Annals of Clinical Microbiology and Antimicrobials

BRIEF REPORT

Open Access

Poor efficacy of the combination of clarithromycin, amikacin, and cefoxitin against *Mycobacterium abscessus* in the hollow fiber infection model

Etienne Vignaud[1], Sylvain Goutelle[2,3], Charlotte Genestet[1,4], Jérôme Guitton[3,5], Sabine Cohen[5], Chloé Bourg[1,4], Aurore Durand[1], Laura Lebouteiller[1], Albin Bernard[1], Caroline Richet[6], Oana Dumitrescu[1,4] and Elisabeth Hodille[1,4*]

Abstract

Background *Mycobacterium abscessus* (MABS) causes difficult-to-treat pulmonary and extra-pulmonary infections. A combination therapy comprising amikacin, cefoxitin, and a macrolide agent is recommended, but its antimicrobial activity and clinical efficacy is uncertain. Inducible resistance to macrolides (macrolides-iR) has been associated with poor clinical response in pulmonary infections, whilst for extra-pulmonary infections data are scarce.

Objectives Herein, the aim was to evaluate the effect of the amikacin, cefoxitin, and clarithromycin combination against macrolides-iR MABS in a hollow-fiber infection model.

Methods The hollow-fiber system was inoculated with *M. abscessus* subsp. *abscessus* type strain ATCC 19977 and treated during 10 days with the antibiotics combination. Two level of macrolide concentrations were evaluated mimicking the pharmacokinetics profiles of free (i.e. unbound) drug in blood and lung.

Results Using blood concentrations, the combination failed to prevent bacterial growth. Using lung concentrations, the combination had a limited but significant effect on bacterial growth from day 2 to day 10. Moreover, increasing clarithromycin concentrations stabilized the amikacin-tolerance level: amikacin minimal inhibitory concentration of amikacin-tolerant strains increased over time using blood concentrations while it remained stable using lung concentrations.

Conclusions Our finding confirms the low activity of the amikacin, cefoxitin, and clarithromycin combination against macrolide-iR MABS infection, and suggest the influence of clarithromycin concentrations on response. The low concentration of clarithromycin in blood may hamper efficacy for the treatment of extra-pulmonary MABS infection. Consequently, it should not be considered as an active molecule in the chosen antibiotic combination, as recently recommended for pulmonary infections.

Keywords *Mycobacterium abscessus*, Hollow-fiber system, Hollow-fiber infection model, Clarithromycin, Amikacin, Cefoxitin, Pulmonary infection, Extra-pulmonary infection

*Correspondence:
Elisabeth Hodille
elisabeth.hodille@chu-lyon.fr
Full list of author information is available at the end of the article

Introduction

Mycobacterium abscessus (MABS) complex is a rapid-growing, facultative intra-cellular, antibiotic-resistant non-tuberculous Mycobacteria causing difficult-to-treat pulmonary and various extra-pulmonary infections. Among these, inoculations are responsible of skin and soft tissue or bone and joint infections, while severe disseminated infections are mainly observed in immunocompromised patients (HIV infected or undergoing immunosuppressive therapy) [1, 2]. A combination therapy is recommended and usually comprises amikacin, cefoxitin, and a macrolide agent [3, 4]. MABS complex includes subspecies carrying the *erm*(41) gene, conferring inducible resistance to macrolides (macrolides-iR) [5], which has been associated with poor clinical outcomes in pulmonary infections [6]. Recent guidelines of MABS complex pulmonary infections treatment suggest using different macrolide-containing combinations depending on the presence of macrolides-iR [4], while extra-pulmonary infection guidelines have not been updated [1, 3]. The efficacy of the amikacin, cefoxitin, and macrolide combination has also been questioned using a hollow-fiber infection model (HFIM) mimicking optimal pulmonary antibiotic concentrations [7]. Nevertheless, no in vitro model has ever mimicked extra-pulmonary antibiotic concentrations whereas clarithromycin is known to concentrate in epithelial lining fluid (at least tenfold) and in phagocytes including alveolar macrophages [8, 9].

