AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Editor's Pick | Antimicrobial Chemotherapy | Commentary

The metallo-β-lactamases strike back: emergence of taniborbactam escape variants

Pranita D. Tamma,[1] **Jose M. Munita**[2]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Metallo-β-lactamases (MBLs) have evolved relatively rapidly to become an international public health threat. There are no clinically available β-lactamase inhibitors with activity against MBLs. This may change with the introduction of cefepime-taniborbactam. Herein, we review three manuscripts (S. I. Drusin, C. Le Terrier, L. Poirel, R. A. Bonomo, et al., Antimicrob Agents Chemother 68:e01168-23, 2024, https://doi.org/10.1128/aac.01168-23; C. Le Terrier, C. Viguier, P. Nordmann, A. J. Vila, and L. Poirel, Antimicrob Agents Chemother 68:e00991-23, 2024, https://doi.org/10.1128/aac.00991-23; D. Ono, M. F. Mojica, C. R. Bethel, Y. Ishii, et al., Antimicrob Agents Chemother 68:e01332-23, 2024, https://doi.org/10.1128/aac.01332-23) in which investigators describe elegant experiments to explore MBL/taniborbactam interactions and modifications to MBLs, in response, to reduce the affinity of taniborbactam. Challenges with MBL inhibition will not disappear; rather, they will evolve commensurate with advancements in medicinal chemistry.

KEYWORDS metallo-β-lactamases, NDM, MBL, VIM, β-lactamase inhibitors, IMP

Metallo-β-lactamases (MBLs) are adept at evolving and have developed a formidable international footprint (1). They are divided into three subclasses (i.e., B1, B2, and B3) based broadly on differences in amino acid sequences at active sites, loop architecture, substrate profiles, and zinc content and ligands (2, 3). However, MBLs are highly divergent even within subclasses (2). The B1 subclass of MBLs is arguably the most clinically relevant; B1 MBLs are largely plasmid borne and can readily be transferred between bacterial strains (2). New Delhi Metallo-β-lactamases (NDM), Verona Integron Metallo-β-lactamases (VIM), and Imipenemases (IMP) are the most problematic members of the B1 subclass.

MBLs present a major public health threat through their production by both Enterobacterales and non-fermenting Gram-negative organisms. This is exemplified by bla_{NDM} which was first identified in 2008 in *Escherichia coli* and *Klebsiella pneumoniae* isolates from a Swedish patient returning from New Delhi, India (4). Since then, NDMs have become widespread around the world—achieving a reach far beyond health care settings—and have spread to virtually every continent (5). Similarly, bla_{VIM} was first identified in 1997 in Verona, Italy and has also spread internationally. As an example, VIM-producing *Pseudomonas aeruginosa* comprises upward of 20% of carbapenem-resistant *P. aeruginosa* in Latin America—paralleling estimates from Italy (6).

Several attributes make MBLs particularly concerning when compared to the serine carbapenemases (e.g., *Klebsiella pneumoniae* carbapenemases). First, MBLs have the ability to readily hydrolyze virtually all β-lactam antibiotics, with the notable exception of aztreonam. Second, their spread has been largely unfettered—mostly due to inadequate infection prevention measures—but also in part because of the lack of clinically available β-lactamase inhibitors with activity against MBLs. Third, MBLs are identified in both environmental and nosocomial reservoirs, making them somewhat ubiquitous.

Editor Cesar A. Arias, Houston Methodist Academic Institute, Houston, Texas, USA

Address correspondence to Pranita D. Tamma, ptamma1@jhmi.edu.

The authors declare no conflict of interest.

See the funding table on p. 3.

For the Le Terrier et al. article discussed, see https://doi.org/10.1128/aac.00991-23.
For the Drusin et al. article discussed, see https://doi.org/10.1128/aac.01168-23.
For the Ono et al. article discussed, see https://doi.org/10.1128/aac.01332-23.

Published 4 January 2024

Additionally, both MBL variants with increasing affinity for zinc, higher stability under zinc-limiting conditions, or lower zinc requirements are emerging, enabling them to thrive even in settings of relative zinc scarcity, which is common in states of human infection (7–11). To this point, the NDM-15 variant has evolved to function with a single zinc-binding site, rather than the more typical dual zinc-binding sites (8). Finally, new MBL variants are being described at an impressive frequency, highlighting the remarkable adaptability of these enzymes. To date, more than 60, 80, and 100 variants have been described in the NDM, VIM, and IMP families, respectively (12).

