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Biochemical Characterization of β-Lactamases from *Mycobacterium abscessus* Complex and Genetic Environment of the β-Lactamase-Encoding Gene

Ana Ramírez,[1] Melina Ruggiero,[2] Carlos Aranaga,[3] Angel Cataldi,[4] Gabriel Gutkind,[2] Jacobus H. de Waard,[5] María Araque,[1] and Pablo Power[2]

The objectives of this study were to determine the kinetic parameters of purified recombinant Bla_{Mab} and Bla_{Mmas} by spectrophotometry, analyze the genetic environment of the *bla*$_{Mab}$ and *bla*$_{Mmas}$ genes in both species by polymerase chain reaction and sequencing, furthermore, in silico models of both enzymes in complex with imipenem were obtained by modeling tools. Our results showed that Bla_{Mab} and Bla_{Mmas} have a similar hydrolysis behavior, displaying high catalytic efficiencies toward penams, cephalothin, and nitrocefin; none of the enzymes are well inhibited by clavulanate. Bla_{Mmas} hydrolyzes imipenem at higher efficiency than cefotaxime and aztreonam. Bla_{Mab} and Bla_{Mmas} showed that their closest structural homologs are KPC-2 and SFC-1, which correlate to the mild carbapenemase activity toward imipenem observed at least for BlaMmas. They also seem to differ from other class A β-lactamases by the presence of a more flexible Ω loop, which could impact in the hydrolysis efficiency against some antibiotics. A −35 consensus sequence (TCGACA) and embedded at the 3′ end of MAB_2874, which may constitute the *bla*$_{Mab}$ and *bla*$_{Mmas}$ promoter. Our results suggest that the resistance mechanisms in fast-growing mycobacteria could be probably evolving toward the production of β-lactamases that have improved catalytic efficiencies against some of the drugs commonly used for the treatment of mycobacterial infections, endangering the use of important drugs like the carbapenems.

Introduction

MYCOBACTERIUM ABSCESSUS COMPLEX is an emerging pathogen whose clinical incidence has significantly increased worldwide in the last decade.[1] It represents the most frequently encountered rapidly growing mycobacteria in human pathology, being mainly responsible for skin and soft tissue infections associated with medical and cosmetic procedures, as well as lung disease, particularly in patients with cystic fibrosis or chronic lung disease.[1]

The current nomenclature for *M. abscessus* complex comprises two subspecies, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*,[2] although a recent comparative genomic study supports the three previously recognized species, *M. abscessus*, *Mycobacterium massiliense*, and *Mycobacterium bolletii*.[3] For clarity, in this article, we will therefore differentiate the *M. abscessus* group in these three species.

Treatment of infections caused by *M. abscessus* complex is difficult, because these mycobacteria are intrinsically resistant to the classical antituberculous drugs and also to most of the antibiotics currently available.[4,5] The recommended therapeutic options include amikacin, linezolid, tigecycline, and the parenteral β-lactams, imipenem and cefoxitin. The latter have moderate *in vitro* activity. However, the *in vivo* efficacy of these antibiotics has been questioned.[1,4–6] Currently, clarithromycin is not recommended as first-line drug, due to the functional *erm*(41) gene conferring inducible resistance to macrolides detected in *M. abscessus* and *M. bolletii*.[4,6–8]

Natural resistance of *M. abscessus* to β-lactams is likely to result from the combination of several mechanisms. That is, impermeability of the cell wall, changes in the predominant transpeptidase involved in peptidoglycan synthesis, and β-lactamase production.[4,5]

[1]Universidad de Los Andes, Facultad de Farmacia y Bioanálisis, Laboratorio de Microbiología Molecular, Mérida, Venezuela.
[2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Resistencia Bacteriana, Buenos Aires, Argentina.
[3]Instituto Venezolano de Investigaciones Científicas, Centro de Microbiología y Biología Celular, Caracas, Venezuela.
[4]Instituto Nacional de Tecnología Agropecuaria, Centro de Investigación en Ciencias Veterinarias y Agronómicas, Instituto de Biotecnología, Castelar, Buenos Aires, Argentina.
[5]Universidad Central de Venezuela, Instituto de Biomedicina, Laboratorio de Tuberculosis, Caracas, Venezuela.