Herein, the aim was therefore to evaluate the efficacy of amikacin, cefoxitin, and clarithromycin combination against *Mycobacterium abscessus* (MABS) in a HFIM mimicking human blood and lung concentrations of macrolides. Giving the low penetration of amikacin into macrophages, the study focused on MABS extracellular component.

Materials and methods

Minimal inhibitory concentrations (MIC) of *M. abscessus* subsp. *abscessus* (MABS) type strain ATCC 19977 were determined by microdilution [9, 10]. A hollow-fiber system (HFS) [11], composed of a 20 mL polysulfone fiber cartridge (FiberCell Systems®, New Market, MD, USA) connected to a central reservoir and a DUET pump (FiberCell Systems®), and containing Cation-adjusted-Muller-Hinton (CAMH) culture medium (Merck Millipore®, Burlington, MA, USA), was inoculated with MABS ATCC 19977 in exponential growth to achieve a 10^5–10^6 CFU/mL density at day (D) 0. Then, HFS was treated during 10 days with an amikacin, cefoxitin, and clarithromycin combination. Two level of macrolide concentrations were evaluated, mimicking the pharmacokinetics profiles of free (i.e. unbound) drug in blood and lung. Based on previous intravenous administration data, clarithromycin (Mylan, Canonsburg, PA, USA) was injected twice a day to achieve a peak concentration of 1.8 mg/L for the blood regimen [12] and 18 mg/L (tenfold) for the lung regimen [8]. Amikacin (Viatris, Pittsburgh, PA, USA) was injected once a day in the HFS to achieve a peak concentration of 60 mg/L [13] for both regimen. Cefoxitin (PANPHARMA, Luitré-Dompierre, France) was administered as a continuous infusion into the CAMH culture medium to obtain a steady-state concentration of 25 mg/L [14] for both regimen. A growth control without antibiotic (NT) was also evaluated. All experiments were performed at 37 °C.

Antibiotic concentrations achieved in the HFS were measured using an automated immunoassay on the Cobas platform (Roche, Bâle, Switzerland) for amikacin, and high-performance liquid chromatography combined with mass-spectrometry for cefoxitin and clarithromycin. The 3 conditions (NT, lung regimen, and blood regimen) were performed 3 times in independent experiments.

Samples were withdrawn from the HFS cartridge from D0, to D10 of treatment, and cultured on antibiotic-free CAMH agar (BioRad, Hercules, CA, USA) for bacterial count. Results were expressed in absolute values ($\log_{10}$ CFU/mL) and in relative values compared to D0, i.e. CFU count relative from baseline, (% D0 $\log_{10}$ CFU/mL) to account for variability in the initial D0 inoculum. Antibiotic tolerance was quantified by culturing on CAMH agar (Becton–Dickinson) containing 80 mg/L of cefoxitin or 32 mg/L of amikacin, and expressed in absolute values ($\log_{10}$ CFU/mL growing on antibiotic-supplemented media), and in relative values: $\% = 100 \times \frac{\text{Count on antibiotic-supplemented media at DX}\left(\frac{\text{CFU}}{\text{mL}}\right)}{\text{Total count at DX}\left(\frac{\text{CFU}}{\text{mL}}\right)}$.

Cefoxitin and amikacin MIC of strains growing on antibiotic-supplemented media were determined by microdilution [10].

Appropriate tests were performed to compare bacterial growth and antibiotic-resistant subpopulation between NT and treatment conditions using the GraphPad Prism software, version 10.2.3. A p value < 0.05 was considered statistically significant.