Recently, three intriguing manuscripts were published in *Antimicrobial Agents and Chemotherapy,* which describe the role of taniborbactam (13), a bicyclic boronate β-lactamase inhibitor, and the factors that appear to both promote and limit its ability to inactivate some of the most clinically relevant MBLs (14–16). The potential availability of taniborbactam in the near future is very welcome news as we are in dire need of MBL-active β-lactamase inhibitors. However, reports of NDM-variants (e.g., NDM-9) and VIM-variants (e.g., VIM-83) with single amino acid substitutions making them less susceptible to inhibition by taniborbactam temper some enthusiasm (15, 17). Regrettably, taniborbactam is also inefficient at inhibiting IMP-type MBLs, a significant contributor to carbapenemase production in the Far East and Australia (18).

In one of the three aforementioned manuscripts, Drusin and colleagues sought to better elucidate the molecular basis by which certain MBLs (including IMP-1) are not inhibited by taniborbactam (14). Using elegant docking simulations and electrostatic surface calculations, they advanced the notion that "MBL escape variants" can arise from changes in electrostatic features due to single amino acid substitutions in MBL active site loops, leading to ineffective binding of taniborbactam (14). For example, the replacement of glutamic acid for a positively charged lysine in position 149 in NDM-9 decreases the negative charge that interacts with the amine group in the side chain of taniborbactam, reducing the affinity of taniborbactam for NDM-9 (14).

In the second manuscript, Ono et al. investigated the role of amino acid substitutions in K224 (16). K224 appears to serve as an anchoring residue by forming two hydrogen bonds with taniborbactam (19, 20). Through construction of a library of NDM-1 variants at position 224 by site-saturation mutagenesis, they found that a specific variant, K224I, conferred a comparable level of resistance to cefepime-taniborbactam as to cefepime, indicating that the inhibitory activities of taniborbactam were unable to rescue cefepime from this variant NDM (16). The microbiological and biochemical findings were supported by structural modeling and docking simulations. Their simulations reveal how specific single amino acid substitutions in NDM-1 decrease the inhibitory activity of taniborbactam despite only a modest increase in the inhibitor constant Ki.

Finally, Le Terrier and colleagues assessed the inhibitory potential of taniborbactam against a broad range of MBLs from all three subclasses through measurements of minimum inhibitory concentrations of taniborbactam-β-lactam associations, hydrolytic inhibitory concentrations of taniborbactam, and site-directed mutagenesis (15). Fortunately, taniborbactam was able to inhibit the activity of most members of the B1 subclass; notable exceptions being SIM-1 (i.e., Seoul Imipenemase 1) and a few specific NDM and VIM variants (i.e., NDM-9, NDM-30, and VIM-83), which all differ from NDM-1 or VIM-1 by single amino acid substitutions. Additionally, these investigations bolster previous findings, indicating that members of the B3 subclass appear to be able to escape the activity of taniborbactam, and taniborbactam activity against the B2 subclass is variable (19).

The findings of these three investigations highlight the critical need to support the development of new β-lactamase inhibitors with activity against MBLs and the necessity of laboratory-directed studies targeted to understand structure activity relationships as new inhibitors are being developed. However, the quest to identify effective MBL inhibitors poses challenges for several reasons. In contrast to serine β-lactamases, the active site in MBLs is in a shallow groove with only a few contact points to bind inhibitors

(21). Moreover, the large structural diversity across MBLs with relatively low homology among active site residues poses challenges (21). Additionally, MBLs belong to a much larger superfamily of metalloproteins with diverse biological functions beyond β-lactam hydrolysis; members share a common protein fold resulting in similarities at active sites (22). Therefore, achieving the fine balance of developing effective broad spectrum MBL inhibitors that remain selective enough to avoid toxicity though inhibition of off-target enzymes remains challenging.

