

***In vitro* activity of the new  $\beta$ -lactamase inhibitors relebactam and vaborbactam in combination with  $\beta$ -lactams against *Mycobacterium abscessus* complex clinical isolates**

Amit Kaushik, Nicole C. Ammerman, Jin Lee, Olumide Martins, Barry N. Kreiswirth, Gyanu Lamichhane, Nicole M. Parrish, Eric L. Nuermberger

---

**SUPPLEMENTAL MATERIALS**

**Tables**

- **Table S1.** MIC values in 7H9 media of carbapenems and faropenem (a penem) with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.
- **Table S2.** MIC values in 7H9 media of cephalosporins with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.
- **Table S3.** MIC values in CAMHB of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.
- **Table S4.** MIC<sub>50</sub> and MIC<sub>90</sub> values in CAMHB of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.
- **Table S5.** CLSI MIC breakpoint values and interpretive categories of carbapenems and cephalosporins included in this study.

**Figures**

- **Fig. S1.** Growth of MABC isolates in CAMHB and Middlebrook 7H9 broth.
- **Fig. S2.** Disk diffusion assay of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors.

**TABLE S1** MIC values in 7H9 media of carbapenems and faropenem (a penem) with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.

MABC strain	MIC ( $\mu$ g/mL) in 7H9 media for MABC strains of the following (carba)penems alone or in combination with relebactam (rele.) or vaborabactam (vabor.)																				
	Biapenem			Doripenem			Ertapenem			Faropenem			Imipenem			Meropenem			Tebipenem		
	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.
1N	16	4	8	16	4	4	256	16	128	128	32	32	8	4	4	32	4	4	256	8	16
2N	32	8	8	32	8	8	>256	32	32	128	32	32	8	8	8	64	8	8	256	16	32
3N	16	4	4	16	4	4	256	16	32	128	32	32	8	4	4	32	4	4	256	8	32
4N	16	4	8	16	4	4	128	16	32	64	32	32	8	8	8	16	4	4	256	8	16
5N	16	8	8	16	8	4	128	16	16	128	32	32	8	8	8	16	4	4	256	8	16
6N	16	4	4	32	4	4	>256	16	32	256	32	32	8	2	2	16	4	4	256	8	32
11N	64	8	8	64	8	8	>256	64	64	64	32	32	16	16	8	64	8	8	64	8	8
12N	16	4	4	16	4	4	256	16	32	256	32	32	8	4	4	16	4	4	>256	16	32
13N	8	4	8	16	4	4	256	16	16	64	16	16	16	8	8	8	4	4	128	4	8
14N	64	8	16	64	16	16	>256	128	256	256	64	64	16	4	4	64	16	16	256	32	64
19N	16	2	4	16	2	4	256	16	16	128	32	32	4	2	2	16	2	4	256	4	8
201	64	8	8	32	8	16	>256	64	128	>256	64	64	64	8	8	16	8	16	>256	32	128
202	128	16	32	64	8	16	>256	128	256	256	64	64	32	8	32	>256	16	64	>256	16	256
203	64	8	16	64	8	16	>256	64	128	64	32	64	16	8	8	256	16	16	128	16	32
204	128	16	32	128	16	32	>256	256	256	256	64	64	16	8	8	128	32	32	256	64	64
206	16	8	8	16	4	8	256	16	32	128	32	32	8	4	4	16	4	4	256	8	16
208	16	4	8	16	4	4	256	16	32	128	32	32	16	8	8	16	4	4	256	8	32
210	32	4	8	32	4	8	>256	32	64	256	64	64	16	4	8	32	4	8	>256	16	32
211	32	4	8	32	4	8	>256	32	64	256	64	64	8	8	8	32	4	8	256	16	64
212	16	4	4	16	4	8	256	16	16	256	32	32	16	4	8	16	4	8	256	8	16
214	16	4	8	16	4	4	256	16	32	64	32	32	16	8	8	16	4	4	128	8	16
215	32	8	8	32	8	4	256	32	32	128	32	32	16	16	8	32	8	4	256	8	32
216	64	16	16	64	8	8	256	128	64	256	64	64	16	8	8	32	8	8	256	32	64
218	16	4	8	16	4	4	256	32	32	128	64	64	8	8	8	32	4	8	256	16	32
JHH2	64	8	16	256	8	16	256	128	128	128	64	64	32	8	16	128	8	16	256	32	64
JHH4	32	4	4	32	4	4	256	32	32	128	32	32	16	4	4	32	4	4	256	8	16
JHH9	32	4	4	32	4	4	256	16	32	128	32	32	8	4	4	32	4	8	256	8	16
JHHKB	128	16	16	256	16	32	256	128	128	128	64	64	32	16	16	128	16	32	128	32	32
ATCC 19977	16	4	4	16	4	4	>256	16	32	128	32	32	8	4	4	16	4	4	256	8	16

