



# *Mycobacterium abscessus* and $\beta$ -Lactams: Emerging Insights and Potential Opportunities

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$\beta$ -lactams, the most widely used class of antibiotics, are well-tolerated, and their molecular mechanisms of action against many bacteria are well-documented. *Mycobacterium abscessus* (*Mab*) is a highly drug-resistant rapidly-growing nontuberculous mycobacteria (NTM). Only in recent years have we started to gain insight into the unique relationship between  $\beta$ -lactams and their targets in *Mab*. In this mini-review, we summarize recent findings that have begun to unravel the molecular basis for overall efficacy of  $\beta$ -lactams against *Mab* and discuss emerging evidence that indicates that we have yet to harness the full potential of this antibiotic class to treat *Mab* infections.

**Keywords:** *Mycobacterium abscessus*,  $\beta$ -lactams, peptidoglycan, LD-transpeptidase,  $\beta$ -lactamase inhibitor

## INTRODUCTION

Although *Mycobacterium abscessus* (*Mab*) was first discovered in 1953 (Moore and Frerichs, 1953), it was only recently that genomic sequencing differentiated the *Mab* complex into three subspecies: *M. abscessus sensu stricto*, *M. abscessus* subsp. *bolletii*, and *M. abscessus* subsp. *massiliense* (Adekambi et al., 2004, 2006; Viana-Niero et al., 2008). These subspecies exhibit differential susceptibilities to certain antibiotics and differential clinical outcomes.

*Mab* can cause pulmonary disease in addition to skin and soft tissue infections, lymphadenitis, and disseminated disease. *Mab* is sometimes considered a respiratory colonizer; however, in the setting of immunosuppression or structural lung disease, such as cystic fibrosis (CF) and bronchiectasis, *Mab* can cause chronic pulmonary disease. In CF patients, *Mab* infections are often incurable and associated with rapid lung function decline (Griffith et al., 2007; Esther et al., 2010; Benwill and Wallace, 2014). The cure rate for *Mab* lung disease is only 30–50% (Jarand et al., 2011), with a recent review reporting sputum culture conversion rates as low as 25% with antibiotic treatment alone (Diel et al., 2017).

Poor treatment outcomes of *Mab* infection have been ascribed to both innate and acquired drug resistance. *Mab* is intrinsically resistant to multiple antibiotic classes which has been attributed to various factors (Brown-Elliott and Wallace, 2002; Nessar et al., 2012; van Ingen et al., 2012). Acquired resistance has further limited therapeutic options (Flume, 2016). Current treatment regimens are suboptimal, as they require several months of intravenous multidrug therapy with potentially cytotoxic antibiotics and produce poor outcomes (Wallace et al., 1985; Floto et al., 2016).

In this review, we will briefly summarize *Mab* treatment recommendations, discuss unique molecular targets of  $\beta$ -lactams in *Mab*, and highlight emerging insights into how  $\beta$ -lactams may be leveraged to treat individuals infected with *Mab*.

## CURRENT *Mab* TREATMENT RECOMMENDATIONS

The US Cystic Fibrosis Foundation and European Cystic Fibrosis Society recently developed consensus guidelines for management of *Mab* lung disease in CF patients (Floto et al., 2016). Similar to tuberculosis, *Mab* infection is treated with multidrug regimens divided into an intensive phase, followed by a continuation phase. Per recent guidelines, the intensive phase of *Mab* therapy should consist of an oral macrolide, combined with 3–12 weeks of intravenous amikacin, plus at least one of the following: intravenous ceftazidime, imipenem, or tigecycline (Floto et al., 2016). Guidelines for the continuation phase include a daily oral macrolide, inhaled amikacin, and two to three additional oral antibiotics, including minocycline, clofazimine, moxifloxacin, and linezolid.

Macrolides have historically been considered the backbone of treatment against *Mab*. They have relatively low toxicity, are orally bioavailable (Griffith et al., 2007; Floto et al., 2016), and exhibit consistent activity against *Mab in vitro* (Griffith et al., 2007). However, subspecies *abscessus* and *bolletii* harbor a functional *erm(41)* gene, which confers inducible macrolide resistance and can limit the effectiveness of this drug class. In contrast, subspecies *massiliense* carries a non-functional *erm(41)* gene (Nash et al., 2009), thus cannot exhibit inducible macrolide resistance and is associated with improved outcomes on macrolide-based regimens (Koh et al., 2011). Consequently, the CF guidelines recommend subspeciation of *Mab* complex, which many clinical laboratories are not equipped to perform routinely. Therefore, some CF centers prescribe initial treatment regimens comprised of intravenous amikacin plus either ceftazidime or imipenem, rather than a macrolide (Phillely et al., 2016).