Based upon the available preclinical and clinical data and the favorable results of a clinical trial of 436 adults with urinary tract infections (23), cefepime-taniborbactam will likely be the first clinically available β-lactam-β-lactamase inhibitor with targeted activity against the most common MBLs in the B1 subgroup. Of note, patients with MBL-producing infections were excluded from the trial. Taniborbactam may be soon followed by xeruborbactam, another bicyclic boronate (24). Presumably, distinct amino acid substitutions will be identified that will challenge xeruborbactam and other inhibitors in development. Only through meticulous experiments focused on exploring substrate/inhibitor interactions will we be able to define the precise niche of new inhibitor combinations as they are introduced into the clinic. The challenges of overcoming MBL inhibition will not disappear but will evolve commensurate with advancements in medicinal chemistry. We applaud the investigators of the aforementioned studies in *Antimicrobial Agents and Chemotherapy* for their innovative work in advancing the science of MBLs and effective inhibitors.

ACKNOWLEDGMENTS

Neither author has any disclosures. P.D.T. was funded by the National Institutes of Health (R21-AI173475).

AUTHOR AFFILIATIONS

[1]Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[2]Genomics and Resistant Microbes Group, Instituto de Ciencias e Innovación en Medicina, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile

AUTHOR ORCIDs

Pranita D. Tamma http://orcid.org/0000-0002-4143-6324
Jose M. Munita http://orcid.org/0000-0002-7870-1056

FUNDING

Funder	Grant(s)	Author(s)
HHS | National Institutes of Health (NIH)	R21AI173475	Pranita D. Tamma

REFERENCES

1. Mojica MF, Rossi MA, Vila AJ, Bonomo RA. 2022. The urgent need for metallo-beta-lactamase inhibitors: an unattended global threat. Lancet Infect Dis 22:e28–e34. https://doi.org/10.1016/S1473-3099(20)30868-9
2. López C, Delmonti J, Bonomo RA, Vila AJ. 2022. Deciphering the evolution of metallo-beta-lactamases: a journey from the test tube to the bacterial periplasm. J Biol Chem 298:101665. https://doi.org/10.1016/j.jbc.2022.101665
3. Bush K. 2013. The ABCD's of beta-lactamase nomenclature. J Infect Chemother 19:549–559. https://doi.org/10.1007/s10156-013-0640-7
4. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. 2009. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53:5046–5054. https://doi.org/10.1128/AAC.00774-09
5. Dortet L, Poirel L, Nordmann P. 2014. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int 2014:249856. https://doi.org/10.1155/2014/249856
6. Reyes J, Komarow L, Chen L, Ge L, Hanson BM, Cober E, Herc E, Alenazi T, Kaye KS, Garcia-Diaz J, et al. 2023. Global epidemiology and clinical outcomes of carbapenem-resistant *Pseudomonas aeruginosa* and associated carbapenemases (POP): a prospective cohort study. Lancet Microbe 4:e159–e170. https://doi.org/10.1016/S2666-5247(22)00329-9
7. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, Anderson KL, Dattilo BM, Dunman PM, Gerads R, Caprioli RM, Nacken W, Chazin WJ, Skaar EP. 2008. Metal chelation and inhibition of bacterial growth in

