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# Assessment of *in vitro* efficacy of three-antibiotics combinations against *Mycobacterium abscessus*

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## Introduction

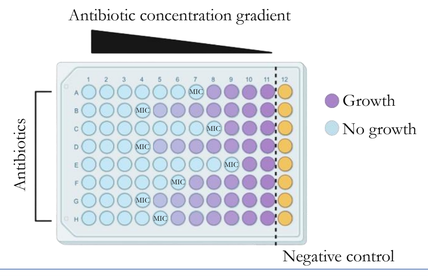
***Mycobacterium abscessus* (Mabs)** is an environmental bacterium that **causes lung infections**, especially in patients with cystic fibrosis. Moreover, Mabs exhibits **resistance** to **many antibiotics** including anti-tuberculous drugs. **Treatment** consists of **prolonged courses of antibiotic combinations** including different molecules. However, antibiotic **resistance** coupled with the **absence of treatment guidelines** leads to **high treatment failure**. This necessitates the **refinement of existing combination therapies** to improve patient treatment. The aim of this study is to **evaluate** the *in vitro* **pharmacodynamic interactions** of **antibiotic combinations** from six molecules frequently used in the treatment of Mabs infections: **amikacin (AMK)**, **azithromycin (AZM)**, **bedaquiline (BDQ)**, **imipenem (IMI)**, **linezolid (LZD)**, **tigecycline (TIG)**.

## Materials and methods

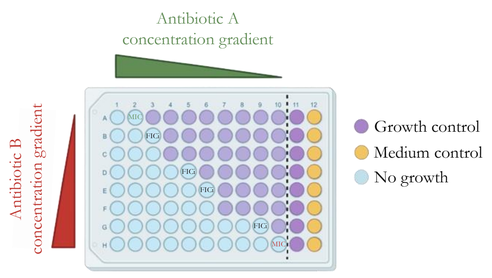
All experiments were performed on the **reference strain** Mabs **ATCC 19977**.

**1** **MICs** were measured in **7H9 medium** at **37 °C** and **read** after **3 days** for **six antibiotics** among the most frequently use:

- amikacin (AMK)
- azithromycin (AZM)
- bedaquiline (BDQ)
- imipenem (IMI)
- linezolid (LZD)
- tigecycline (TIG)




**2** To assess combination the **two-antibiotics combinations**, **checkerboard assays** were performed under the same conditions as MICs and **analysed** using the **FICi method**[1].




**4**

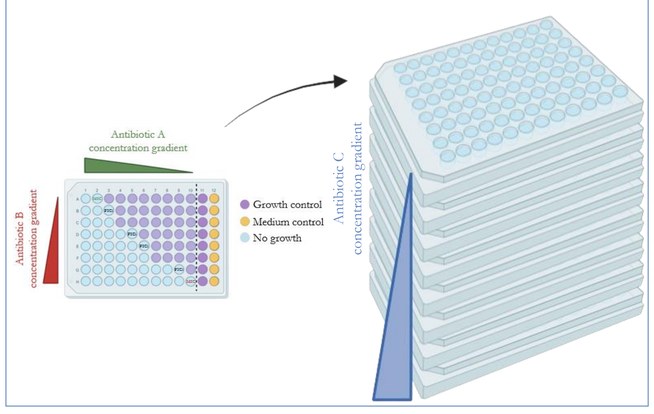
$$FICi_{two\text{-}antibiotics} = MIC_{A/B} / MIC_A + MIC_{B/A} / MIC_B$$

$$FICi_{three\text{-}antibiotics} = MIC_{A/(B/C)} / MIC_A + MIC_{B/(A/C)} / MIC_B + MIC_{C/(A/B)} / MIC_C$$

| | | |
|---|---|---|
| < 0.5 | synergy | < 0.75 |
| 0.5 – 4 | additivity / indifférence | 0.75 – 4 |
| > 4 | antagonism | > 4 |

Calculation of the minimal fractional inhibitory concentration index (FICi_min) for the analysis of the different combinations.