Results

MABS amikacin and cefoxitin MIC were 1 and 8 mg/L, respectively. MABS presented a clarithromycin-iR with increased MIC, from 0.12 mg/L on D3 to > 16 mg/L on D14. The antibiotic concentration profiles achieved human concentration target values (Fig. 1). For amikacin, average peak concentration and area under the concentration–time curve over 24 h (AUC) were 62 mg/L and 329 mg.h/L, respectively, very similar to values reported in humans for a dose of 20 mg/kg [15].). For amikacin,

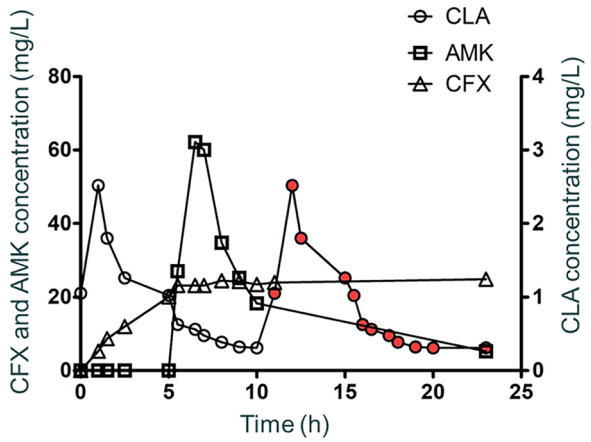

Fig. 1 Kinetics of antibiotic concentrations over time during 24 h in the hollow-fiber system for the blood regimen. *CLA* clarithromycin (red point: point extrapolated from the first peak due to evening injection), *AMK* amikacin, *CFX* cefoxitin. Targets were peak concentration for CLA: 1.8 mg/L, peak concentration for AMK: 60 mg/L, steady-state concentration for CFX: 25 mg/L

average peak concentration and AUC were 2.5 mg/L and 20.4 mg.h/L, respectively, also very similar to results observed in subjects who received 500 mg/12 h over 5 days [16]. The lung regimen was associated with a ten-fold higher exposure in the HFS (data not shown). For cefoxitin, average steady-state concentration was about 25 mg/L. Based on typical cefoxitin clearance reported by Isla et al. (11.5 L/h), a 6000 mg dose administered as a continuous infusion over 24 h would lead to a steady-state concentration of 21.7 mg/L [17]. Thus, our results were consistent with human exposure.

Overall, none of the antibiotic regimens enabled HFS sterilization after D10 of treatment (Fig. 2). The blood regimen failed to produce a bactericidal or bacteriostatic effect over 10 days. It only had a limited but significant inhibitory effect on bacterial growth compared to NT at D3 (− 1.17 $\log_{10}$ CFU/mL, 95% confidence interval, 95%CI [− 2.26; − 0.08]) and D4 (− 1.33 $\log_{10}$ CFU/mL, 95%CI [− 2.16; − 0.49]; Fig. 2a), but had no effect on CFU count relative from baseline, compared to NT (Fig. 2b). The lung regimen also failed to kill bacteria. It exhibited an initial bacteriostatic effect but could not inhibit growth beyond 3 days. It had a limited but significant effect on growth inhibition compared to NT at D5 (− 1.36 $\log_{10}$ CFU/mL, 95%CI [− 0.01; − 2.72]; Fig. 2a). This regimen significantly reduced CFU count relative from baseline compared to NT, from D2 to D10, with a maximum effect at D10 (− 38.0%, 95%CI [− 8.02; − 67.9]; Fig. 2b). Moreover, compared to NT, the reduction in the area under the curve of CFU counts relative from baseline was greater for the lung regimen (− 65%) than for the blood regimen (− 42%; Fig. 2b). Finally, although the CFU counts were not significantly different between both regimens at D10 (in absolute and relative values), there was a trend toward greater CFU counts under the blood regimen, while they were stable under the lung regimen (Fig. 2).

The emergence of MABS strains growing on antibiotic-supplemented media (Fig. 3a–c) was observed in all conditions and could be defined as either tolerant or persistent, but not resistant, since no strain reached the MIC defining the resistance clinical category [18] (Table 1).

In NT, the proportion of antibiotic-tolerant or -persistent strains remained relatively stable over time; 0.01% of the inoculum growing on cefoxitin-supplemented media (Fig. 3d), and between 0.0001–0.001% of the inoculum growing on amikacin-supplemented media (Fig. 3e).

