Prevalence and Contribution of the Carbapenem Resistance *bla*VIM, *bla*KPC, and *bla*SPM Genes in *Pseudomonas aeruginosa*

Sophia A. Smith

Cambridge Center of International Research (CCIR)

Abstract

Antibiotic resistance is an increasingly critical problem for researchers and clinicians. *Pseudomonas aeruginosa* (*P. aeruginosa*), a ubiquitous opportunistic pathogen, poses a particular threat to patients with cystic fibrosis, as it is rapidly becoming extensively resistant to drugs, including carbapenems. This study investigated the prevalence of three carbapenemase genes found in *P. aeruginosa*, *bla*VIM, *bla*SPM, and *bla*KPC, and their associated proteins, outside of *P. aeruginosa*. BLAST was used to identify species possessing genes and proteins with significant homologies to the genes and proteins found in *P. aeruginosa*. Secondary databases and analysis tools, including Interpro, the Worldwide Protein Data Bank (wwPDB), AlphaFold, and Phobius, were then used to investigate the proteins found in those other species. Analysis indicated that several bacterial species express proteins that are likely to function similarly to the proteins found in *P. aeruginosa* and revealed surprising homologies between the *P. aeruginosa* proteins and proteins found in eukaryotic and archaic species. Some species seemed to possess the genes without expressing them, while others seemed to possess despite apparently lacking corresponding genes. It was also revealed that some bacterial species in

addition to *P. aeruginosa* possess and express all three genes and associated proteins or a combination, including species which are not currently considered especially dangerous. The findings of this study suggest it would be prudent to further explore carbapenem resistance in potentially problematic but somewhat neglected pathogens and the acquisition and use of carbapenemase proteins by eukaryotic and archaic species that have no clear need of them.

Keywords: antibiotics, resistance, carbapenemase, bioinformatics, sequence

Introduction

Infections by pathogenic bacteria have been one of the most prevalent causes of severe illness and death throughout human history. Since their discovery in the early 20th century, antibiotics have saved countless lives and are now some of the most frequently used drugs in medicine (Bognár et al, 2024). The emergence and spread of antibiotic resistance has therefore become a major concern for researchers (Bognár et al, 2024). Many bacteria have gained resistance to several or even all available antibiotics, making infections extremely difficult to treat. These multidrug resistant (MDR) pathogens have been widely recognized as a serious threat by global and national organizations, including the World Health Organization (WHO) and the USA's Centers for Disease Control and Prevention (CDC) (Saxena et al, 2023).

Pseudomonas aeruginosa (*P. aeruginosa*) is a ubiquitous, Gram-negative, opportunistic bacteria that can cause infections in the lungs, urinary tract, and open wounds and particularly affects immunocompromised patients. It is among the most common causes of chronic lung

infections in patients with existing chronic conditions such as cystic fibrosis (CF) and a leading cause of life-threatening infections for immunocompromised patients in general, with a mortality rate of up to 40% for all immunocompromised patients and 50% in mechanically ventilated patients (García-Villada et al, 2024; Kim et al, 2024; Swart et al, 2024). It is responsible for a large portion of hospital-acquired infections, accounting for nearly 10% of all nosocomial infections (Kim et al, 2024).

The respiratory systems of CF patients are an ideal environment for colonization by *P. aeruginosa* (Baker et al, 2024). CF is an autosomal recessive condition characterized by mutations in the CF conductance regulator (CFTR) gene which cause defects in the CFTR ion channel, a protein that controls salt transport across epithelial cells (Baker et al, 2024). Ineffective salt transport results in highly viscous sputum, promoting improper mucociliary clearance. Uninhibited by this primary innate defense mechanism of the airway, *P. aeruginosa* can develop thriving colonies that can cause intractable and sometimes deadly infections in CF patients (Baker et al, 2024).