Production of an Ambler class A β-lactamase has already been reported in several *Mycobacterium* species, such as *Mycobacterium tuberculosis* (BlaC), *Mycobacterium fortuitum* (MFO), *Mycobacterium kansasii*, *M. phlei*, *Mycobacterium smegmatis* (BlaA), and *M. abscessus* (Bla_{Mab}). The corresponding genes are chromosomally encoded; and the spectrum of activity is variable depending on the species.[5,9–13]

M. abscessus genome analysis (accession number: CU45 8896.1) reveals the presence of the MAB_2875 gene (*bla*$_{Mab}$) encoding an Ambler class A β-lactamase (Bla_{Mab}).[11] This enzyme has little sequence diversity (≥96% amino acid identity) in 35 sequenced genomes of *M. abscessus* complex isolates. At amino acid level, BlaC and the enzyme encoded by *bla*$_{Mab}$ (Bla_{Mab}) have an identity of 48%. Therefore, these enzymes are distantly related.[10] According to Soroka *et al.*, Bla_{Mab} has a broad hydrolysis spectrum, similar to that of BlaC. However, Bla_{Mab} is not well inhibited by clavulanate, sulbactam, or tazobactam, because these β-lactamase inhibitors are efficiently hydrolyzed by this enzyme,[10] in contrast to BlaC, which is irreversibly acylated by clavulanate.[12]

Recently, it was reported that avibactam, a non-β-lactam β-lactamase inhibitor, is an efficient *in vitro* inhibitor of Bla_{Mab} by the reversible formation of a covalent adduct and is also intracellularly active in the zebrafish model. Therefore, these results strongly suggest that β-lactamase inhibition should be evaluated to provide improved therapeutic options for *M. abscessus* infections.[14]

There is scarce information in the scientific literature on Bla_{Mab} and β-lactamase of *M. massiliense* (Bla_{Mmas}). The purpose of this study was to determine the hydrolysis behavior of β-lactamases from clinical isolates of *M. abscessus* and *M. massiliense*, evaluate the phenotypic expression conferred by these enzymes in *Escherichia coli*, analyze the genetic environment of the *bla*$_{Mab}$ and *bla*$_{Mmas}$ genes in both species, and obtain predicted three-dimensional (3D) models for the enzymes.

Materials and Methods

Bacterial strains and cloning vectors

From a collection of 200 *M. abscessus* complex strains of Tuberculosis Laboratory, Instituto de Biomedicina, Universidad Central de Venezuela, in Caracas, *M. abscessus* LTC1499 and *M. massiliense* LTF756 isolates were randomly selected for further studies. They were isolated from skin infections associated with invasive cosmetic procedures. These strains were identified by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis and sequencing of *erm*(41) and *hsp65* genes, following previously described methodologies.[7,8,15,16] *E. coli* XL1-blue and *E. coli* BL21 (DE3) were used as recipient cells for transformation experiments. Carbenicillin-resistant pUC119 and kanamycin-resistant pET28a plasmid vectors were used for cloning and expression experiments, respectively.

Recombinant DNA methodology

The β-lactamase-encoding genes from both species were amplified from genomic DNA by PCR using primers MAB_2875f (5′-GGATCCATCTCTCGTCGCGCACTTC-3′) and MAB_2875r (5′-AAGCTTTCAAGCGCCGAAGGC CCGC-3′) to produce an 879-bp fragment. The PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen GmbH) and the identity of the gene, as well as the absence of aberrant nucleotides, was checked by double-strand sequencing (Model 3730XL; Applied Biosystems), using the same primers.

Basic recombinant DNA procedures were carried out as described by Sambrook *et al.*[17] The purified amplicon was ligated in a pUC119 vector and transformed in competent *E. coli* XL1-blue, following standard procedures. The resulting recombinant plasmid was digested with *Bam*HI (BioLabs) and *Hind*III (BioLabs), and the released insert was cloned in a pET28a vector. The ligation mixture was used to transform competent *E. coli* BL21 (DE3) cells, and recombinant clones were selected on Luria-Bertani (LB) agar plates supplemented with 30 μg/mL kanamycin. Positive recombinant clones (*E. coli* BL21pET28-*bla*$_{Mab}$ and *E. coli* BL21pET28-*bla*$_{Mmas}$) were screened by PCR with specific primers previously described.