Both regimens led to an early emergence of cefoxitin-tolerant or -persistent strains (Fig. 3b, c), which

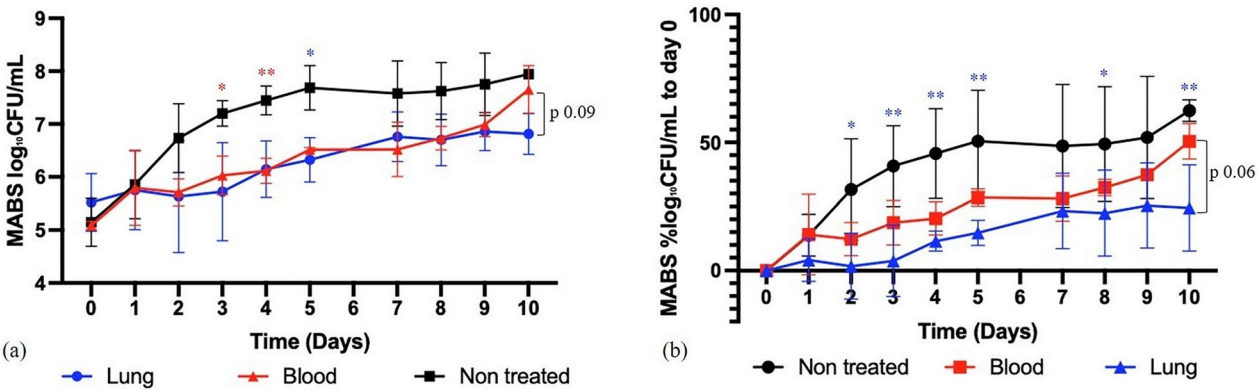

Fig. 2 MABS growth kinetics in the hollow-fiber system for 10 days. In not treated condition (black), under the blood antibiotic regimen (red) and under the lung antibiotic regimen (blue). **a** Absolut count, $\log_{10}$ CFU/mL. **b** in relative values compared to D0, % D0 $\log_{10}$ CFU/mL. *, $p < 0.05$; **, $p < 0.01$ (comparison between blood regimen and not treated condition). *, $p < 0.05$; **, $p < 0.01$ (comparison between lung regimen and not treated condition). Mean ± SD of 3 independent experiments for each condition