Ceftazidime and imipenem are currently the only two  $\beta$ -lactams included in the guidelines for treatment of *Mab* infections. This antibiotic class has been largely understudied against *Mab* and may be a potential untapped resource in combating this highly-resistant microbe.

## MECHANISM OF ACTION OF $\beta$ -LACTAMS AGAINST *Mab*

$\beta$ -lactams are the most widely-used antibiotic class to treat bacterial infections (Hamad, 2010) and their safety and efficacy profiles have been well-established. There are five subclasses of  $\beta$ -lactams currently available in the clinical setting: penicillins, cephalosporins, monobactams, carbapenems, and penems.  $\beta$ -lactams have been studied extensively for treatment of drug-resistant *Mycobacterium tuberculosis (Mtb)* infections, which is summarized elsewhere (Story-Roller and Lamichhane, 2018). Certain  $\beta$ -lactam subclasses also exhibit activity against *Mab* (Lavollay et al., 2014; Kaushik et al., 2015; Lefebvre et al., 2016). While initial insights into the molecular mechanism of action of  $\beta$ -lactams against mycobacteria were gleaned largely from *Mtb*, recent studies have begun to elucidate the relationship between *Mab* and  $\beta$ -lactams (Lavollay et al., 2014; Lefebvre et al., 2016; Kumar et al., 2017a).

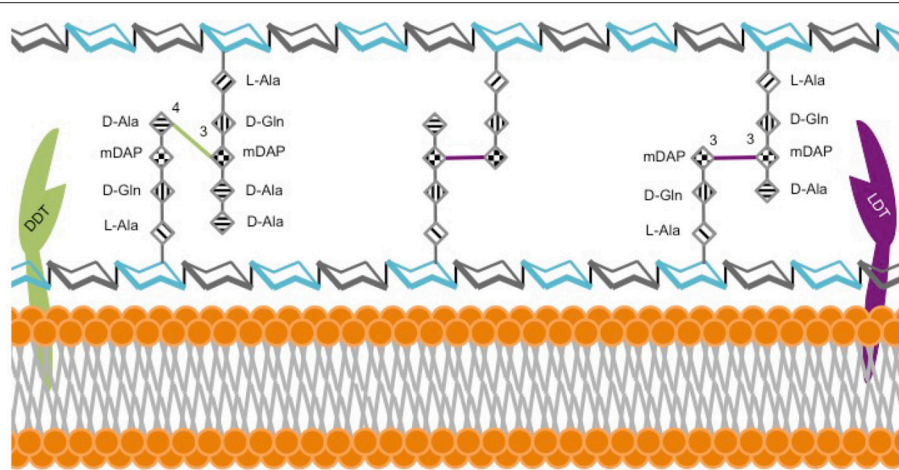
$\beta$ -lactams exert their activity by inhibiting synthesis of an essential component of the bacterial cell wall, the peptidoglycan (PG) (Hartmann et al., 1972). The building block of PG is a disaccharide with a stem peptide comprised of four or five amino acids; specifically *N*-acetyl-glucosamine-*N*-acetyl-muramic acid-L-alanyl-D-glutaminyl-*meso*-diaminopimelyl-D-alanyl-D-alanine in *Mab* (Lavollay et al., 2011). Polymerization of disaccharides by transglycosylases and stem peptides by transpeptidases produces a three-dimensional macromolecule, the PG (Figure 1).

The dominant model of PG architecture was largely established by studies using model organisms, such as *E. coli*. According to this historical model, the final step of PG synthesis is catalyzed by D,D-transpeptidases (DDT), also known as penicillin binding proteins, which link the 4th amino acid of one stem peptide to the 3rd amino acid of the adjacent stem peptide, thereby generating a 4  $\rightarrow$  3-linked peptide network. However, as early as 1974, it became clear that the chemical architecture of mycobacterial PG, and therefore the enzymes necessary for its synthesis, were distinct from those described in the historical model. This study reported that stem peptides in the PG of *M. smegmatis*, *Mtb*, and *M. bovis* BCG were predominantly cross-linked with non-canonical linkages between the 3rd amino acid of one peptide and the 3rd amino acid of another (Wietzerbin et al., 1974). That same year, this group also demonstrated that the enzyme, L,D-transpeptidase (LDT), generated these 3  $\rightarrow$  3 linkages in *Streptococcus faecalis* (Coyette et al., 1974).