Genetic environment of bla_{Mab} and bla_{Mmas}

The genetic organization of *bla*$_{Mab}$ and *bla*$_{Mmas}$ was investigated by PCR and by sequencing the regions surrounding these genes. The primers used for this purpose were 2874-75f (5′-ACCCGACCACCCAGTACAAG-3′) and 2874-75r (5′-GCGTCGCATCACACAGTTC-3′) (amplicon size, 676 bp) and 2875-76f (5′-GACGGCGATCTGGATACCTC-3′) and 2875-76r (5′-GCAGTCGGAACGGATCATC-3′) (amplicon size, 747 bp). PCR conditions for both the 2874–75 and 2875–76 amplifications were 5 min at 95°C, followed by 35 cycles at 95°C for 60 sec, 60°C for 60 sec, and 72°C for 60 sec.

Putative gene promoter sequences (−35) were recognized using the BPROM program (http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb).

DNA sequencing and analysis

PCR products were purified using the AccuPrep Gel Extraction Kit (Bioneer) and sequenced in both strands with the corresponding forward and reverse primers on an Applied Biosystems 3730XL Genetic Analyzer (Macrogen, Inc.). Sequences were analyzed using the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/) and the European Bioinformatics Institute (www.ebi.ac.uk/) analysis tools. Sequences were compared with *M. abscessus* ATCC 19977 (GenBank accession number CU458896.1).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of β-lactams were determined in cation-adjusted Mueller–Hinton medium by the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, using 96-well microtiter plates,[18] which were incubated for 3 days at 30°C. *Staphylococcus aureus* ATCC 29213 and *E. coli* ATCC 25922 were used as control strains.

For *E. coli* BL21pET28-*bla*$_{Mab}$ and *E. coli* BL21pET28-*bla*$_{Mmas}$, the MIC was determined by a modification of the methodology described above, by supplementation of the cultures with 0.5 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) for β-lactamase induction.

Production and purification of β-lactamases

Cultures of *E. coli* BL21pET28-*bla*$_{Mab}$ and *E. coli* BL21pET28-*bla*$_{Mmas}$ were grown in LB broth supplement with 30 μg/mL kanamycin and incubated at 37°C until an optical density of 0.8 at 600 nm. Induction was performed with 0.5 mM IPTG at 16°C for 18 hr. Cells were harvested by centrifugation at 5,000 rpm at 4°C and lyzed by sonication. Clear supernatants were loaded onto a HisTrap HP affinity column (GE Healthcare) connected to an AKTA purifier (GE Healthcare) and eluted with 250 mM imidazole in 50 mM Tris-NaCl, pH 7.5. The eluted fractions were dialyzed against 1% phosphate buffer, pH 7.4 and the enzymes (Bla$_{Mab}$ and Bla$_{Mmas}$) were preserved at −20°C.

Determination of kinetic parameters

Steady-state kinetic parameters were determined using a T80 UV/Vis spectrophotometer (PG Instruments Ltd.). Reactions were performed in a total volume of 500 μL at 25°C. For good substrates, the steady-state kinetic parameters (K_m and k_{cat}) were determined under initial rate as described previously,[19] with nonlinear least-squares fitting of the data (Henri Michaelis–Menten equation) using GraphPad Prism 5.03 for Windows (GraphPad Software).

In cases of low K_m values, or for poor substrates and inactivators, apparent K_m values were determined as competitive inhibitor constants (K_i) by monitoring the residual activity of the enzyme in the presence of the drug and 150 μM ampicillin as a reporter substrate, while the k_{cat} for poor substrates was determined by analyzing the complete hydrolysis time courses.[20] Corrected K_i (considered as the observed or apparent K_m) value is finally determined using the equation: $K_i = K_{i\ obs}/(1 + [S]/K_{m(S)})$, where $K_{m(S)}$ and [S] are the reporter substrate's K_m and fixed concentration used, respectively.