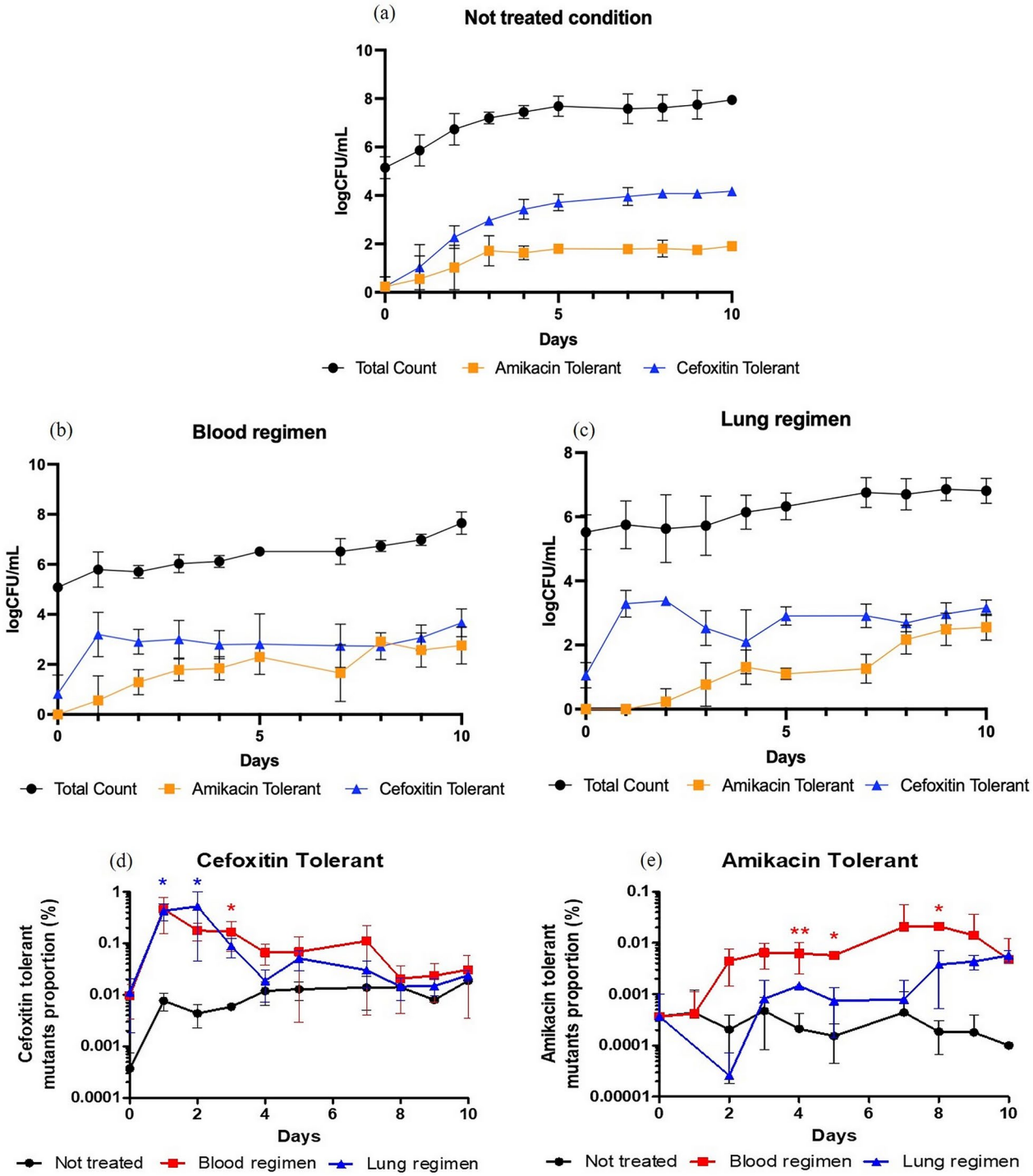

Fig. 3 Antibiotic-tolerant or -persistent MABS growth kinetics in the hollow-fiber system for 10 days. **a**–**c** Growth kinetics in absolute count (log_{10} CFU/mL) of MABS total count (black), amikacin-tolerant or -persistent MABS (orange), cefoxitin-tolerant or -persistent MABS (blue) in the hollow-fiber system for 10 days, for the not treated condition (**a**), blood regimen (**b**) and the lung regimen (**c**), (**d**), (**e**): Growth kinetics of cefoxitin-tolerant or -persistent MABS (d) and amikacin-tolerant or -persistent MABS (**e**) expressed in proportion (%), in the hollow-fiber system for 10 days, for the not treated condition (black), blood regimen (red) and lung regimen (blue). *, $p < 0.05$, **, $p < 0.01$; (comparison between blood regimen and not treated condition). *, $p < 0.05$; (comparison between lung regimen and not treated conditions). Mean ± SD of 3 independent experiments for each condition

Table 1 MIC (mg/L) of amikacin and cefoxitin for MABS growing on antibiotic-supplemented medium

Time (Days)	MIC (mg/L) for MABS growing on amikacin-supplemented media			MIC (mg/L) for MABS growing of cefoxitin-supplemented media		
	Not treated	Blood	Lung	Not treated	Blood	Lung
2	1	1	1	8	8	8
3	2	1	1	8	8	8
4	1	1	1	8	8	8
5	2	2	1	8	8	8
7	2	2	1	8	8	8
8	4	4	1	8	8	8
9	4	4	1	8	8	8
10	4	4	1	8	8	8

MIC for MABS growing on antibiotic-supplemented medium according to time and treatment (not treated, under the blood regimen, or under the lung regimen)
MIC minimal inhibitory concentration

reached > 0.1% of the inoculum at D1, a proportion significantly greater than in NT (Fig. 3d). The proportion gradually decreased over time; at D10, there was no difference between NT and both regimens (Fig. 3d). In all conditions, cefoxitin MIC of the bacteria growing on the cefoxitin-supplemented media were stable (8 mg/L, Table 1).