**3** To assess **three-antibiotics combinations**, **checkerboard assays** were performed under the same conditions as MICs and **analysed** using the **FICi method**[2].




## Results

**1** As seen on **Table 1**, the **MICs** of **all the antibiotics** were **below** to the **T(ECOFF)** proposed by **EUCAST, when available**[3].

**Table 1:** Results of MICs measurements for the reference strain Mabs ATCC 19977.

| Single antibiotic | MICs *(mg/L)* | T(ECOFF) *(mg/L)* |
|---|---|---|
| AMK | 8 | 64 |
| AZM | 8 - 16 | |
| BDQ | 0.5 | |
| IMI | 2 - 4 | 64 |
| LZD | 16 | 64 |
| TIG | 0.5 - 1 | 2 |

**2** Among the fifteen **two-antibiotics combinations** tested in checkerboards **no antagonism** were observed (FICi_min > 4). **Twelve** combinations were **additive** (0.5 < FICi_min < 4). And **three** were **synergistic** (FICi_min < 0.5) [1] **(Table 2)**.

**Table 2:** Minimal FIC index for all two-antibiotics combinations against Mabs (in green, synergistic combinations with FICi < 0.5). Experiments were repeated between 3 and 5 times.

| Two-antibiotics combination | Range of FICi_min |
|---|---|
| AMK - AZM | 0.56 - 3 |
| AMK - BDQ | 0.5 - 0.75 |
| AMK - IMI | 0.375 - 0.75 |
| AMK - LZD | 0.625 - 1.25 |
| AMK - TIG | 1.25 - 3 |
| AZM - BDQ | 0.5 - 1 |
| AZM - IMI | 0.515 - 1 |
| AZM - LZD | 0.156 - 0.375 |
| AZM - TIG | 0.25 - 0.5 |
| BDQ - IMI | 1 - 1.5 |
| BDQ - LZD | 0.75 - 1.5 |
| BDQ - TIG | 0.53 - 1 |
| IMI - LZD | 0.25 - 0.5 |
| IMI - TIG | 0.75 - 1.015 |
| LZD - TIG | 0.5 - 0.625 |

**3** To design **three-antibiotics combinations, synergistic (e.g. AZM-LZD, AZM-TIG and IMI-LZD) two-antibiotics combinations** containing a shared antibiotic **was taken** as a **starting point. AZM-LZD-IMI** presented a FICi_min of 0.19, and **AZM-LZD-TIG** presented a FICi_min of 0.25.

The isobologram analysis **(Figure1)** revealed that, cross the entire range of antibiotic concentrations tested 73% of the data points for the AZM-LZD-IMI combination and 65% for the AZM-LZD-TIG combination fell below the synergy isobole threshold of 0.75[2].