For irreversible inhibitors, the inactivation rate constant, k_{inact}, was measured directly by time-dependent inactivation of the β-lactamases in the presence of different concentrations of the tested inhibitor, a fixed concentration of enzyme, and 200 μM nitrocefin as reporter. The observed rate constant for inactivation (k_{obs}) was determined by nonlinear least-squares fitting of the data using OriginPro 8.0.[21]

Tested drugs, as well as the wavelengths and extinction coefficients used, were the same as those previously described.[22]

Protein structure prediction and 3D modeling

A prediction of the α-helix content of *bla*$_{Mab}$ and *bla*$_{Mmas}$ was performed with Agadir software,[23] and theoretical 3D models for the enzymes were predicted using the Swiss-Model tool (http://swissmodel.expasy.org). Simulation modeling of Bla$_{Mab}$ and Bla$_{Mmas}$ in association with imipenem was achieved with AutoDock Vina,[24] using SFC-1 structure as template (PDB 4EQI). All models were visualized with the PyMOL Molecular Graphics System, version 1.6.

Results and Discussion

Identification of strains based on the erm*(41) and* hsp65 *gene sequences*

Based on the analysis of promoter sequences of the *erm*(41) gene, the LTC1499 and LTF756 strains were identified as *M. abscessus* and *M. massiliense*, respectively (GenBank accession numbers KP702822 and KP702846, respectively).[16] In addition, the *hsp65* gene of LTC1499 and LTF756 strains had an identity of 100% and 99%, respectively, with the *hsp65* gene of *M. abscessus* ATCC 19997 (GenBank accession numbers CU458896.1).

Antimicrobial susceptibility

Antimicrobial susceptibility is shown in Table 1. Both clinical isolates were resistant to all tested β-lactams, except imipenem, for which *M. abscessus* LTC1499 and *M. massiliense* LTF756 were moderately susceptible and susceptible, respectively. These results are in agreement with the previously reported phenotypic features of these species.[5,6,10]

MIC values for *E. coli* BL21pET28-*bla*$_{Mab}$ and *E. coli* BL21pET28-*bla*$_{Mmas}$ clones are also shown in Table 1. Bla$_{Mab}$ and Bla$_{Mmas}$ were functional in *E. coli* BL21, and resistance profiles were in agreement with the kinetic parameters determined *in vitro*. These results are similar to those reported by Soroka *et al.*[10]

TABLE 1. *IN VITRO* SUSCEPTIBILITY OF CLINICAL ISOLATES OF SPECIES OF *MYCOBACTERIUM ABSCESSUS* COMPLEX AND DERIVED *ESCHERICHIA COLI* BL21 RECOMBINANT CLONES

	MIC (μg/mL)						
Antibiotic	E. coli *ATCC 25922*[a]	S. aureus *ATCC 29213*[a]	M. abscessus *LTC1499*	Mycobacterium massiliense *LTF756*	E. coli *BL21/ pET28*-bla$_{Mab}$	E. coli *BL21/ pET28*-bla$_{Mmas}$	E. coli *BL21/ pET28*
Amoxicillin	4	nd	32	256	>128	>128	2
Penicillin	ND	1.0	>512	>512	>256	>256	16
Piperacillin/tazobactam	2/4	4/4	>256	>256	8	4	2
Cephalothin	16	0.5	>256	>256	>128	128	4
Cefoxitin	4.0	4.0	64	128	4	4	4
Ceftazidime	0.5	8.0	>128	>128	0.006	0.006	0.006
Cefotaxime	0.12	2.0	>128	>128	0.006	0.006	0.006
Imipenem	0.5	0.06	8	4	0.5	0.5	0.5
Aztreonam	0.25	nd	>32	32	0.003	0.003	0.003

[a]Control strains and the MIC values obtained were within the range established by the CLSI to validate the assay.
CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration; nd, not determined.

PCR screening, sequencing of MAB_2875, and cloning

A ~850-bp amplicon was obtained by PCR screening with MAB_2875-specific primers for both clinical isolates. DNA sequencing of both amplicons revealed 99% nucleotidic identity compared to the reference sequence of *M. abscessus* ATCC 19997.