Though *P. aeruginosa*'s behavior during an infection is not yet fully understood, it has been shown to utilize a colonization process with two phases of rapid reproduction separated by a lag phase (Swart et al, 2024). In lung infections, *P. aeruginosa* replicates in the mucus layer first, exploiting the nutrients found there and removing the body's first layer of protection (Swart et al, 2024). It then moves to the basolateral side of the epithelial layer, where it resumes rapid growth and tissue destruction (Swart et al, 2024).

Infections caused by *P. aeruginosa* are ordinarily treated with antibiotics, but *P. aeruginosa* is quickly developing increasing resistance to drugs, assisted by the overuse and

misuse of antibiotics and noncompliance to treatment regimens (Pan et al, 2024; Moule et al, 2024). In 2017, the WHO included *P. aeruginosa* in a list of antibiotic-resistant pathogens that required the most attention from researchers and drug developers, and the WHO's 2024 update of the list categorizes *P. aeruginosa* in the "high"-threat group (Kim et al, 2024; WHO, 2024). *P. aeruginosa* strains possess various mechanisms of resistance to antibiotics, including overexpression of efflux pumps, modification or loss of porins, virulence factors, quorum sensing systems, and the ability to form biofilms (Miller & Arias, 2024; Pan et al, 2024; Qin et al, 2022; Rossi et al, 2020; Liu et al, 2024).

Biofilms are colonies of microorganisms encased in a self-produced protective matrix (Liu et al, 2024). Biofilms can contribute heavily to persistent infections, especially in CF patients, as they can slow the absorption of antibiotics, allow for increased horizontal gene transfer, and harbor persistors (Liu et al, 2024; Rossi et al, 2020; Ciofu et al, 2022). *P. aeruginosa* biofilms have been shown to inactivate the complement system by secreting proteolytic enzymes and can switch their metabolism to fermentation to survive in anaerobic conditions in the lungs of CF patients (Ciofu et al, 2022). *P. aeruginosa* obtained from biofilm infections in CF patients also demonstrated greater expression of genes involved in antibiotic resistance than biofilm bacteria grown in vitro (Ciofu et al, 2022).

Among *P. aeruginosa*'s antibiotic resistance genes are genes that code for carbapenemases, enzymes that inhibit antibiotics in the carbapenem class (Pottier et al, 2023). Carbapenemase genes have particular significance for researchers, as carbapenems are one of only eight classes of antimicrobials that are currently used against *P. aeruginosa*, and carbapenem resistance is quickly gaining prevalence (Pottier et al, 2023). One 10-year study

detected carbapenemase genes in 84 of the 180 isolates that were obtained from patients (Pottier et al, 2023).

This study investigates three carbapenemase genes found in *P. aeruginosa*: *bla*VIM, *bla*KPC, and *bla*SPM. The genes were retrieved from primary databases, and their prevalence and the prevalence of their corresponding proteins outside of *P. aeruginosa* were assessed. Secondary databases were also used to collect further information about those genes and proteins showing significant homology with the genes and proteins found in *P. aeruginosa*.

Results

Retrieving *P. aeruginosa*'s blaVIM, blaSPM, and blaKPC Gene Entries

The *P. aeruginosa bla*VIM (accession number MG797448), *bla*KPC (accession number JX682705), and *bla*SPM (accession number KC710242) gene entries were retrieved from GENBANK, ENA, and DDBJ (**Table 1**). With the exception of slightly later submission dates on ENA for *bla*VIM and *bla*KPC, there was no variation in the sequence or metadata between the files from the three databases.

Table 1.

