In vitro efficacy of relebactam versus avibactam against *Mycobacterium abscessus* complex

James Harrison[a*], John A. Weaver[a], Maya Desai[b], Jonathan A.G. Cox[a]

[a]Aston University, Birmingham, UK; [b]Birmingham Children's Hospital, Birmingham Women's and Children's NHS Foundation Trust, UK, *j.harrison11@aston.ac.uk

1. Introduction

- *Mycobacterium abscessus* is an opportunistic human pathogen that is an increasing global health threat, capable of causing pulmonary disease or skin and soft tissue infections. Of particular susceptibility to these infections are those who suffer from cystic fibrosis (CF) or bronchiectasis[1]. *M. abscessus* is largely drug resistant, resulting in front-line antimicrobial treatments being lengthy and relatively unsuccessful, resulting in low patient compliance and high morbidity[2]. This is exacerbated by the existence of multiple subspecies within the *M. abscessus* complex, which have a range of additional antimicrobial resistance profiles that must be identified for successful treatment[3].
- A major mechanism of antimicrobial resistance employed by *M. abscessus* is the intrinsic β-lactam resistance provided by expression of an endogenous class A β-lactamase, Bla_{Mab}, which hydrolyses common antibiotics such as penicillin and renders them ineffective[4]. Therefore, inhibition of Bla_{Mab} can result in improved efficacy of β-lactams against *M. abscessus*[4]. The non-β-lactam based β-lactamase inhibitors **relebactam (REL)** and **avibactam (AVI)** have previously been shown to efficiently block β-lactam hydrolysis in *M. abscessus*[5]. Here, we assess the efficacy of these compounds with their respective commercial partner β-lactams **imipenem (IMI)** and **ceftazidime (CAZ)**, as well as exploring the most effective triplicate combination of these drugs with **meropenem (MER)**.

2. Methods

- *M. abscessus* NCTC 13031, 15944 subsp. *abscessus,* DC088A subsp. *bolletii,* DC088D subsp. *massiliense* were grown in Middlebrook 7H9 media with 10 % (v/v) Albumin-Dextrose-Catalase supplement, 1 % (w/v) glycerol and 0.05 % (w/v) Tween80 and incubated at 37 °C.
- Broth microdilution assays (or checkerboard assays) were performed, briefly, 96-well plates were prepared by serial dilution of MER, CAZ or IMI along the x-axis and either CAZ/AVI, IMI/REL, IMI/AVI, AVI or REL along the y-axis, depending upon the compound combination being analysed. M. abscessus isolates were diluted to OD600nm= 0.1 before addition to experimental wells at a final volume of 100 µL (n=4). Plates were sealed and incubated at 37 °C for 96 h, with spectrophotometric plate reads at 570 nm at every 24 h. Minimum Inhibitory Concentrations (MIC) were retroactively plotted as absorbance vs time (h).
- Minimum Bactericidal Concentrations (MBC) were identified as lack of growth after plating each experimental well onto Middlebrook 7H11 and incubating at 37 °C for 48 h.

3. Results

Assessment of β-lactam/β-lactamase inhibitor formulations against *M. abscessus*

- The ratios of IMI:REL and CAZ:AVI were assessed at both of the commercially available ratios 2:1 and 4:1 (respectively), as well as at 1:1 and 1:2 against *M. abscessus*.
- IMI:REL was inhibitory at all ratios (Fig 1), however bactericidal activity was observed for 2:1 at 3 µg/mL IMI, 1.5 µg/mL REL, with higher concentrations of REL unable to reduce the minimum IMI concentration any further.
- CAZ:AVI had no inhibitory or bactericidal activity at any ratio (Fig 1).