The inserts from recombinant plasmids pET28-bla_{Mab} and pET28-bla_{Mmas} were sequenced in full size for both strands. Analysis of pET28-bla_{Mab} for coding regions confirmed the presence of bla_{Mab} and bla_{Mmas}. Encoded β-lactamases differ from each other in only two amino acids: Glu38 and Thr140 in Bla_{Mab} are replaced by Val38 and Ala140 in Bla_{Mmas} (according to Ambler's class A β-lactamase numbering scheme). Another difference is a 14-residue deletion at the C-terminus in Bla_{Mmas}. Nevertheless, these differences are located in regions of the folded proteins that do not seem to have major influence in both the enzyme activity and structural properties.

Genetic environment of bla_{Mab} *and* bla_{Mmas}

Figure 1 shows the architecture of the bla_{Mab} and bla_{Mmas} encoding genes and neighboring sequences covering ~1,500 bp. One hundred nucleotides downstream the bla_{Mab} and bla_{Mmas} genes, there is a partial open reading frame (281 bp) of the MAB_2876 gene that encodes a probable GTP pyro-phosphokinase with 99% nucleotide identity with the reference strain's gene (GenBank accession number CU458896.1). Partial sequence of the MAB_2874 gene (247 bp), encoding a putative peptidyl-prolyl cis/trans isomerase, is located upstream the *bla* gene.

We also deduced a putative −35 consensus sequence (TCGACA) separated by 19-bp from the putative −10 sequence (GGCCAAGAT) and embedded at the 3′ end of MAB_2874, which may constitute the bla_{Mab} and bla_{Mmas} promoter.

DNA sequences were deposited in GenBank under accession numbers KT159981, KU244311 (*M. abscessus* LTC1499) and KT159982, KU244312 (*M. massiliense* LTF756).

Bla_{Mab} *and* Bla_{Mmas} *hydrolytic activity*

A total of 1.08 mg/mL ($3.68.10^{-5}$ M) and 1.34 mg/mL ($4.83.10^{-5}$ M) of Bla_{Mab} and Bla_{Mmas}, respectively, were obtained after purification. The main kinetic parameters of both β-lactamases are shown in Table 2. According to its activity spectrum, Bla_{Mab} showed high catalytic efficiency ratios (k_{cat}/K_m) for all tested penams, cephalothin and nitrocefin, the two latter being the best substrates. The same behavior was observed for Bla_{Mmas}, with the exception of ampicillin, for which it displayed a threefold lower k_{cat}/K_m ratio compared to Bla_{Mab} (characterized by high K_m and low k_{cat}). The most poorly hydrolyzed antibiotics by both β-lactamases were cefuroxime, cefotaxime, and aztreonam.

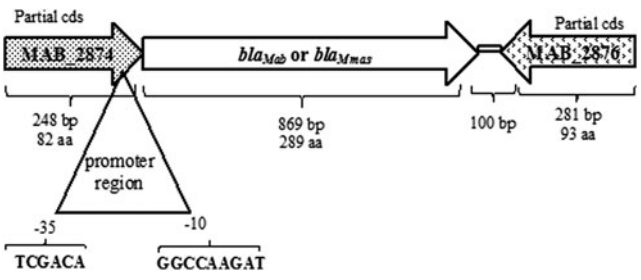

FIG. 1. Schematic representation of the bla_{Mab} gene and neighboring sequences.

The differences (two to nine times lower) observed in our data in comparison to previously reported k_{cat}/K_m values for Bla_{Mab} with penicillin, ampicillin, cefuroxime, and cefotaxime[10] may be considered within the expected error margin. These observed variations could reflect differences in enzyme preparation procedures and assay conditions, which may influence protein's purity, folding, and metal content.

In contrast, the catalytic efficiency toward aztreonam was 310 times lower compared to previously reported results, due to low k_{cat} (0.06 sec^{-1}) and K_m (400 μM) values.[10] These results are in agreement with the MICs of aztreonam in recombinant clones (Table 1) and suggest that the monobactam behaves as a very poor substrate or even as an inhibitor. The observed resistance in the mycobacteria isolates is probably due to the coexistence of additional resistance mechanisms.