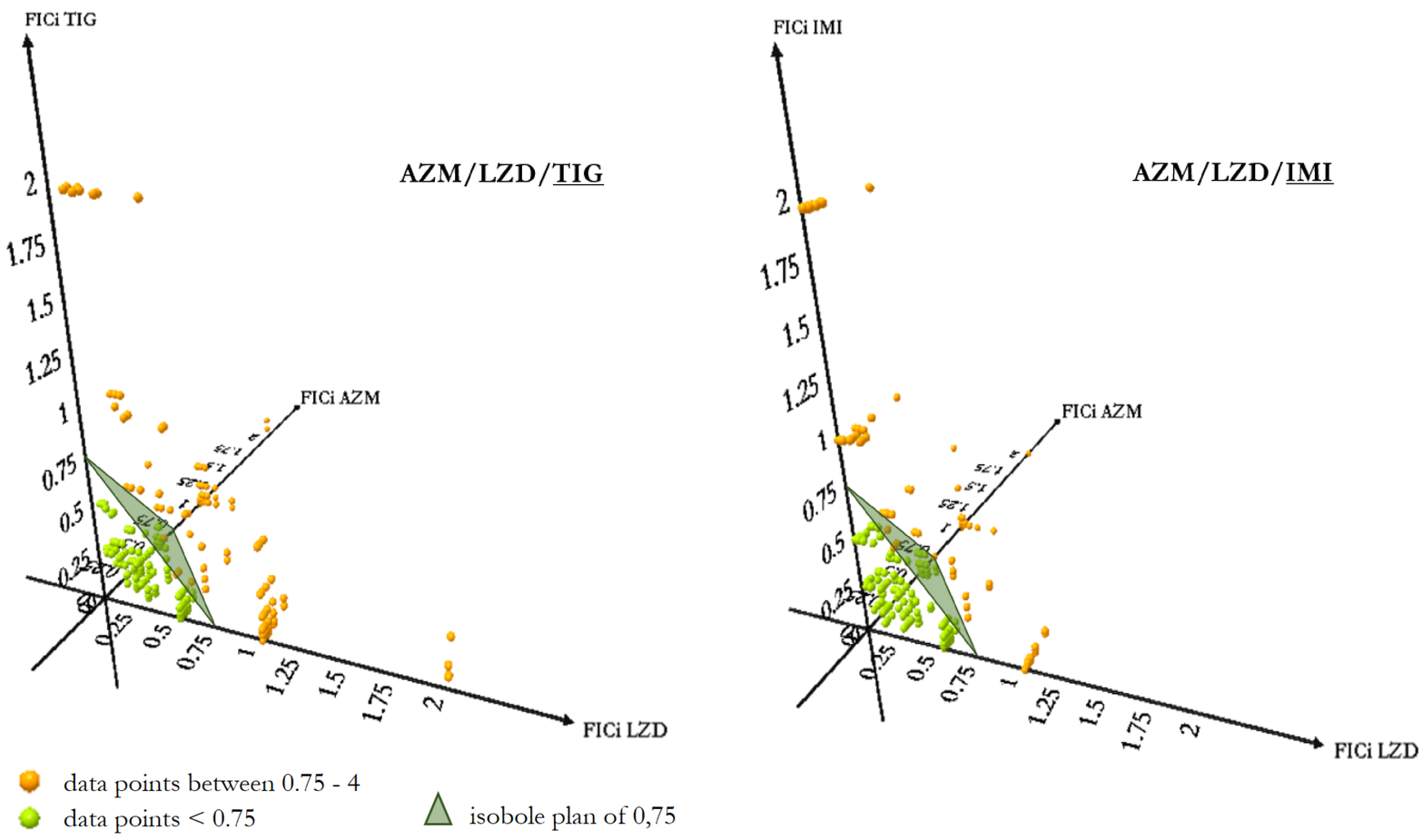



**Figure 1 :** Isobologram analysis of the FIC values for the two most promising three-antibiotics combinations containing AZM and LZD. The dots represent the experimental values of calculated FICi for each triplet of concentrations. Green dots are considered as synergistic since they are below the isobole plan of 0.75, orange dots are considered as additive since they are between the isobole plans of 0.75 and 4.

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## Conclusion

As for now, our results have **never** shown **antagonism** regardless of the antibiotic combinations. **Synergism** was observed for azithromycin-linezolid **(AZM-LZD)**, and azithromycin-tigecycline **(AZM-TIG)**, and **imipenem-linezolid (IMI-LZD)**. Two **triple combinations** were evaluated **based on** the results of **double combinations** and demonstrated **synergy** for **AZM-LZD-IMI** and **AZM-LZD-TIG**. These combinations will be **further investigated** by time-kill curves experiments to predict **dosing regimen** that achieve **bactericidal action** on **Mabs**.

**References:**

**1.** Odds, 2003. DOI: 10.1093/jac/dkg301.

**2.** Bhusal et al., 2005. DOI: 10.1016/j.ijantimicag.2005.05.005.

**3.** EUCAST MIC distribution for M. abscessus, 2024.