The first direct evidence demonstrating that stem peptides in *Mab* PG are predominantly cross-linked by 3  $\rightarrow$  3 linkages was reported in 2011 (Lavollay et al., 2011). Subsequently, five putative LDTs, Ldt<sub>Mab1–5</sub>, were identified in *Mab* (Mattoo et al., 2017) and the first crystal structure of one of these enzymes, Ldt<sub>Mab2</sub>, was described (Kumar et al., 2017a). These studies confirmed that *Mab* utilizes both LDTs and DDTs to generate 3  $\rightarrow$  3 and 4  $\rightarrow$  3 linkages between stem peptides, respectively (Figure 1). The majority of linkages in *Mab* are 3  $\rightarrow$  3, which suggests that LDTs are at least as important as DDTs for synthesis of its PG. Several studies report that genes involved in PG synthesis and remodeling are largely conserved across mycobacteria, implying a similar PG chemical composition, architecture, and metabolism (Sanders et al., 2014; Mattoo et al., 2017). A review of PG biosynthesis in *Mtb* by Pavelka et al. is recommended for further insight into mycobacterial PG biology (Pavelka et al., 2014).

## LDTs ARE PREFERENTIALLY INHIBITED BY CARBAPENEMS AND CEPHALOSPORINS

$\beta$ -lactams mimic the C-terminal end of the native stem peptide of PG, bind to the active site of transpeptidases, and irreversibly inhibit their enzymatic activity (Park and Strominger, 1957). As the historical model considered DDTs to be the only enzymes that synthesized PG, they were assumed to be the sole targets of  $\beta$ -lactams. The discovery of LDTs (Mainardi et al., 2005) prompted inquiry into whether  $\beta$ -lactams also interacted with this enzyme



**FIGURE 1 |** Model of *M. abscessus* peptidoglycan. The hexagonal structures depict sugars *N*-acetylglucosamine (gray) and *N*-acetylmuramic acid (cyan). L-alanine (L-Ala), D-glutamine (D-Gln), meso-diaminopimelic acid (m-DAP) and D-alanine (D-Ala).

class. Subsequent studies have demonstrated that LDTs and DDTs of mycobacteria differ in their binding affinities to  $\beta$ -lactam subclasses and are consequently inhibited by different subclasses to varying degrees (Dubee et al., 2012; Kumar et al., 2017b). DDTs are effectively bound and inhibited by all  $\beta$ -lactam subclasses, whereas *Mab* LDTs are preferentially bound and inhibited by carbapenems and to a lesser extent by cephalosporins (Kumar et al., 2017a,b).

Although the crystal structures of LDTs of *Mab* bound to  $\beta$ -lactams are not yet available, several groups have reported crystal structures of LDTs of *Mtb* bound to carbapenems and penems (Kim et al., 2013; Li et al., 2013; Bianchet et al., 2017; Kumar et al., 2017b; Steiner et al., 2017). As LDTs and DDTs of *Mab* are differentially inhibited by  $\beta$ -lactam subclasses, comprehensive inhibition of PG synthesis will likely require simultaneous administration of multiple  $\beta$ -lactams belonging to different subclasses to optimally inhibit the two enzyme classes.

## FACTORS THAT DETERMINE POTENCY OF $\beta$ -LACTAMS AGAINST *Mab*

The major molecular factors limiting effectiveness of  $\beta$ -lactams against *Mab* are  $\beta$ -lactamase activity and the bacterial cell wall. Factors that commonly affect other antibiotic classes, including poor permeability of the cellular envelope, low affinity of antibiotic targets, drug efflux pumps, and chromosomally-encoded neutralizing enzymes, have been elegantly summarized elsewhere (Nessar et al., 2012; van Ingen et al., 2012).

