In vitro evaluation of novel bi- or tri-antibiotic combinations against clinical isolates of *Mycobacterium abscessus*

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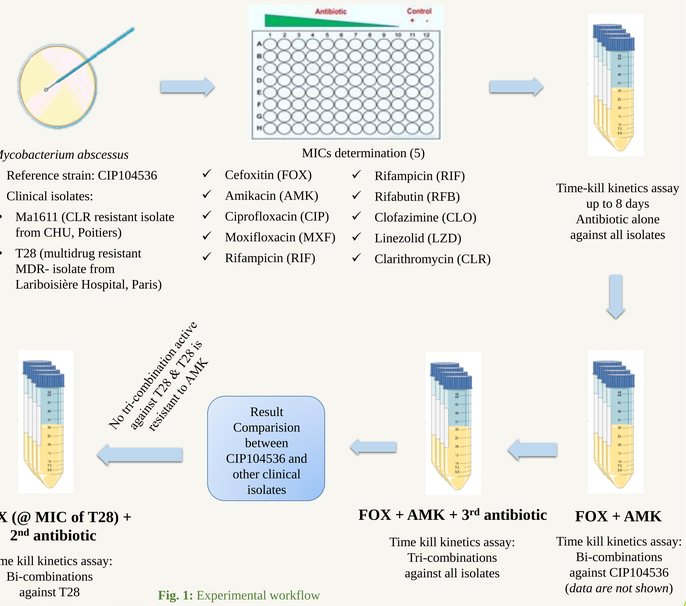
Introduction

- *Mycobacterium abscessus* (Mab) is an emerging pathogen, intrinsically resistance to many antimycobacterial drugs (1).
- Current treatment is limited to combination of intravenous amikacin (AMK), and cefoxitin (FOX) with oral clarithromycin (CLR). In most cases, it is associated with treatment failure (2).
- FOX is known to degrade *in vitro* with the degradation half-life of 1.5 days which may lead to misinterpretation of time-kill results (article under revision with AAC).
- Recent reports demonstrate intrinsic resistance to CLR in Mab clinical isolates. Fluoroquinolones, rifamycins, linezolid or clofazimine can be added when standard therapy is ineffective (3,4).

Purpose

- To evaluate the *in vitro* efficacy of several combinations against clinical isolates of Mab including FOX and AMK and replacing CLR to avoid the induced resistance.

Materials/Methods



Mycobacterium abscessus

- ✓ Reference strain: CIP104536
- ✓ Clinical isolates:
  - Ma1611 (CLR resistant isolate from CHU, Poitiers)
  - T28 (multidrug resistant MDR- isolate from Lariboisière Hospital, Paris)

- ✓ Cefoxitin (FOX)
- ✓ Amikacin (AMK)
- ✓ Ciprofloxacin (CIP)
- ✓ Moxifloxacin (MXF)
- ✓ Rifampicin (RIF)
- ✓ Rifampicin (RIF)
- ✓ Rifabutin (RFB)
- ✓ Clofazimine (CLO)
- ✓ Linezolid (LZD)
- ✓ Clarithromycin (CLR)

Fig. 1: Experimental workflow

Materials/Methods

Table I: Antibiotics used for combination time-kill assay, containing cefoxitin and amikacin combined with 3rd antibiotic

	3rd antibiotic	concentrations used for 3rd antibiotic
FOX*† + AMK*	+ CIP	4 mg/L
	+ MXF	4 mg/L
	+ LZD	8 mg/L
	+ CLO	2 mg/L
	+ RFB	2 mg/L
	+ RIF	16 mg/L
	+ CLR	1 mg/L

* FOX and AMK concentrations were 8 mg/L and 32 mg/L respectively

† 50% amount of FOX was added each 24h up to 8 days to compensate the FOX degradation

Results

Table II: Susceptibility data for different Mab isolates tested by broth microdilution (4)

Antibiotics	MIC breakpoints			MICs (mg/L)		
	S	I	R	CIP 104536	Ma1611	T28
FOX	<16	32	>128	8	8	64
AMK	<16	32	>64	32	16	>1024
CIP	<1	2	>4	4	4	8
MXF	<1	2	>4	4	4	16
LZD	<8	16	>32	8	8	256
RIF			>1	16	16	256
CLR	<2	4	>8	1	16	>256
CLO	ND			2	16	4
RFB				2	2	16

S, susceptible; I, intermediate; R, resistance; ND, not determined

- ✓ Clinical isolate T28 was resistant to all antibiotics
- ✓ CIP104536 and Ma1611 were susceptible to intermediate against all tested antibiotics.