Relebactam and vaborabactam were each used at a fixed concentration of 4  $\mu$ g/ml.

Assay medium was Middlebrook 7H9 broth supplemented with 0.5% (v/v) glycerol and 10% (v/v) Middlebrook OADC enrichment; MIC values were determined after 72 hours of incubation at 30°C.

**TABLE S2** MIC values in 7H9 media of cephalosporins with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.

MABC strain	MIC ( $\mu\text{g/mL}$ ) in 7H9 media for MABC strains of the following cephalosporins alone or in combination with relebactam (rele.) or vaborabactam (vabor.)																								
	Cefdinir			Cefepime			Cefotaxime			Cefoxitin			Cefpodoxime			Ceftaroline			Ceftriaxone			Cefuroxime			
	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	
1N	64	16	16	16	16	16	128	32	32	32	32	32	32	>256	32	64	>256	16	32	>256	32	32	128	8	16
2N	64	16	8	32	32	32	128	32	32	32	32	32	256	64	64	>256	32	32	>256	64	64	128	16	16	
3N	64	16	16	16	16	16	128	32	32	32	32	32	>256	32	64	>256	16	32	>256	32	32	128	8	16	
4N	64	16	16	32	32	16	128	32	32	32	32	32	256	64	64	>256	16	32	>256	32	32	128	8	16	
5N	64	16	16	32	16	32	64	32	32	16	16	16	256	32	32	>256	16	16	>256	32	32	128	8	8	
6N	128	16	32	32	8	16	256	64	64	32	32	32	>256	64	64	>256	16	64	>256	64	128	256	16	16	
11N	64	16	16	64	64	64	64	32	16	32	32	32	256	64	64	>256	32	32	>256	32	32	128	8	16	
12N	128	32	32	16	16	8	128	64	64	32	16	16	>256	64	64	>256	16	32	>256	64	64	256	16	16	
13N	32	16	16	64	64	64	64	32	16	32	16	16	128	64	64	256	16	16	256	32	32	64	8	8	
14N	64	8	32	64	16	16	64	32	32	64	64	64	256	64	64	256	256	128	128	64	64	16	16	16	
19N	64	16	16	32	8	16	128	32	32	32	32	32	256	32	64	>256	16	32	>256	32	64	256	16	32	
201	64	16	16	64	16	16	256	64	128	32	32	32	>256	128	128	>256	32	128	>256	64	128	>256	16	32	
202	32	16	16	128	16	32	256	64	64	64	64	64	256	64	64	>256	16	32	>256	64	64	>256	32	32	
203	256	64	64	32	16	32	32	16	16	32	32	32	128	32	32	256	16	32	128	16	16	32	8	8	
204	256	32	128	32	16	16	64	32	32	64	64	32	128	64	64	>256	64	64	256	64	64	64	16	16	
206	32	16	16	32	32	16	64	32	32	16	32	16	128	32	32	256	8	8	256	16	16	128	8	8	
208	64	32	32	32	32	16	128	32	32	32	32	32	>256	64	64	>256	16	32	>256	32	64	128	8	16	
210	128	32	32	32	8	16	256	32	64	32	32	32	>256	64	64	>256	16	32	>256	64	64	128	16	16	
211	64	16	16	32	8	8	128	32	64	32	32	32	>256	64	64	>256	16	32	>256	64	64	128	16	16	
212	128	32	32	32	16	16	128	32	32	16	16	16	256	32	32	256	8	8	>256	32	64	128	8	8	
214	128	32	32	32	32	32	64	32	32	32	16	16	256	32	32	>256	8	16	>256	16	32	64	8	8	
215	64	16	16	32	32	32	128	32	32	32	32	32	>256	64	64	>256	16	32	>256	32	32	128	8	8	
216	128	32	32	64	64	64	256	64	64	32	32	32	>256	64	128	>256	32	32	>256	128	128	>256	32	32	
218	128	32	32	64	64	64	256	64	64	32	32	32	>256	64	128	>256	32	32	>256	128	128	>256	32	32	
JHH2	32	16	16	64	32	32	64	64	64	128	32	64	64	128	64	256	32	64	>256	64	128	256	32	32	
JHH4	128	32	64	16	16	16	64	32	32	32	32	32	128	32	32	>256	16	16	>256	32	32	128	16	16	
JHH9	128	16	32	32	16	16	128	32	32	32	32	32	256	32	32	>256	16	32	>256	32	32	256	16	32	
JHHKB	64	16	16	64	64	64	64	32	32	64	32	32	256	64	64	>256	64	32	>256	128	64	128	32	32	
ATCC 19977	64	32	32	32	16	16	256	64	64	32	32	32	>256	64	64	>256	32	32	>256	64	64	256	16	32	