### $\beta$ -Lactamases

The potent activity of the chromosomally-encoded  $\beta$ -lactamase, Bla<sub>Mab</sub>, is primarily responsible for poor efficacy of  $\beta$ -lactams against *Mab* (Soroka et al., 2014).  $\beta$ -lactamases hydrolyze the  $\beta$ -lactam ring, thereby inactivating these antibiotics (Kasik et al., 1971). Not only does Bla<sub>Mab</sub> degrade several  $\beta$ -lactams with significantly higher efficiency than BlaC of *Mtb*, Bla<sub>Mab</sub>

is not effectively inhibited by common  $\beta$ -lactamase inhibitors (BLI) clavulanate, tazobactam, and sulbactam (Soroka et al., 2017); agents that inhibit BlaC of *Mtb* (Wang et al., 2006). The observation that these BLIs do not reduce the minimum inhibitory concentration (MIC) of  $\beta$ -lactams against *Mab* in a whole-cell assay (Kaushik et al., 2017) is additional confirmation that  $\beta$ -lactamase activity in *Mab* is more robust than in *Mtb*. Subspecies *massiliense* harbors an additional  $\beta$ -lactamase, Bla<sub>Mmas</sub> (Ramirez et al., 2017).

Bla<sub>Mab</sub> is inactivated by avibactam (Dubee et al., 2015a), a recently-developed BLI whose core chemical composition differs from older BLIs and lacks a  $\beta$ -lactam ring (Coleman, 2011). Observations that avibactam reduces the MIC of several  $\beta$ -lactams against *Mab* provides further validation of its efficacy against both the Bla<sub>Mab</sub> protein and whole-cell *Mab* (Dubee et al., 2015a; Kaushik et al., 2017; Lefebvre et al., 2017). A recent study showed avibactam not only inhibits  $\beta$ -lactamases but also inhibits LDTs (Edoo et al., 2018). A recombinant *Mab* strain lacking *bla<sub>Mab</sub>* exhibited increased sensitivity to  $\beta$ -lactams and was rendered susceptible to amoxicillin and ceftaroline (Lefebvre et al., 2016). This study also observed that  $\beta$ -lactams plus avibactam exhibited similar efficacy against the parental *Mab* strain as compared to each drug alone against  $\Delta$ *bla<sub>Mab</sub>*, suggesting that avibactam fully inhibits Bla<sub>Mab</sub>. While Bla<sub>Mab</sub> and Bla<sub>Mmas</sub> hydrolyze penicillins and cephalosporins with similar efficacy, Bla<sub>Mmas</sub> also exhibits mild carbapenemase activity, a potential concern as it suggests an evolutionary movement toward  $\beta$ -lactamases with extended spectra (Ramirez et al., 2017). This study also noted that Bla<sub>Mmas</sub> is structurally similar to other acquired carbapenemases normally found in gram negative bacteria, such as KPC-2 and SFC-1. Avibactam activity against Bla<sub>Mmas</sub> has not yet been assessed and further study is warranted.

### Cell Wall

Mycobacteria possess an unusually thick cell wall composed of layers of complex hydrophobic molecules including fatty acids,

mycolic acids, lipoproteins, glycopeptidolipids (GPL), and largely insoluble PG and arabinogalactan layers. Although poorly understood in *Mab*, epigenetic factors generating differential levels of these molecules, especially GPLs, are associated with two distinct colony morphotypes—rough and smooth—within a clonal population. The rough morphotype tends to be associated with higher rates of antimicrobial resistance, including against  $\beta$ -lactams (Cangelosi et al., 1999; Greendyke and Byrd, 2008; Lavollay et al., 2014). Additionally, glycosylation of lipoproteins limits permeability of the cell wall to antibiotics that inhibit PG synthesis (Becker et al., 2017). Cell wall porins are also partially responsible for  $\beta$ -lactam resistance, as they allow transport of small hydrophilic molecules across the membrane, which interact with targets within the cytoplasm to potentially activate expression of drug resistance genes (Nguyen and Thompson, 2006; Nessar et al., 2012).