Comparison of P. aeruginosa blaVIM, blaKPC, and blaSPM gene entries from the three primary databases NCBI, ENA, and DDBJ

	GENBANK	ENA	DDBJ
*bla*VIM (FU308)	Accession: MG797448 Date: 18 March 2018	Accession: MG797448 Date: 22 March 2018	Accession: MG797448 Date: 18 March 2018
*bla*SPM (54)	Accession: KC710242 Date: 19 March 2013 Length: 719 bp	Accession: KC710242 Date: 19 March 2013 Length: 719 bp	Accession: KC710242 Date: 19 March 2013 Length: 719 bp
*bla*KPC (617)	Accession: JX682705 Date: 20 November 2012 Length: 736 bp	Accession: JX682705 Date: 21 November 2012 Length: 736 bp	Accession: JX682705 Date: 20 November 2012 Length: 736 bp

BLASTn/p Analysis of the *bla*VIM, *bla*SPM, and *bla*KPC Genes and Associated Proteins Across Bacterial Species

Tables 2a–2f summarize BLASTn and BLASTp results for *P. aeruginosa*, including the genus and species and the query coverage, percent identity, and E-value scores for results with significant homologies to *P. aeruginosa*, up to a "threshold" of similarity.

Table 2a.

Summary of BLASTn results showing nucleotide sequences in other bacterial species producing significant homology to P. aeruginosa's blaVIM gene (MG797448)

Genus	Species	Query Cover	Percent Identity	E-value
Pseudomonas	*P. aeruginosa, P. putida, P.*	100%	100.00%	0.0
Ralstonia	*R. pickettii*	79–100%	100.00%	0.0
Klebsiella	*K. pneumoniae, K. oxytoca*	100%	96.20–100.00%	0.0
Acinetobacter	*A. seifertii, A. baumannii, A.*	100%	99.58–100.00%	0.0
Enterobacter	*E. cloacae, E. hormaechei*	100%	96.20–100.00%	0.0
Citrobacter	*C. freundii, C. cronae, C.*	100%	96.20–100.00%	0.0
Serratia	*S. marcescens*	100%	100.00%	0.0

Stutzerimonas	*S. stutzeri*	100%	100.00%	0.0
Ectopseudomonas	*E. mendocina, E. oleovorans*	100%	99.58–100.00%	0.0
Stenotrophomonas	*S. maltophilia*	100%	100.00%	0.0
Escherichia	*E. coli*	100%	99.79–100.00%	0.0
Elizabethkingia	*E. meningoseptica*	100%	100.00%	0.0
Alcaligenes	*A. faecalis*	80–100%	95.99–100.00%	0.0
Achromobacter	*A. xylosoxidans, A.*	100%	95.99–100.00%	0.0
Sphingomonas	*S. abaci*	100%	100.00%	0.0
Providencia	*P. rettgeri, P. stuartii, P.*	100%	95.99–100.00%	0.0
Proteus	*P. mirabilis*	38–100%	94.94–96.20%	2e-80 – 0.0
Aeromonas	*A. caviae, A. hydrophila*	66–100%	95.59--96.20%	1e-161 – 0.0
Vibrio	*V. cholerae, V. alginolyticus*	100%	93.25–96.20%	0.0
Morganella	*M. morganii*	100%	95.59–96.20%	0.0
Leclercia	*L. adecarboxylata*	100%	95.59%	0.0
Raoultella	*R. ornithinolytica, R.*	70–100%	95.99–98.49%	3e-162 – 0.0
Kluyvera	*K. cryocrescens*	100%	95.59%	0.0
Salmonella	*S. enterica*	15–100%	95.59%	2e-24 – 0.0
Nocardia	*N. farcinica*	78%	100.00%	0.0
Bacillus	No species listed	12–77%	98.36%	1e-16 – 6e-180
Staphylococcus	No species listed	75%	98.32%	6e-175 – 2e-174
Burkholderia	*B. cenocepacia*	73%	98.55%	6e-170 – 2e-169

Table 2b.