Relebactam and vaborbactam were each used at a fixed concentration of 4  $\mu\text{g/ml}$ .

Assay medium was Middlebrook 7H9 broth supplemented with 0.5% (v/v) glycerol and 10% (v/v) Middlebrook OADC enrichment; MIC values were determined after 72 hours of incubation at 30°C.

**Table S3** MIC values in CAMHB of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates.

MABC strain	Incubation time (days)	MIC ( $\mu$ g/mL) in CAMHB for MABC strains of the following $\beta$ -lactams alone or in combination with relebactam (rele.) or vaborabactam (vabor.)																				
		Doripenem			Ertapenem			Imipenem			Meropenem			Cefdinir			Cefepime			Cefuroxime		
		Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.	Alone	Rele.	Vabor.
1N	4.5	32	16	16	256	128	64	16	8	8	64	16	16	256	16	16	256	64	64	256	32	32
2N	6.0	16	16	8	256	64	32	16	8	8	64	8	16	32	8	8	64	64	64	64	32	32
3N	4.5	64	16	16	256	64	128	16	16	4	64	16	16	128	32	16	256	64	64	>256	64	64
4N	4.5	128	32	16	256	64	64	32	16	16	64	32	32	128	64	32	128	64	64	256	64	64
5N	4.5	32	16	16	128	64	64	16	8	8	32	16	16	32	16	16	128	64	64	64	16	16
6N	4.5	64	16	16	256	128	128	32	16	8	64	32	16	128	16	32	128	64	64	256	128	64
11N	6.0	16	16	8	256	128	64	8	8	8	32	16	8	32	32	16	64	64	64	64	64	64
12N	4.5	64	32	16	>256	256	64	64	32	8	64	64	32	128	64	32	256	64	64	>256	64	64
13N	4.5	32	8	8	128	64	64	8	4	4	64	16	16	4	8	4	64	64	64	64	32	16
14N	4.5	16	16	8	128	64	32	16	8	8	64	64	8	16	16	32	64	64	64	64	64	32
19N	4.5	32	16	16	256	64	64	16	8	8	32	16	16	32	8	8	128	64	64	128	16	32
201	4.5	128	64	16	>256	128	128	32	32	4	128	64	64	128	64	64	256	128	128	128	64	128
202	4.5	64	8	32	64	64	128	16	8	4	64	32	16	128	32	32	256	64	32	256	64	32
203	4.5	64	32	8	256	64	32	8	4	4	64	32	16	32	16	8	64	64	32	64	64	16
204	4.5	64	32	16	128	128	64	8	8	8	64	64	32	32	32	8	64	64	64	128	64	64
206	4.5	8	8	8	256	32	16	8	4	4	16	8	16	32	8	8	256	64	64	256	8	8
208	4.5	64	16	16	256	64	64	16	8	8	64	16	16	32	8	16	128	64	64	128	16	16
210	4.5	64	16	16	>256	128	128	32	8	8	128	32	16	64	32	32	128	64	64	64	64	64
211	4.5	128	16	16	>256	256	32	32	8	8	64	32	32	256	64	32	256	128	64	64	128	64
212	4.5	32	16	16	>256	128	64	16	8	8	32	16	8	64	16	16	256	64	64	>256	128	64
214	4.5	32	8	16	256	32	64	16	16	8	32	16	16	16	8	8	64	64	64	32	8	32
215	4.5	16	8	8	128	32	16	4	4	2	16	8	8	32	8	4	64	64	32	64	16	8
216	4.5	64	32	32	128	64	256	32	32	4	32	32	32	64	32	16	128	64	32	128	32	64
218	4.5	16	8	8	32	16	64	8	2	4	32	4	8	16	16	8	64	32	32	16	32	16
JHH2	4.5	32	32	16	256	32	64	32	8	8	64	16	16	128	16	8	256	32	64	256	16	32
JHH4	4.5	32	16	16	256	64	64	16	16	8	32	16	16	32	8	8	128	64	64	256	32	16
JHH9	4.5	64	16	16	256	128	64	32	8	8	64	16	16	64	16	16	128	64	64	128	64	32
JHHKB	6.0	16	8	8	>256	128	256	8	8	4	64	8	8	32	4	16	64	32	64	32	4	4
ATCC 19977	3.0	32	8	16	256	64	64	16	4	8	32	8	16	128	32	16	128	64	64	256	32	64