## ACTIVITY OF $\beta$ -LACTAMS AGAINST *Mab*

We identified thirty-five studies with documented MIC ranges of  $\beta$ -lactams against clinical isolates of *Mab* globally (Table 1). These data serve to highlight the high degree of variability in observed MIC ranges among clinical isolates, even within each study, and this variability is partially why standardized treatment regimens against *Mab* are often not practical in the clinical setting. Imipenem and ceftazidime were the most commonly tested  $\beta$ -lactams and nearly all studies included *Mab* strains that were resistant to these agents based on established MIC breakpoints (Woods et al., 2011). Only in four studies were all strains susceptible or intermediate to ceftazidime (Lee et al., 2012; Lavollay et al., 2014; Singh et al., 2014; Jeong et al., 2017). Two studies performed subspeciation and observed that all strains of subspecies *massiliense* and/or *bolletii* were either susceptible or intermediate to imipenem, whereas subspecies *abscessus* exhibited higher MICs to this drug (Lavollay et al., 2014; Singh et al., 2014). The reason for this is not currently known. Although seventeen studies also evaluated additional  $\beta$ -lactams, it is evident that this antibiotic class is largely understudied against *Mab*.

## FURTHER POTENTIATION OF $\beta$ -LACTAMS AGAINST *Mab* BY BLIs

Several studies have investigated the ability of BLIs to potentiate  $\beta$ -lactams against *Mab*, both *in vitro* and *in vivo*. The combination of amoxicillin and avibactam effectively reduced abscess formation and prolonged survival of zebrafish infected with *Mab* reference strain ATCC 19977 compared to amoxicillin alone (Dubee et al., 2015a). A subsequent study found that a combination of imipenem and avibactam also prolonged zebrafish survival compared to imipenem alone (Lefebvre et al., 2017). Avibactam also decreases the MIC of ceftaroline against *Mab* (Dubee et al., 2015b). Combinations of carbapenems and avibactam against clinical isolates of *Mab* showed that avibactam reduced MICs to therapeutically-achievable levels (Kaushik et al., 2017). The greatest MIC reductions were noted with tebipenem,

ertapenem, and panipenem; demonstrating that avibactam can successfully overcome  $\beta$ -lactamase activity and further suggests that carbapenems, especially those developed after imipenem, such as doripenem, biapenem and tebipenem, have untapped potential for use against *Mab* (Kaushik et al., 2017).

## SYNERGY STUDIES WITH $\beta$ -LACTAMS AND OTHER DRUGS

As combination regimens are essential for clinical management of *Mab* infections, several studies have evaluated antibiotic synergy against *Mab* with mixed results (Cremades et al., 2009; Shen et al., 2010; Bastian et al., 2011; Choi et al., 2012; van Ingen et al., 2012; Oh et al., 2014; Singh et al., 2014; Ferro et al., 2016; Mukherjee et al., 2017; Aziz et al., 2018; Pryjma et al., 2018; Schwartz et al., 2018; Zhang et al., 2018). *In vitro* studies have shown variable synergy of  $\beta$ -lactams in combination with other drugs. One study found no evidence of synergy among combinations of either imipenem or ertapenem with various other antibiotics (Cremades et al., 2009). However, another study reported high levels of synergy against *Mab* clinical isolates when clofazimine and amikacin were combined with several  $\beta$ -lactam subclasses (Schwartz et al., 2018). In a final study, rifampin combined with either doripenem or biapenem significantly reduced the MICs of both drugs to within therapeutic levels, compared with each carbapenem alone (Kaushik et al., 2015).

## DUAL $\beta$ -LACTAMS FOR *Mab*

Given that different subclasses of  $\beta$ -lactams target distinct aspects of mycobacterial cell wall biosynthesis, *Mab* regimens that contain two  $\beta$ -lactams from different subclasses may have high efficacy in *Mab*. As mentioned above, mycobacterial DDTs are inhibited by all  $\beta$ -lactams, whereas LDTs are preferentially inhibited by carbapenems and cephalosporins (Kumar et al., 2017a,b). A combination of cefdinir and doripenem was observed to be synergistic against *Mab* 19977 (Kumar et al., 2017a), demonstrating that dual  $\beta$ -lactams have therapeutic potential against *Mab*. This promising finding warrants further investigation into the effects of dual  $\beta$ -lactams against clinical isolates of *Mab*, further potentiation with BLIs, and additional *in vivo* studies.