Another interesting finding was the relatively rapid hydrolysis observed for both enzymes (Bla_{Mab} and Bla_{Mmas}) toward piperacillin (ureido-penicillin), usually considered as ''stable'' β-lactams.[9]

Cefoxitin, considered as one of the most active β-lactams against *M. abscessus* infections,[6,10] gave unmeasured catalytic efficiencies due to negligible k_{cat} values and fairly good affinities ($K_m = 62$ and 154 μM for Bla_{Mab} and Bla_{Mmas}, respectively), in agreement to previous results indicating that the methoxy group of cefoxitin may be similarly critical to protect this drug from being hydrolyzed by Bla_{Mab}. This would account for the antibacterial activity and *in vivo* efficacy of cefoxitin against β-lactamase–producing *M. abscessus*.[10] In addition, both β-lactamases gave undetectable hydrolysis of ceftazidime in complete time course kinetics, even after using high amounts of enzyme. Therefore, both k_{cat} and k_{cat}/K_m could not be determined under these conditions, and only K_m values were obtained by the reporter substrate method, suggesting that both enzymes have a very poor activity toward this oxyimino-cephalosporin. Molecular modeling studies and structural analyses show that substitutions of the β-lactam ring by a methoxy group block the activity of class A β-lactamases.[25]

Interestingly, Bla_{Mmas} was found to display a moderate activity toward imipenem. The catalytic efficiency (k_{cat}/K_m) toward imipenem was nearly 10-fold higher than that for cefotaxime and aztreonam, and it is characterized by low K_m and k_{cat} values. For other mycobacterial β-lactamases such as BlaC (*M. tuberculosis*), carbapenems are slow substrates that acylate the enzyme but are only slowly deacylated and can therefore act also as potent inhibitors.[12,26] *In vivo* studies showed that meropenem, in combination with the β-lactamase inhibitor clavulanate, behaves as a bactericidal agent against clinical TB strains that are phenotypically extensively drug resistant (XDR-TB).[26]

In contrast, clavulanic acid behaved as a weak inhibitor of Bla_{Mab} and Bla_{Mmas} compared to other class A β-lactamases, due to relatively high K_i values and low inhibition rate constants k_{inact}, yielding low inhibition efficiency values (k_{inact}/K_i).

TABLE 2. MAIN KINETIC PARAMETERS OF BLA_{MAB} AND BLA_{MMAS}

	Bla_{Mab} (M. abscessus *LTC1499*)				*Bla_{Mmas}* (M. massiliense *LTF756*)			
Substrate	K_m (μM)	k_{cat} (sec^{-1})	k_{cat}/K_m ($\mu M^{-1}.sec^{-1}$)	*Relative* k_{cat}/K_m (%)	K_m (μM)	k_{cat} (sec^{-1})	k_{cat}/K_m ($\mu M^{-1}.sec^{-1}$)	*Relative* k_{cat}/K_m (%)
Ampicillin	482 ± 63	115 ± 7	0.24 ± 0.05	19	774 ± 214	65 ± 10	0.08 ± 0.02	8
Benzyl-penicillin	510 ± 77	230 ± 14	0.45 ± 0.09	35	1,109 ± 128	313 ± 22	0.28 ± 0.05	29
Piperacillin	194 ± 15	81 ± 4	0.42 ± 0.05	33	108 ± 18	48 ± 4	0.44 ± 0.12	46
Nitrocefin	29 ± 4	72 ± 2	2.49 ± 0.39	196	93 ± 31	89 ± 9	0.96 ± 0.38	100
Cephalothin	20 ± 3	27 ± 1	1.27 ± 0.24	**100**	17 ± 3	16 ± 1	0.96 ± 0.22	**100**
Cefuroxime[a]	7 ± 0.8	0.1 ± 0.02	0.01 ± 0.002	0.8	5 ± 0.7	0.02 ± 0.007	0.005 ± 0.0017	0.5
Ceftazidime[a]	73 ± 21	nd	nd	—	52 ± 12	nd	nd	—
Cefotaxime[a]	135 ± 40	0.04 ± 0.008	0.0003 ± 0.0001	0.2	69 ± 7	0.03 ± 0.002	0.0004 ± 0.00005	0.04
Cefoxitin[a]	62 ± 12	nd	nd	—	154 ± 24	nd	nd	—
Aztreonam[a]	400 ± 120	0.06 ± 0.008	0.0002 ± 0.00007	0.02	122 ± 36	0.06 ± 0.008	0.0005 ± 0.00018	0.05
Imipenem[a]	43 ± 8	nd	nd	—	49 ± 8	0.15 ± 0.02	0.003 ± 0.0004	0.3
Inhibitor	k_{inact} (sec^{-1})	K_i (μM)	k_{inact}/K_i ($\mu M^{-1}.sec^{-1}$)		k_{inact} (sec^{-1})	K_i (μM)	k_{inact}/K_i ($\mu M^{-1}.sec^{-1}$)	
Clavulanic acid	0.0086 ± 0.0014	2.3 ± 0.7	0.0037 ± 0.0014		0.0068 ± 0.0004	2.23 ± 0.33	0.003 ± 0.0007	