Summary of BLASTn results showing nucleotide sequences in other bacterial species producing significant homology to P. aeruginosa's blaSPM gene (KC710242)

Genus	Species	Query Cover	Percent Identity	E-value
Pseudomonas	*P. aeruginosa*	24–100%	99.16–100.00%	2e-81 – 0.0
Acinetobacter	*A. baumannii*	28–100%	99.51–100.00%	2e-95 – 0.0
Escherichia	*E. coli*	38%	100.00%	6e-133 – 2e-136

Klebsiella	*K. pneumoniae*	26%	100.00%	7e-88 – 3e-90
Pseudobacteriovorax	*P. antillogorgiicola*	65%	64.63%	4e-09
Raoultella	*R. planticola*	30%	66.97%	0.003
Serratia	*S. marcescens*	30%	66.97%	0.003
Citrobacter	*C. freundii*	30%	66.97%	0.003
Ornithobacterium	*O. rhinotracheale*	8%	80.00%	0.012
Riemerella	*R. columbina*	8%	80.00%	0.012

Table 2c.

Summary of BLASTn results showing nucleotide sequences in other bacterial species producing significant homology to P. aeruginosa's blaKPC gene (JX682705)

Genus	Species	Query Cover	Percent Identity	E-value
Escherichia	*E. coli*	100%	100.00%	0.0
Raoultella	*R. ornithinolytica*	100%	100.00%	0.0
Klebsiella	*K. pneumoniae, K.*	100%	100.00%	0.0
Proteus	*P. mirabilis*	100%	100.00%	0.0
Citrobacter	*C. freundii, C. youngae, C.*	100%	100.00%	0.0
Pseudomonas	*P. aeruginosa*	100%	100.00%	0.0
Serratia	*S. marcescens*	100%	100.00%	0.0
Morganella	*M. morganii*	91–100%	99.86–100.00%	0.0
Enterobacter	*E. hormaechei, E. cloacae, E.*	100%	100.00%	0.0
Salmonella	*S. enterica*	100%	100.00%	0.0
Aeromonas	*A. caviae, A. hydrophila, A. veronii, A. taiwanensis, A. allosaccharophila, A. media, A. salmonicida*	33–100%	99.86–100.00%	0.0
Providencia	*P. huaxiensis, P. stuartii, P.*	38–100%	100.00%	0.0
Achromobacter	No species listed	53–100%	100.00%	0.0
Acinetobacter	*A. baumannii*	100%	99.73–100.00%	0.0
Pantoea	No species listed	53–100%	100.00%	0.0

Hafnia	*H. paralvei*	53–100%	99.86–100.00%	0.0
Chromobacterium	*C. piscinae, C. haemolyticum,*	52%	68.77–78.48%	4e-56 – 0.0
Brucella	*B. intermedia*	35%	100.00%	0.0
Burkholderia	*B. cenocepacia, B. ubonensis,*	28–39%	68.92–100.00%	3e-39 – 0.0
Rugamonas	No species listed	52%	67.90%	3e-51
Pseudoduganella	*P. aquatica, P. albidiflava, P.*	47%	68.02–77.33%	9e-42 – 1e-69
Bordetella	*B. flabilis*	44%	68.88%	1e-50
Duganella	*D. dendranthematis*	44%	67.16–67.37%	5e-42 – 1e-42
Massilia	*M. endophytica*	38–42%	69.44–70.00%	4e-50 – 1e-55
Janthinobacterium	*J. lividum, J. svalbardensis, J.*	28–39%	68.80–71.54%	9e-52 – 1e-42
Variovorax	*V. paradoxus, V.*	38%	68.55–68.86%	4e-43 – 1e-42

Table 2d.

Summary of BLASTp results showing amino acid sequences in other bacterial species producing significant homology to P. aeruginosa's blaVIM gene (MG797448)

Genus	Species	Query Cover	Percent Identity	E-value
Pseudomonas	*P. aeruginosa, P.*	10%	96.82–100.00%	9e-99 – 1e-97
Klebsiella	*K. pneumoniae, K.*	10%	99.36–100.00%	8e-99 – 1e-97
Enterobacter	*E. cloacae, E.*	10%	96.82–100.00%	9e-96 – 1e-98
Serratia	*S. marcescens*	10%	96.82–100.00%	8e-96 – 2e-98
Gammaproteobacteria	No species listed	10%	97.45–100.00%	4e-97 – 2e-96
Citrobacter	*C. cronae, C. freundii*	10%	99.36–100%	3e-98 – 1e-97
Escherichia	*E. coli*	10%	96.82–100.00%	9e-97 – 1e-97
Morganella	*M. morganii*	10%	97.45–99.36%	7e-96 – 1e-97
Ectopseudomonas	*E. mendocina*	10%	99.36%	4e-97
Elizabethkingia	*E. meningoseptica*	10%	100.00%	1e-95
Ralstonia	*R. pickettii*	8%	100.00%	8e-76
Norcardia	*N. farcinica*	8%	100.00%	4e-75
Shigella	*S. flexneri*	10%	99.36%	2e-97