## PRECLINICAL MODELS AND CLINICAL TRIALS

At least two groups have taken initiatives to develop animal models of *Mab* infection (Lerat et al., 2014; Obregon-Henao et al., 2015). Two studies have assessed efficacy of antibiotic treatment of mice infected with *Mab*, one of which included a  $\beta$ -lactam, ceftazidime. Lerat et al. assessed regimens containing clarithromycin, amikacin, or ceftazidime monotherapy vs. a three-drug combination in nude mice infected with *Mab* ATCC 19977. Ceftazidime monotherapy was equally effective as triple therapy, resulting in prolonged survival and reduced splenic bacillary

**TABLE 1 |** MIC range (ug/mL) for β-lactam antibiotics against clinical isolates of *M. abscessus*.

Description of <i>M. abscessus</i> clinical isolates	BIA	DOR	ERT	FAR	IPM	MEM	PAN	TEB	FEP	CMZ	FOX	CRO	AMC	References
3 isolates from US (TX)					8-64						16-512			Woods et al., 2000
8 isolates from Japan					8-16	8-16	4-16				16->32		>32	Ito et al., 2003
92 isolates from Taiwan					1->64	8->64					8-256			Yang et al., 2003
48 isolates from South Korea					1-64						16-128			Lee et al., 2007
167 isolates from Taiwan					<0.5->64					<1->32	<2->256			Huang et al., 2008
74 isolates from Korea					4->16						<16-64			Park et al., 2008
45 isolates from South Korea					2-64						4-128			Jeon et al., 2009
108 isolates from US (UT)		16->32	32->32		2->32	32->32								Chihara et al., 2010
40 isolates from Taiwan					1-256						16-256			Huang et al., 2010
3 isolates from India (Mumbai)					64						256	64	32	Set et al., 2010
37 isolates from US (TX)					4->16									Brown-Elliott et al., 2012
86 clinical isolates from Japan (63 subsp. abscessus, 23 massiliense)					2-64									Harada et al., 2012
6 isolates from Taiwan					4-64									
177 isolates from UK					32-64				>32		32-64	>64	>64	Lee et al., 2012
					<4->16				>32		<16->128	<8->64	16->32	Broda et al., 2013
					<4-16						8-16			Lavollay et al., 2014
					<4						8-32			
					<4-8						8-32			
143 isolates from Japan (90 subsp. abscessus, 53 bolletii)					2-64				16->256				8->256	Yoshida et al., 2013
70 isolates from China					1-64				1->256			>32	2->256	
30 isolates from Brazil (6 subsp. abscessus, 24 bolletii)					1-64						16-128			Zhuo et al., 2013
70 isolates from China (Beijing) (45 subsp. abscessus, 25 bolletii)					<0.5-16						32-256	>64	>64	Candido et al., 2014
14 isolates from Taiwan (4 subsp. abscessus, 10 bolletii)					16-32						32-256	>64	>64	Nie et al., 2014
67 isolates from France (42 subsp. abscessus, 21 massiliense, 24 bolletii)					16-64				32->32		4-64			
					4-32				>32		16->256			
					4-8						32-128	>64	>64	Lee et al., 2014
					4-16						32-64	>64	>64	Singh et al., 2014
											2-64			
											2-8			
											2-64			

(Continued)

**TABLE 1 |** Continued

Description of <i>M. abscessus</i> clinical isolates	BIA	DOR	ERT	FAR	IPM	MEM	PAN	TEB	FEP	CMZ	FOX	CRO	AMC	References
38 isolates from Australia (20 subsp. abscessus, 18 massiliense)					8->64 4->64				>32 >32		32->128 32->128	64->64 64->64	64->64 >64	Chua et al., 2015
3 isolates from US (MD)	6.25-12.5	3, 12-6, 25	>25	40-80	>25	>25	>80	40-80						Kaushik et al., 2015
55 isolates from China						<4->32				<16->64	<16->128		<8->32	Pang et al., 2015
313 isolates from Singapore					4->64						4->128			Tang et al., 2015
22 isolates from China					0.5-256						8-256			Li et al., 2016
78 isolates from US (TX) (67 subsp. abscessus, 11 massiliense)		4	4		8-16 8-16	8-16 8-16								Brown-Elliott et al., 2016
30 isolates from Iran					1-256	1-64					2-256			Heidarreh et al., 2016
165 isolates from France					4->64				16->32		8-128	8->64	2->64	Mougari et al., 2016
13 isolates from Japan				>2	2-16	8-64		4->4	32->64	8-64				Hatakeyama et al., 2017
20 isolates from South Korea (10 subsp. abscessus, 10 massiliense)					2-32 8-64						16-32 16-64			Jeong et al., 2017
28 isolates from US (MD)	16-128	8-128	64->256	64-256	4-32	8-128	32-256	128->256						Kaushik et al., 2017
67 isolates from Taiwan (28 subsp. abscessus, 38 massiliense, 1 bollettii)					8->64 4->64 32						16-128 16->128 64			Lee et al., 2017
28 isolates from US (MD)					4->64				16->32		16->128	32->64	32->64	Schwartz et al., 2018
64 isolates from US (FL)					<4->16						<16->128		16->32	Sfeir et al., 2018