Both regimens resulted in a progressive emergence of amikacin-tolerant or -persistent strains over time. Nevertheless, the proportion of bacteria growing on amikacin-supplemented media was greater under the blood regimen, reaching > 0.01% of the inoculum, which was significantly different from NT (at D4, D5, and D8). Under the lung regimen, the emergence of amikacin-tolerant or -persistent strains was delayed (Fig. 3e). Moreover, amikacin MICs of MABS growing on the amikacin-supplemented media were different between the two regimens: MICs increased over time for the blood regimen (2 and 4 mg/L at D5 and D8, respectively) while it remained stable under the lung regimen until D10 (1 mg/L; Table 1). For MABS with two-fold increase of amikacin MIC, DNA was extracted and *rrs* locus was amplified and sequenced as previously described [19] but no mutations were detected.

Discussion

As previously found in a HFIM mimicking lung exposure [7], the present study confirmed that the amikacin, cefoxitin, and clarithromycin combination fails to produce a relevant antibacterial effect (bactericidal or bacteriostatic) against MABS using both blood and lung concentrations of clarithromycin. However, the lung regimen better inhibited MABS growth compared to the blood regimen. Moreover, increased clarithromycin concentrations achieved in lungs [8] appeared to prevent the development of amikacin-tolerance or -persistence. This activity of high-concentration clarithromycin, in addition to its immunomodulatory effect previously described [4], may support the relevance of its use in the antibiotic combination against MABS pulmonary infections, even with clarithromycin-iR. However, new macrolide-containing drug combinations should be evaluated considering the poor efficacy of the present one.

Ferro et al. reported the emergence of antibiotic-resistant mutants in HFIM over time, notably an increase in cefoxitin-resistant mutants from D14 (although no MIC value was mentioned) [7]; we did not observe true antibiotic-resistance emergence but rather antibiotic-tolerance or -persistence, defined as the ability of a subpopulation to survive exposure to a bactericidal drug concentration without an increase in the MIC [20]. However, as minimum duration killing (MDK) experiments were not performed, we could not differentiate between tolerance and persistence mechanisms [21]. It has been shown that tolerance and persistence often promote the development of resistance [22]; it is thus possible that the development of the cefoxitin-tolerant or -persistent subpopulations may subsequently enable the emergence of the cefoxitin-resistant mutants. Moreover, it could be interesting to further explore the underlying mechanisms of either antibiotic tolerance or persistence by performing MDK experiments and whole genome sequencing of bacteria growing on antibiotic-supplemented media. A recently published study found that a mutation in *serB2,* a gene involved in L-serine biosynthesis, resulted in the increased emergence of MABS cross-tolerance to cefoxitin and moxifloxacin, through activation of a WhiB7-dependant adaptive stress response [23].

Our study has several limitations. First, the HFS model we used only explored extracellular compartment, although MABS may also be responsible of intracellular infections. Second, though relevant in the context

of chronic lung infections, biofilms were not modelled in our system. Third, while clarithromycin is known to have an active metabolite (14-OH clarithromycin), this metabolite was not available as a reagent and so could not be added to clarithromycin in the HFS. The addition of the metabolite could increase the activity of the drug. Further research is necessary to examine this question. Fourth, the duration of HFIM (10 days), may not be sufficient to accurately consider the effects of antibiotic treatment on chronic lung infection. However, this duration may be relevant to study the efficacy of antibiotic treatment in more acute infections such as bacteremia, where the bactericidal effect must be rapid. In this case, we showed herein a very weak antibacterial effect of the amikacin, cefoxitin, and clarithromycin combination, insufficient to inhibit bacterial growth. In addition, there was no benefit in preventing antibiotic-tolerance or persistence, suggesting that these effects directly depend on the efficacy of the antibacterial effect of clarithromycin as previously showed for other bacterial pathogens treated with protein synthesis inhibitory agents [24]. Thus, the value of using clarithromycin in the antibiotic regimen against MABS with macrolides-iR should be critically reappraised in the absence of increased clarithromycin concentration at the site of infection. We think that the low concentration of clarithromycin in blood may hamper efficacy for the treatment of extra-pulmonary (especially disseminated) MABS infections and should not be considered as an active molecule in the chosen antibiotic combination, as recently recommended for lung infections [4]. Further investigations are urgently needed to find the best combination of antibiotics, which is rapidly and effectively bactericidal against MABS in disseminated extra-pulmonary infections.