tissue abscesses. Science 319:962–965. https://doi.org/10.1126/science.1152449
8. Cheng Z, Thomas PW, Ju L, Bergstrom A, Mason K, Clayton D, Miller C, Bethel CR, VanPelt J, Tierney DL, Page RC, Bonomo RA, Fast W, Crowder MW. 2018. Evolution of New Delhi metallo-beta-lactamase (NDM) in the clinic: effects of NDM mutations on stability, zinc affinity, and mono-zinc activity. J Biol Chem 293:12606–12618. https://doi.org/10.1074/jbc.RA118.003835
9. Bahr G, González LJ, Vila AJ. 2022. Metallo-beta-lactamases and a tug-of-war for the available zinc at the host-pathogen interface. Curr Opin Chem Biol 66:102103. https://doi.org/10.1016/j.cbpa.2021.102103
10. González LJ, Bahr G, González MM, Bonomo RA, Vila AJ. 2023. In-cell kinetic stability is an essential trait in metallo-beta-lactamase evolution. Nat Chem Biol 19:1116–1126. https://doi.org/10.1038/s41589-023-01319-0
11. Bahr G, Vitor-Horen L, Bethel CR, Bonomo RA, González LJ, Vila AJ. 2018. Clinical evolution of New Delhi metallo-beta-lactamase (NDM) optimizes resistance under Zn(II) deprivation. Antimicrob Agents Chemother 62:e01849-17. https://doi.org/10.1128/AAC.01849-17
12. National Library of Medicine. n.d. National center for biotechnology information. Available from: https://ncbi.nlm.nih.gov/pathogens/refgene
13. Hamrick JC, Docquier J-D, Uehara T, Myers CL, Six DA, Chatwin CL, John KJ, Vernacchio SF, Cusick SM, Trout REL, Pozzi C, De Luca F, Benvenuti M, Mangani S, Liu B, Jackson RW, Moeck G, Xerri L, Burns CJ, Pevear DC, Daigle DM. 2020. VNRX-5133 (taniborbactam), a broad-spectrum inhibitor of serine- and metallo-beta-lactamases, restores activity of cefepime in *Enterobacterales* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 64:e01963-19. https://doi.org/10.1128/AAC.01963-19
14. Drusin SI, Le Terrier C, Poirel L, Bonomo RA, Vila AJ, Moreno DM. 2024. Structural basis of metallo-β-lactamase resistance to taniborbactam. Antimicrob Agents Chemother 68:e0116823. https://doi.org/10.1128/aac.01168-23
15. Le Terrier C, Viguier C, Nordmann P, Vila AJ, Poirel L. 2024. Relative inhibitory activities of the broad-spectrum β-lactamase inhibitor taniborbactam against metallo-β-lactamases. Antimicrob Agents Chemother 68:e0099123. https://doi.org/10.1128/aac.00991-23
16. Ono D, Mojica MF, Bethel CR, Ishii Y, Drusin SI, Moreno DM, Vila AJ, Bonomo RA. 2024. Structural role of K224 in taniborbactam inhibition of NDM-1. Antimicrob Agents Chemother 68:e01332-23. https://doi.org/10.1128/aac.01332-23
17. Le Terrier C, Gruenig V, Fournier C, Nordmann P, Poirel L. 2023. NDM-9 resistance to taniborbactam. Lancet Infect Dis 23:401–402. https://doi.org/10.1016/S1473-3099(23)00069-5
18. Matsumura Y, Peirano G, Motyl MR, Adams MD, Chen L, Kreiswirth B, DeVinney R, Pitout JDD. 2017. Global molecular epidemiology of IMP-producing *Enterobacteriaceae*. Antimicrob Agents Chemother 61:e02729-16. https://doi.org/10.1128/AAC.02729-16
19. Krajnc A, Brem J, Hinchliffe P, Calvopiña K, Panduwawala TD, Lang PA, Kamps J, Tyrrell JM, Widlake E, Saward BG, Walsh TR, Spencer J, Schofield CJ. 2019. Bicyclic boronate VNRX-5133 inhibits metallo- and serine-beta-lactamases. J Med Chem 62:8544–8556. https://doi.org/10.1021/acs.jmedchem.9b00911
20. Liu B, Trout REL, Chu G-H, McGarry D, Jackson RW, Hamrick JC, Daigle DM, Cusick SM, Pozzi C, De Luca F, Benvenuti M, Mangani S, Docquier J-D, Weiss WJ, Pevear DC, Xerri L, Burns CJ. 2020. Discovery of taniborbactam (VNRX-5133): a broad-spectrum serine- and metallo-beta-lactamase inhibitor for carbapenem-resistant bacterial infections. J Med Chem 63:2789–2801. https://doi.org/10.1021/acs.jmedchem.9b01518
21. Bahr G, González LJ, Vila AJ. 2021. Metallo-beta-lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design. Chem Rev 121:7957–8094. https://doi.org/10.1021/acs.chemrev.1c00138
22. Daiyasu H, Osaka K, Ishino Y, Toh H. 2001. Expansion of the zinc metallo-hydrolase family of the beta-lactamase fold. FEBS Lett 503:1–6. https://doi.org/10.1016/s0014-5793(01)02686-2
23. McGovern PC, Wagenlehner F, Gasink L, Moeck G, McLeroth PL, Beth M, Dane A, Henkel T. 2022. CERTAIN-1: a phase 3 study of cefepime-taniborbactam efficacy and safety in the treatment of complicated urinary tract infections (cUTI), including acute pyelonephritis (AP). Open Forum Infect Dis 9:ofact492. https://doi.org/10.1093/ofid/ofac492.022
24. Lomovskaya O, Tsivkovski R, Totrov M, Dressel D, Castanheira M, Dudley M. 2023. New boronate drugs and evolving NDM-mediated beta-lactam resistance. Antimicrob Agents Chemother 67:e0057923. https://doi.org/10.1128/aac.00579-23