Relebactam and vaborbactam were each used at a fixed concentration of 4  $\mu$ g/ml.

Assay medium was Mueller-Hinton II broth, cation-adjusted; MIC values were determined after incubation at 30°C for the duration indicated.

**TABLE S4** MIC<sub>50</sub> and MIC<sub>90</sub> values in CAMHB of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors against 28 MDR MABC clinical isolates

$\beta$ -lactam tested	MICs ( $\mu$ g/mL) in CAMHB for MABC clinical isolates								
	Alone			With relebactam			With vaborbactam		
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<b>Parenteral carbapenems</b>									
Doripenem	8 - 128	32	128	8 - 64	16	32	8 - 32	16	16
Ertapenem	32 - 256	256	256	16 - 256	64	128	16 - 256	64	128
Imipenem	4 - 64	16	32	2 - 32	8	32	2 - 16	8	8
Meropenem	16 - 128	64	64	4 - 64	16	64	8 - 64	16	32
<b>Oral cephalosporins</b>									
Cefdinir	4 - 256	32	128	4 - 64	16	64	4 - 64	16	32
Cefuroxime	16 - 256	128	256	4 - 128	32	128	4 - 128	32	64
<b>Parenteral cephalosporin</b>									
Cefepime	64 - 256	128	256	32 - 128	64	64	32 - 128	64	64

Relebactam and vaborbactam were each used at a fixed concentration of 4  $\mu$ g/ml.

Assay medium was Mueller-Hinton II broth, cation-adjusted; MIC values were determined after incubation at 30°C for the duration indicated in **Table S3**.

MIC values for each strain are provided in **Table S3**.