BIA, biapenem; DOR, doripenem; ERT, ertapenem; FAR, faropenem; IPM, imipenem; MEM, meropenem; PAN, panipenem; TEB, tebipenem; FEP, ceftipime; CMZ, cefmetazole; FOX, cefoxitin; CRO, ceftriaxone; AMC, amoxicillin.

loads compared to untreated controls (Lerat et al., 2014). Several clinical trials assessing efficacy of non- $\beta$ -lactam antibiotics against NTMs have been undertaken (clinicaltrials.gov). To date, there are no published clinical trials that have specifically investigated  $\beta$ -lactams for the treatment of *Mab*; however, we are hopeful that an increasing awareness of  $\beta$ -lactams as viable treatment options may lead to clinical trials with this class in the future.

## FUTURE DIRECTIONS AND CONCLUSIONS

There is a dearth of research exploring  $\beta$ -lactams as potential treatments for *Mab*. Given the increasing prevalence of highly drug-resistant *Mab* isolates leading to poor clinical outcomes, new therapeutic approaches are needed to adequately treat these infections. Given our understanding of the differential mechanisms of  $\beta$ -lactam subclasses, and the ability of certain BLIs to overcome  $\beta$ -lactamase activity, currently-available  $\beta$ -lactams are a largely untapped resource for *Mab* treatment. Of the  $\beta$ -lactam subclasses, carbapenems/penems have the greatest activity against *Mab*, followed by cephalosporins, then penicillins. As noted above (Kumar et al., 2017a), it is likely that combinations of different  $\beta$ -lactam subclasses are required to fully inhibit PG synthesis in *Mab*. This insight may partially explain why prior studies evaluating  $\beta$ -lactams individually have not shown significant efficacy against this microbe. Further investigation may identify novel treatments utilizing combinations of  $\beta$ -lactams that optimally inhibit the distinct enzymatic targets present in *Mab*.

Appropriate selection of companion BLIs is another area in which  $\beta$ -lactams can be potentiated for use against *Mab*. Several studies have demonstrated efficacy of the BLI avibactam in inhibiting Bla<sub>Mab</sub> activity, which is a major factor contributing to the high MIC of most  $\beta$ -lactams against *Mab*. However, avibactam is currently only available as a coformulated combination with ceftazidime, which itself does not appear to

have activity against *Mab* (Dubee et al., 2015a; Kaushik et al., 2017). If avibactam were to be made available as an individual formulation, this would significantly increase its clinical usefulness, as regimens could be tailored to combine it with any  $\beta$ -lactam shown to be effective against a particular microbe or strain. Recently, two novel carbapenem-BLI combinations have been developed. These are meropenem-vaborbactam, which was recently FDA-approved for use against gram-negative organisms, and imipenem-relebactam, which is currently in phase II clinical trials (Zhanel et al., 2018). There are no published studies assessing efficacy of these BLIs against *Mab*, but their coformulation with carbapenems may confer greater potential for clinical use and further studies with these drugs are certainly warranted. It is possible that  $\beta$ -lactam-BLI combinations will become integral to effective treatment of drug-resistant *Mab* in the future. Additional animal studies as well as clinical trials with this drug class will be essential for the development of novel treatment regimens with improved clinical outcomes. Furthermore, repurposing already FDA-approved  $\beta$ -lactams for use against *Mab* may allow for expedited clinical implementation of regimens that show promise in preclinical models.

## AUTHOR CONTRIBUTIONS

ES-R, EM, KC, and GL discussed relevant literature. ES-R and KC focused on clinical aspects of the literature and EM focused on the basic biology. ES-R, EM, KC, and GL wrote the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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