Abbreviations

CAMH	Cation-adjusted-Muller-Hinton
CFU	Colony-forming unit
D	Day
HFIM	Hollow-fiber infection model
HFS	Hollow-fiber system
MABS	*Mycobacterium abscessus*
Macrolides-iR	Inducible resistance to macrolides
MIC	Minimal-inhibitory concentration
NT	Growth control without antibiotic

Acknowledgements
We thank Shanez Haouari (DRS, Hospices Civils de Lyon) for help in manuscript preparation.

Author contributions
Conception: SG, OD, EH; design of the work: EV, SG, AD, LL, AB, OD, EH; acquisition: EV, CG, JG, SC, CB, AD, LL, AB, CR, EH; analysis: EV, CG, JG, SC, AD, LL, AB, CR, EH; interpretation of data: EV, SG, CG, JG, SC, AD, LL, AB, CR, EH; drafted the work: EV, CG, EH; revised the work: SG and OD.

Funding
This work was supported by the internal "Jeunes Chercheurs" grant 2021 operated by the Hospices civils de Lyon.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Laboratoire des Mycobactéries, Institut des Agents Infectieux, Laboratoire de Biologie Médicale Multi-Site, Hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon, France. [2]Service de Pharmacie, Groupement Hospitalier Nord, Hospices Civils de Lyon, Lyon, France. [3]UMR CNRS 5558, Laboratoire de Biométrie et Biologie Evolutive & ISPB, Faculté de Pharmacie de Lyon, Université Claude Bernard Lyon 1, Lyon, France. [4]CIRI-Centre International de Recherche en Infectiologie, Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Inserm U1111, CNRS UMR5308, Lyon, France. [5]Laboratoire de Biochimie et de Pharmacologie-Toxicologie, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Pierre Bénite, France. [6]Laboratoire de Biochimie, Hôpital de la Croix Rousse, Hospices Civils de Lyon, Lyon, France.

Received: 10 September 2024 Accepted: 7 January 2025
Published online: 30 January 2025

References

1. To K, Cao R, Yegiazaryan A, Owens J, Venketaraman V. General overview of nontuberculous mycobacteria opportunistic pathogens: *Mycobacterium avium* and *Mycobacterium abscessus*. J Clin Med. 2020;9:2541.
2. Lee M-R, Sheng W-H, Hung C-C, Yu C-J, Lee L-N, Hsueh P-R. *Mycobacterium abscessus* complex infections in humans. Emerg Infect Dis. 2015;21:1638–46.
3. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175:367–416.
4. Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ, Andrejak C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: an official ATS/ERS/ESCMID/IDSA clinical practice guideline. Eur Respir J. 2020;56:2000535.
5. Tortoli E, Kohl TA, Brown-Elliott BA, Trovato A, Leão SC, Garcia MJ, et al. Emended description of *Mycobacterium abscessus*, *Mycobacterium abscessus* subsp. abscessus and *Mycobacterium abscessus* subsp. bolletii and designation of *Mycobacterium abscessus* subsp. massiliense comb. Nov. Int J Syst Evol Microbiol. 2016;66:4471–9.
6. Pasipanodya JG, Ogbonna D, Ferro BE, Magombedze G, Srivastava S, Deshpande D, et al. Systematic review and meta-analyses of the effect of chemotherapy on pulmonary *Mycobacterium abscessus* outcomes and disease recurrence. Antimicrob Agents Chemother. 2017;61:e01206-e1217.
7. Ferro BE, Srivastava S, Deshpande D, Pasipanodya JG, van Soolingen D, Mouton JW, et al. Failure of the Amikacin, Cefoxitin, and Clarithromycin combination regimen for treating pulmonary *Mycobacterium abscessus* infection. Antimicrob Agents Chemother. 2016;60:6374–6.
8. Honeybourne D, Kees F, Andrews JM, Baldwin D, Wise R. The levels of clarithromycin and its 14-hydroxy metabolite in the lung. Eur Respir J. 1994;7:1275–80.
9. Rodvold KA, Gotfried MH, Danziger LH, Servi RJ. Intrapulmonary steady-state concentrations of clarithromycin and azithromycin in healthy adult volunteers. Antimicrob Agents Chemother. 1997;41:1399–402.