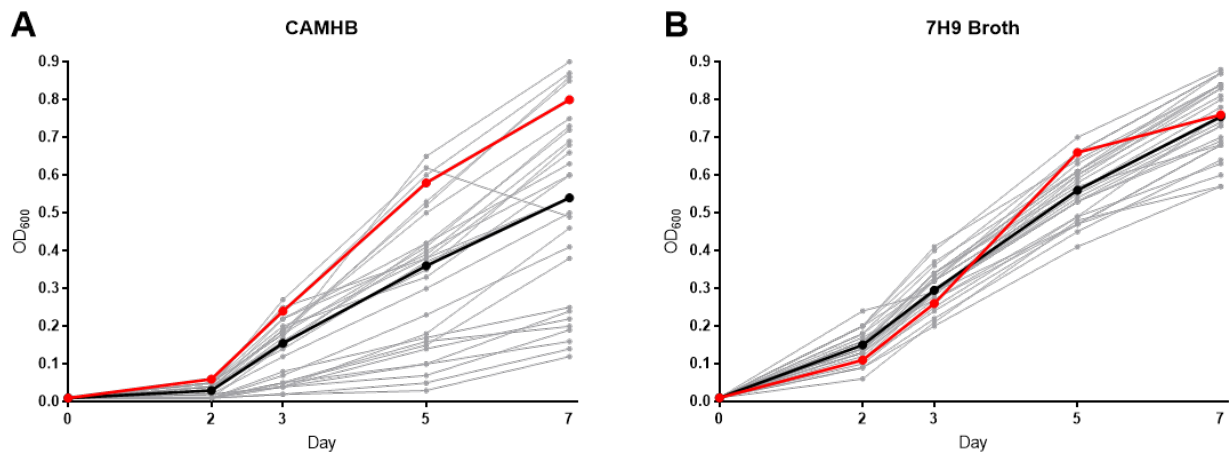
Cefuroxime is available in both oral and parenteral formulations.

**TABLE S5** CLSI MIC breakpoint values and interpretive categories of carbapenems and cephalosporins included in this study.