All relative catalytic efficiencies (%) were referred to cephalothin (boldface value).
[a]K_m constants were determined as K_i obs by competitive assays with reporter substrates.

Clavulanate is a mechanism-based inhibitor of BlaC; therefore, Bla_{Mab}, Bla_{Mmas}, and BlaC have opposite behaviors with respect to their interaction with β-lactamase inhibitors.[10,12]

Recently, it was reported that avibactam is an efficient inhibitor, and combinations with imipenem and cefoxitin could be considered as potential therapeutic options for *M. abscessus* infections, which should be studied in more detail.[14] In addition, it has been reported that the combination of avibactam with ceftaroline can be used for treatment of pulmonary infections due to clarithromycin-resistant *M. abscessus*.[27]

Bla_{Mab} *and* Bla_{Mmas} *structure prediction and 3D modeling*

Figure 2 shows the predicted 3D models of Bla_{Mab} and Bla_{Mmas} β-lactamases from *M. abscessus* LTC1499 and *M. massiliense* LTF756, respectively. The overall folding, and the conserved residues, which constitute the active site of both enzymes, seem to be equivalent to that for class A β-lactamases, being the most relevant difference the probable presence of a more flexible Ω loop, which could have influence in the accommodation and further hydrolysis of some antibiotics.

The 3D models of both enzymes showed that the closest structural homologs are the carbapenemases KPC-2 (50% amino acid identity with both Bla β-lactamases) and SFC-1 (52% amino acid identity with both enzymes), which correlate to the mild carbapenemase activity toward imipenem observed at least for Bla_{Mmas} (Table 2). KPC-2 and SFC-1 are Ambler class A enzymes capable of hydrolyzing penicillins, cephalosporins, aztreonam, and carbapenems (imipenem, meropenem, doripenem, and ertapenem). In addition, these enzymes are weakly inhibited by clavulanic acid and tazobactam.[28,29]