10. Clinical and laboratory standards institute. M24—susceptibility testing of Mycobacteria, Nocardia spp, and other aerobic actinomycetes. 3rd edn. 2018.
11. Sadouki Z, McHugh TD, Aarnoutse R, Ortiz Canseco J, Darlow C, Hope W, et al. Application of the hollow fibre infection model (HFIM) in antimicrobial development: a systematic review and recommendations of reporting. J Antimicrob Chemother. 2021;76:2252–9.
12. Base de données publique des médicaments. Résumé des caractéristiques du produit—ZECLAR 0.5 g, poudre pour solution à diluer pour perfusion. https://base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=61433564&typedoc=R#RcpPropPharmacocinetiques. Accessed 19 Jul 2024.
13. Base de données publique des médicaments. Résumé des caractéristiques du produit—AMIKACINE VIATRIS 1 g, poudre pour solution injectable en flacon. https://base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=62516778&typedoc=R#RcpPropPharmacocinetiques. Accessed 19 Jul 2024.
14. Base de données publique des médicaments. Résumé des caractéristiques du produit—CEFOXITINE PANPHARMA 1 g, poudre pour solution injectable (IV). https://base-donnees-publique.medicaments.gouv.fr/affichageDoc.php?specid=69084488&typedoc=R#RcpPropPharmacocinetiques. Accessed 19 Jul 2024.
15. The European Committee on Antimicrobial Susceptibility, EUCAST. Amikacin: Rationale for EUCAST Clinical Breakpoints. 2024. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Rationale_documents/Amikacin_Rationale_Document_v_3.1_20240901.pdf. Accessed 22 Nov 2024.
16. Davey PG. The pharmacokinetics of clarithromycin and its 14-OH metabolite. J Hosp Infect. 1991;19(Suppl A):29–37.
17. Isla A, Trocóniz IF, de Tejada IL, Vázquez S, Canut A, López JM, et al. Population pharmacokinetics of prophylactic cefoxitin in patients undergoing colorectal surgery. Eur J Clin Pharmacol. 2012;68:735–45.
18. Clinical and laboratory standards institute. M62—performance standards for susceptibility testing of Mycobacetria, Nocardia spp., and other aerobic actinomycetes. 1st edn. 2018.
19. Prammananan T, Sander P, Brown BA, Frischkorn K, Onyi GO, Zhang Y, et al. A single 16S ribosomal RNA substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in *Mycobacterium abscessus* and *Mycobacterium chelonae*. J Infect Dis. 1998;177:1573–81.
20. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, et al. Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol. 2019;17:441–8.
21. Brauner A, Fridman O, Gefen O, Balaban NQ. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol. 2016;14:320–30.
22. Sulaiman JE, Lam H. Evolution of bacterial tolerance under antibiotic treatment and its implications on the development of resistance. Front Microbiol. 2021;12: 617412.
23. Bernard C, Liu Y, Larrouy-Maumus G, Guilhot C, Cam K, Chalut C. Altered serine metabolism promotes drug tolerance in *Mycobacterium abscessus* via a WhiB7-mediated adaptive stress response. Antimicrob Agents Chemother. 2024;68:e01456-e1523.
24. Herbert S, Barry P, Novick RP. Subinhibitory clindamycin differentially inhibits transcription of exoprotein genes in *Staphylococcus aureus*. Infect Immun. 2001;69:2996–3003.

Publisher's Note