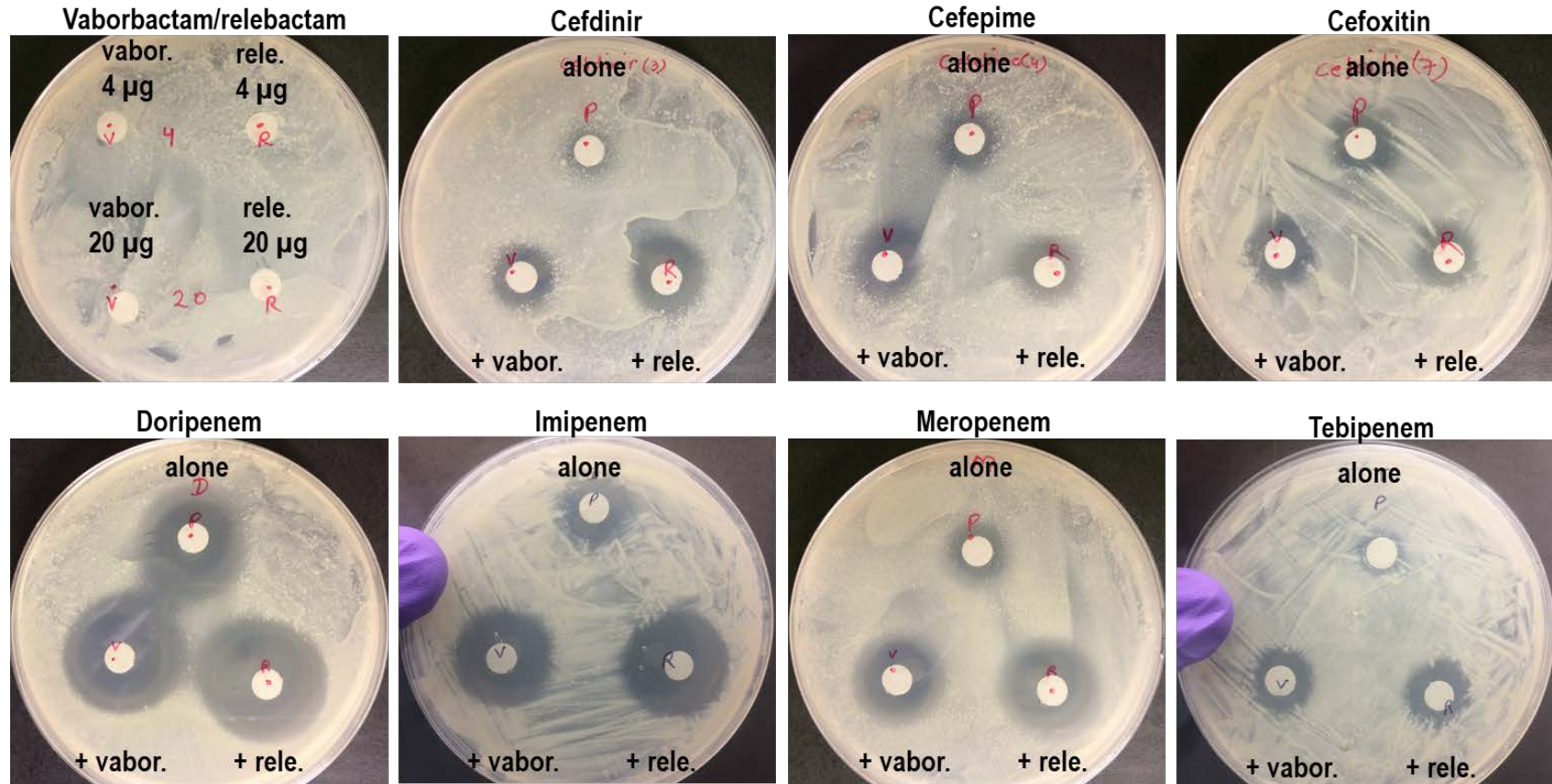
$\beta$ -lactam	CLSI interpretive categories and MIC breakpoints ( $\mu\text{g/ml}$ ) for the following organisms:								
	Rapidly growing mycobacteria			<i>Enterobacteriaceae</i>			Anaerobes		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
<u>Carbapenems</u>									
Doripenem				$\leq 1$	2	$\geq 4$	$\leq 2$	4	$\geq 8$
Ertapenem				$\leq 0.5$	1	$\geq 2$	$\leq 4$	8	$\geq 16$
Imipenem	$\leq 4$	8-16	$\geq 32$	$\leq 1$	2	$\geq 4$	$\leq 4$	8	$\geq 16$
Meropenem	$\leq 4$	8-16	$\geq 32$	$\leq 1$	2	$\geq 4$	$\leq 4$	8	$\geq 16$
<u>Cephalosporins</u>									
Cefdinir				$\leq 1$	2	$\geq 4$			
Cefepime				$\leq 2$	4-8	$\geq 16$			
Cefoxitin	$\leq 16$	32-64	$\geq 128$	$\leq 8$	16	$\geq 32$	$\leq 16$	32	$\geq 64$
Cefotaxime				$\leq 1$	2	$\geq 4$	$\leq 16$	32	$\geq 64$
Cefpodoxime				$\leq 2$	4	$\geq 8$			
Ceftaroline				$\leq 0.5$	1	$\geq 2$			
Ceftriaxone				$\leq 1$	2	$\geq 4$	$\leq 16$	32	$\geq 64$
Cefuroxime (oral)				$\leq 4$	8-16	$\geq 32$			
Cefuroxime (parenteral)				$\leq 8$	16	$\geq 32$			

Information obtained from CLSI guidelines (22, 31).

Breakpoints are not available for biapenem, faropenem, and tebipenem.



**FIG. S1** Growth of MABC isolates in **A)** CAMHB and **B)** Middlebrook 7H9 broth. The cultures were adjusted to a starting  $OD_{600}$  of 0.01, and the  $OD_{600}$  was monitored during 7 days of incubation at 30°C. The growth curve for the ATCC 19977 strain is shown in red. The growth curves for the individual clinical isolates are shown in gray, and the median growth curve for clinical isolates is shown in black.



**FIG. S2** Disk diffusion assay of  $\beta$ -lactams with and without  $\beta$ -lactamase inhibitors. Paper disks containing 20  $\mu$ g carbapenems (doripenem, imipenem, meropenem, or tebipenem), 20  $\mu$ g cephalosporins (cefdinir, cefepime, cefoxitin) and/or  $\beta$ -lactamase inhibitors (4 or 20  $\mu$ g alone; 4  $\mu$ g in combination) were incubated with *M. abscessus* strain ATCC 19977 for 4 days at 37°C.