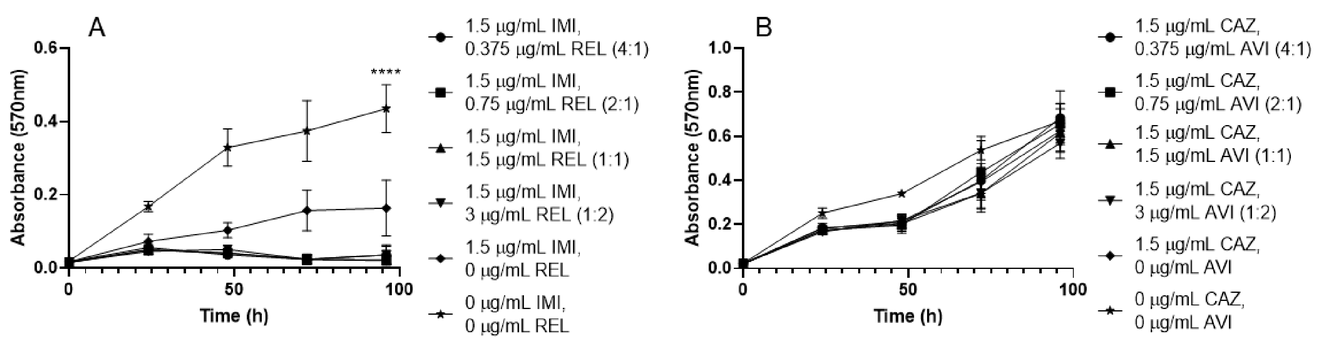

Figure 1: Growth curves of *M. abscessus* NCTC 13031 with varying β-lactam/β-lactamase inhibitor combination ratios. A) Varying ratios of imipenem (IMI) and relebactam (REL) (p=<0.0001, n=4). B) Varying ratios of ceftazidime (CAZ) and avibactam (AVI) (p=0.5287, n=4).

Triplicate combinations of β-lactam/β-lactamase inhibitor against *M. abscessus*

- Combinations of IMI/REL (2:1) with MER, CAZ/AVI (4:1) with MER and IMI/AVI (2:1) with MER were assessed against members of the *M. abscessus* complex.
- IMI/REL/MER combinations were bactericidal at 0.75 µg/mL/0.375 µg/mL/1 µg/mL (Fig 2).
  - Alone, neither IMI/REL or MER were bactericidal at these concentrations.
- The combination of CAZ/AVI/MER sterilised each subspecies at 0.75 µg/mL/0.1875 µg/mL/2 µg/mL (Fig 3). This activity was beyond that of CAZ/AVI or MER alone.
  - However, *M. abscessus* subsp. *massiliense* required 4 µg/mL MER at the same concentrations of CAZ and AVI (0.75 µg/mL/0.1875 µg/mL) to be lethal.
- The non-commercially available combination of IMI/AVI with MER was bactericidal at 0.375 µg/mL/0.1875 µg/mL/0.5 µg/mL (Fig 4).
  - IMI/AVI and MER alone at these concentrations did not retain this activity.

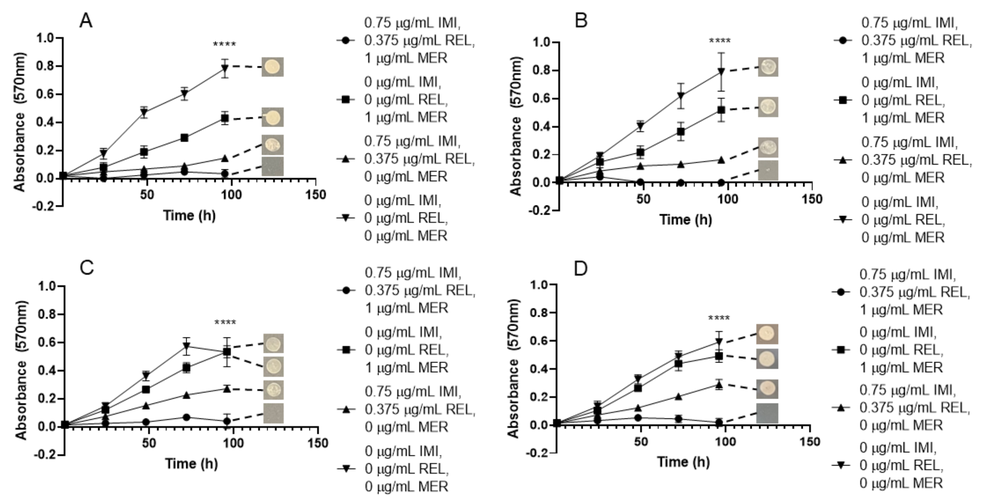

Figure 2: Growth curves of *M. abscessus* subspecies with IMI/REL vs MER. Endpoint solid media bacterial re-growth is shown at the end of each curve. A) *M. abscessus* NCTC 13031 (p=<0.0001, n=4). B) *M. abscessus* subsp. *abscessus* (p=<0.0001, n=4). C) *M. abscessus* subsp. *bolletii* (p=<0.0001, n=4). D) *M. abscessus* subsp. *massiliense* (p=<0.0001, n=4). Each subspecies has an MIC of 0.75 µg/mL IMI, 0.375 µg/mL REL and 1 µg/mL MER.