Effect of several antibiotics alone and in tri-combination against CIP104536

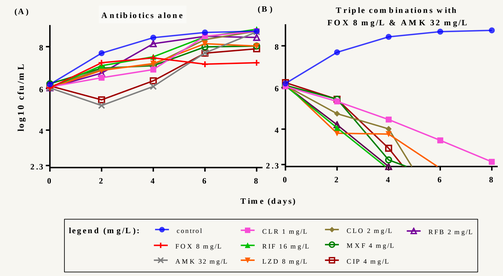


Fig. 2: (A) Activity of several antibiotics alone and (B) Tri-combination of cefoxitin and amikacin with a 3rd antibiotic.

Results

Comparision of the effect of several tri-combination against reference strain CIP104536 and clinical isolates at day 4 and day 8

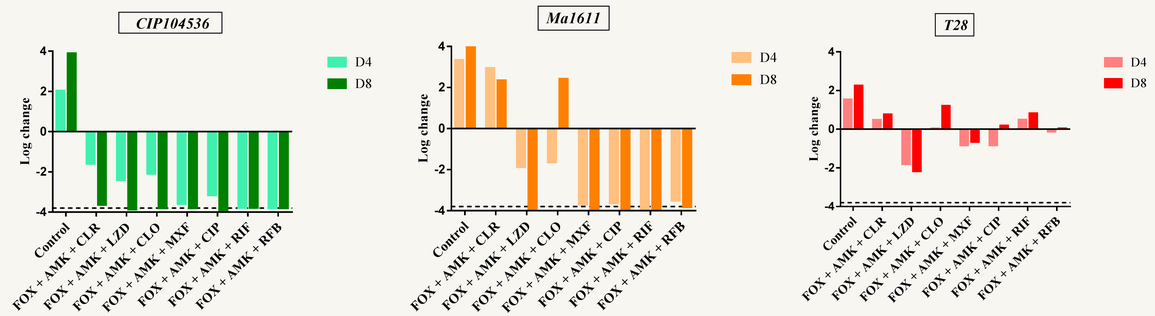


Fig. 3: In vitro activity of various tri-combinations against Mab (A) CIP104536 (B) Ma1611 and (C) T28. Log change was calculated using respective positive control data. For Comparision purpose, results are compared at day 4 and day 8.

- ✓ Tri-combinations including CLO or CIP were also active against CIP104536 but inactive against Ma1611.
- ✓ Almost all tested triple combinations were inactive against T28 except for tri-combination with LZD & MXF.

Effect of several antibiotics alone and in bi-combination against MDR clinical isolate T28

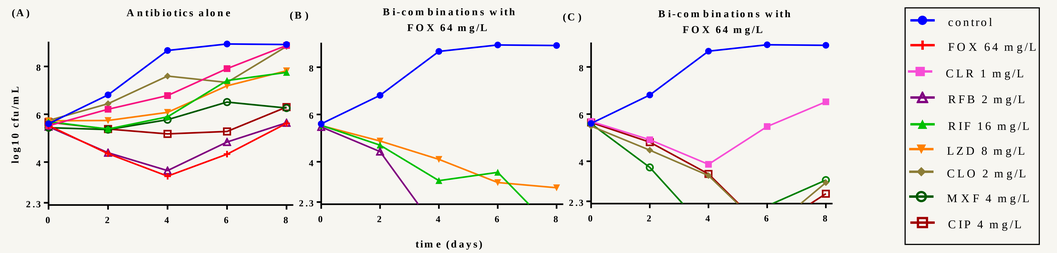


Fig. 4: Effect of various combinations on M. abscessus T28. (A) Activity of several screened antibiotics alone, (B) cefoxitin in combination with linezolid, rifampicin and rifabutin, and (C) cefoxitin in combination with clarithromycin, clofazimine, moxifloxacin and ciprofloxacin. 50% amount of FOX was compensated each 24h up to 8 days to maintain the FOX amount constant throughout the experiment.

- ✓ T28 was highly resistant to AMK because of A1408G mutation of the *rrs* gene encoding rRNA 16S. FOX alone was not much efficient, so it was used in bi-combinations. FOX with RFB was the most active bi-combination against T28.

Conclusion & perspective

- ✓ Tri-combinations were highly efficient against Mab CIP104536 and intermediate to susceptible clinical isolate Ma1611 but not against MDR isolate T28.
- ✓ The synergy between FOX and rifamycins suggests a potent role of this combinations that may warrant further optimization of treatment regimen for the treatment of *M. abscessus* pulmonary infections.

References

1) Nessar *et al.*, *JAC*, 2012
2) Lee *et al.*, *Emerg Infect Dis*, 2015
3) Oh *et al.*, *JAC*, 2014
4) Griffith *et al.*, *IDSA*, 2007
5) NCCLS, 2003

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