µL from selected dilutions were spotted onto Campylobacter agar. After 5 days of incubation under microaerophilic conditions at 37°C, the colonies number was counted to follow bacterial growth over time.

Results

In monotherapy as well as in combination, at 2 x MIC concentrations the bactericidal effects were relatively weak. In combination, the effect observed generally corresponded to the effect of the most effective antibiotic in monotherapy (cf. highlighted examples on figures 1 and 2). Moreover, No bacterial regrowth was observed during TKC.

Figure 1. The effect of AMOX alone or in combination with LEV with different concentrations on J99, C1 and C23. The effects obtained in combination for some pairs of concentrations have been highlighted as an example (AMX: red and LEV: blue)

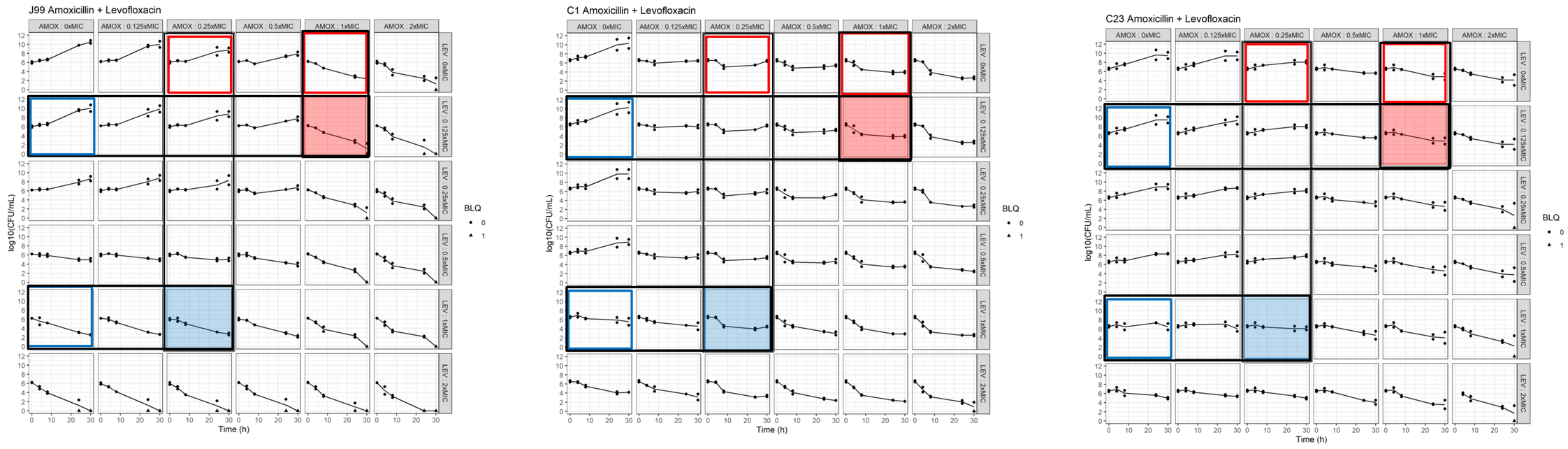
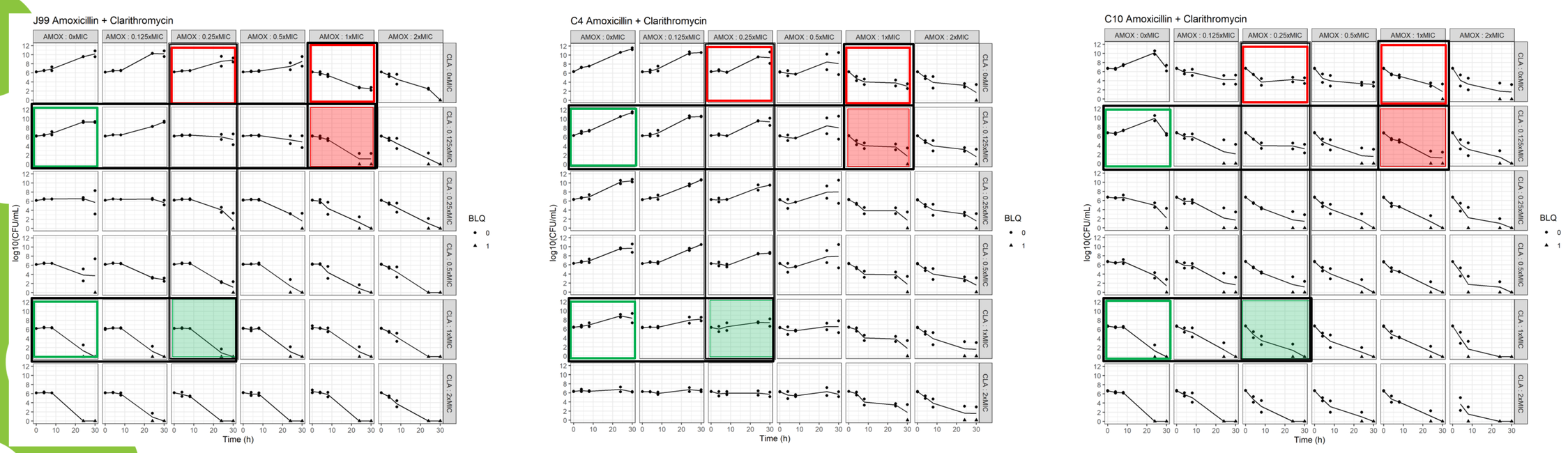


Figure 2. The effect of AMOX alone or in combination with CLA with different concentrations on J99, C4 and C10. The effects obtained in combination for some pairs of concentrations have been highlighted as an example (AMX: red and CLA: green)



Conclusion

The *in vitro* effects observed in combinations at static concentrations corresponded to those of the most effective antibiotic in monotherapy. However, in clinical practice, variations in antibiotic concentrations over time suggest that it is still preferable to use combinations.

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