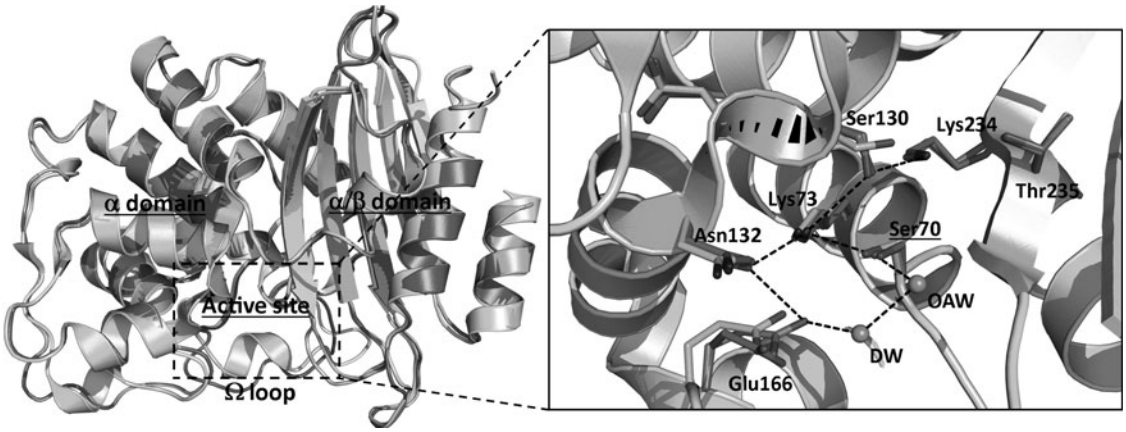

FIG. 2. 3D models of Bla β-lactamases from *Mycobacterium abscessus* (Bla_{Mab}) and *Mycobacterium massiliense* (Bla_{Mmas}). *Left panel*: overall structure of the β-lactamases, showing the conservation of both α and α/β domains and the presence of the Ω loop. *Right panel*: detail of the active site of Bla β-lactamases, showing the putative hydrogen bonding network between essential residues and the hypothetical positions of both the oxyanion hole (OAW) and deacylating water (DW) molecules, based on the structure of SFC-1 β-lactamase (PDB 4EQI). 3D, three dimensional.

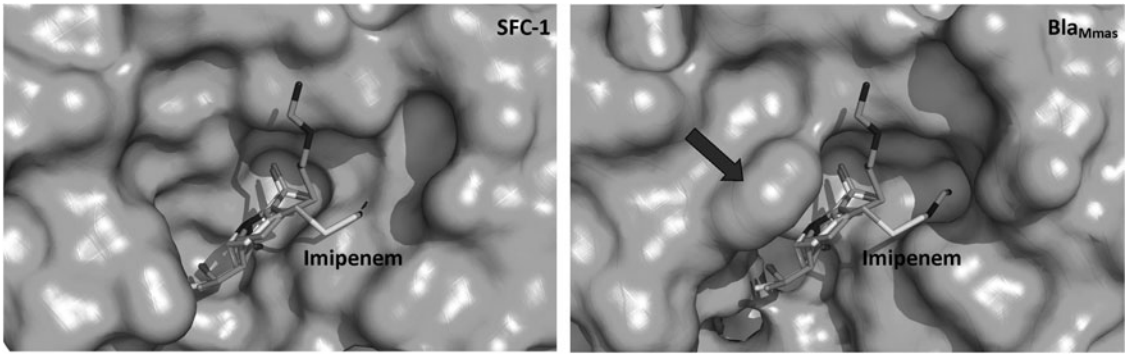

FIG. 3. Simulation models of the association of imipenem with SFC-1 carbapenemase (*left*) and Bla_{Mmas} from *M. massiliense* LTF756 (*right*). The β-lactamases are shown as surface models for better visualization of dimensions of the active site cavities. In Bla_{Mmas}, an *arrow* shows a reduction in the active site's entrance. Imipenem was modeled in the two most favorable conformations for both β-lactamases.

Structural models of both *Bla* enzymes from mycobacteria in association with imipenem, in the two most favorable conformations, were compared with SFC-1 carbapenemase (Fig. 3). According to these models, it seems that the β-lactamases from *Mycobacterium* offer a more restricted entry to the active site cavity in contrast to SFC-1, partly due to a local accumulation of bulky residues pointing toward its entrance. These structural differences could partially explain the differences observed in the kinetic profiles, and it could be also correlated with the stronger carbapenemase activity in SFC-1 in comparison to mycobacteria β-lactamases.

Conclusions

In this study, we demonstrate that the hydrolytic behavior of Bla_{Mab} and Bla_{Mmas} varies in comparison with β-lactamases from *Mycobacterium* species, and even from other non-mycobacterial class A β-lactamases. In addition, Bla_{Mab} and Bla_{Mmas} showed structural homology with carbapenemases like KPC-2 and SFC-1, which could be associated with the mild carbapenemase activity observed at least for Bla_{Mmas}.

Our results suggest that the resistance mechanisms in fast-growing mycobacteria could be probably evolving toward the production of β-lactamases having improved catalytic efficiencies against some of the drugs commonly used for the treatment of mycobacterial infections, endangering the use of important drugs like the carbapenems.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Ana Ramírez, MSc
Laboratorio de Microbiología Molecular
Facultad de Farmacia y Bioanálisis
Universidad de Los Andes
Prolongación Av. Humberto Tejera
Edif. Gonzalo González
Mérida 5101
Venezuela

E-mail: ramirezana@ula.ve