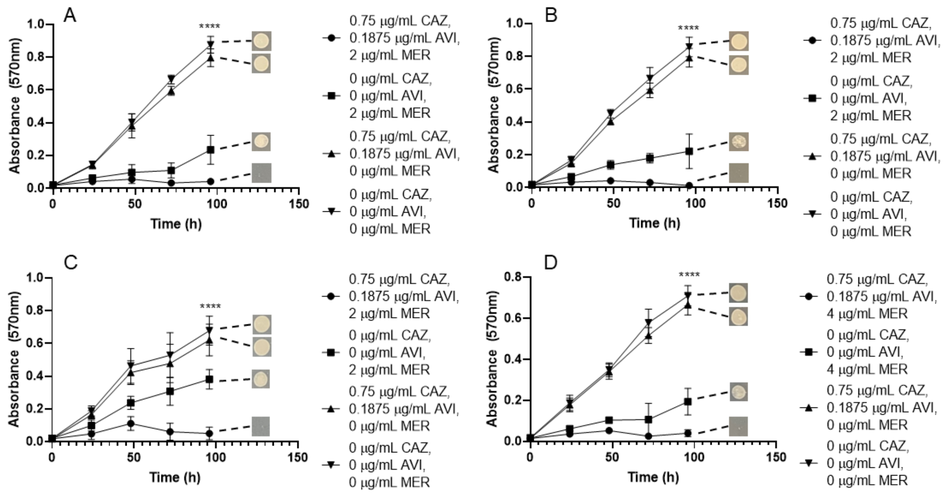

Figure 3: Growth curves of *M. abscessus* subspecies with CAZ/AVI vs MER. Endpoint solid media bacterial re-growth is shown at the end of each curve. A) *M. abscessus* NCTC 13031 (p=<0.0001, n=4). B) *M. abscessus* subsp. *abscessus* (p=<0.0001, n=4). C) *M. abscessus* subsp. *bolletii* (p=<0.0001, n=4). D) *M. abscessus* subsp. *massiliense* (p=<0.0001, n=4). Each subspecies had an MIC of 0.75 µg/mL CAZ, 0.1875 µg/mL AVI and 2 µg/mL MER (*M. abscessus* subsp. *massiliense* required 4 µg/mL MER at the same CAZ and AVI concentrations).

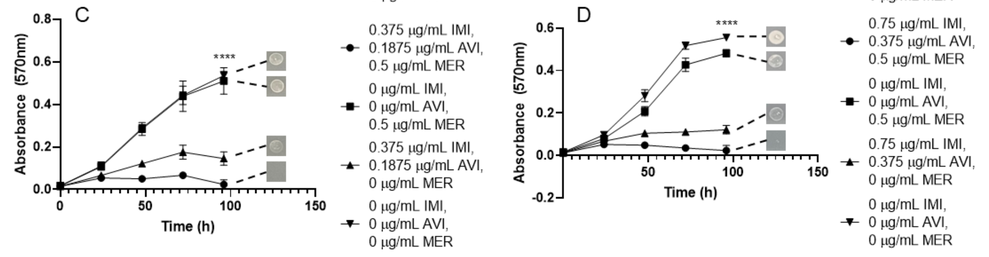

Figure 4: Growth curves of *M. abscessus* subspecies with IMI/AVI vs MER. Endpoint solid media bacterial re-growth is shown at the end of each curve. A) *M. abscessus* NCTC 13031 (p=<0.0001, n=4). B) *M. abscessus* subsp. *abscessus* (p=<0.0001, n=4). C) *M. abscessus* subsp. *bolletii* (p=<0.0001, n=4). D) *M. abscessus* subsp. *massiliense* (p=<0.0001, n=4). Each subspecies has an MIC of 0.75 µg/mL IMI, 0.1875 µg/mL AVI and 0.5 µg/mL MER

4. Conclusions

- IMI, AVI + MER and IMI, REL + MER are the best triplicate combinations tested.
- These combinations yielded low MIC/MBC values of 0.375 µg/mL IMI, 0.1875 µg/mL AVI, 0.5 µg/mL MER and 0.75 µg/mL IMI, 0.375 µg/mL REL and 1 µg/mL MER.
- But there is excessive additional antibiotic burden with AVI usage, because of the addition of CAZ which has no effect. This is not the case with REL as it is pre-formulated with IMI, an existing front-line treatment.
- **Therefore, the use of IMI, REL + MER against members of the *M. abscessus* complex is an appropriate choice based upon patient quality of life and sterilisation of *M. abscessus* infections.**

5. Read the paper

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Acknowledgements

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