Bacillus	No species listed	8%	99.17%	7e-73
Staphylococcus	No species listed	8%	99.15%	6e-71
Raoultella	*R. ornithinolytica*	8–10%	96.82–99.14%	7e-95
Burkholderia	*B. cenocepacia*	8%	99.13%	1e-68
Acinetobacter	*A. baumannii, A. pittii*	9–10%	97.45–98.52%	4e-94 – 3e-94
Alcaligenes	*A. faecalis*	8-10%	97.45–98.41%	3e-95
Providencia	*P. rettgeri, P. stuartii,*	10%	96.18–97.45%	5e-95 – 1e-94
Proteus	*P. mirabilis*	10%	96.18–96.82%	2e-94 – 1e-95
Kluyvera	*K. cryocrescens*	10%	96.82%	2e-93
Vibrio	No species listed	10%	91.72%	5e-91
Marinobacter	No species listed	10%	83.44%	1e-80
Kangiella	No species listed	10%	77.71%	3e-73
Bowmanella	*B. dentrificans*	10%	68.79–70.06%	3e-66 – 2e-67

Table 2e.

Summary of BLASTp results showing amino acid sequences in other bacterial species producing significant homology to P. aeruginosa's blaSPM gene (KC710242)

Genus	Species	Query Cover	Percent Identity	E-value
Pseudomonas	*P. aeruginosa*	11-13%	94.86–97.33%	9e-124 – 1e-150
Acinetobacter	*A. baumannii*	11-13%	96.23–96.76%	9e-131 – 7e-157

Table 2f.

Summary of BLASTp results showing amino acid sequences in other bacterial species producing significant homology to P. aeruginosa's blaKPC gene (JX682705)

Genus	Species	Query Cover	Percent Identity	E-value
Klebsiella	*K. pneumoniae, K. aerogenes,*	14–15%	95.06–98.41%	1e-176 – 5e-178
Serratia	*S. marcescens*	14%	98.41%	8e-178

Luteolibacter	*L. ambystomatis*	14%	98.41%	8e-178
Pseudomonas	*P. aeruginosa*	14%	98.01–98.41%	8e-177 – 1e-177
Escherichia	*E. coli*	14%	98.41%	1e-176 – 2e-177
Gammaproteobacteria	No species listed	14%	98.01%	1e-176 – 2e-177
Aeromonas	*A. caviae*	14%	98.41%	2e-177
Enterobacter	No species listed	14%	98.01%	1e-176

Tables 3a–b list species that BLAST results indicate possess all three genes and express all three proteins investigated in this study, and species that possess all three genes and the BlaVIM and BlaKPC proteins (since only one species outside of *P. aeruginosa* was found to express the BlaSPM protein), respectively.

Table 3a.

Species possessing blaVIM, blaSPM, and blaKPC genes and expressing BlaVIM, BlaSPM, and BlaKPC proteins

Genus	Species
Acinetobacter	*A. baumannii*

Table 3b.

Species possessing blaVIM, blaSPM, and blaKPC genes and expressing BlaVIM and BlaKPC proteins

Genus	Species
Klebsiella	*K. pneumoniae*
Serratia	*S. marcescens*
Escherichia	*E. coli*

Analysis of the BlaVIM, BlaSPM, and BlaKPC Proteins Using Interpro, the Worldwide Protein Data Bank, AlphaFold, and Phobius

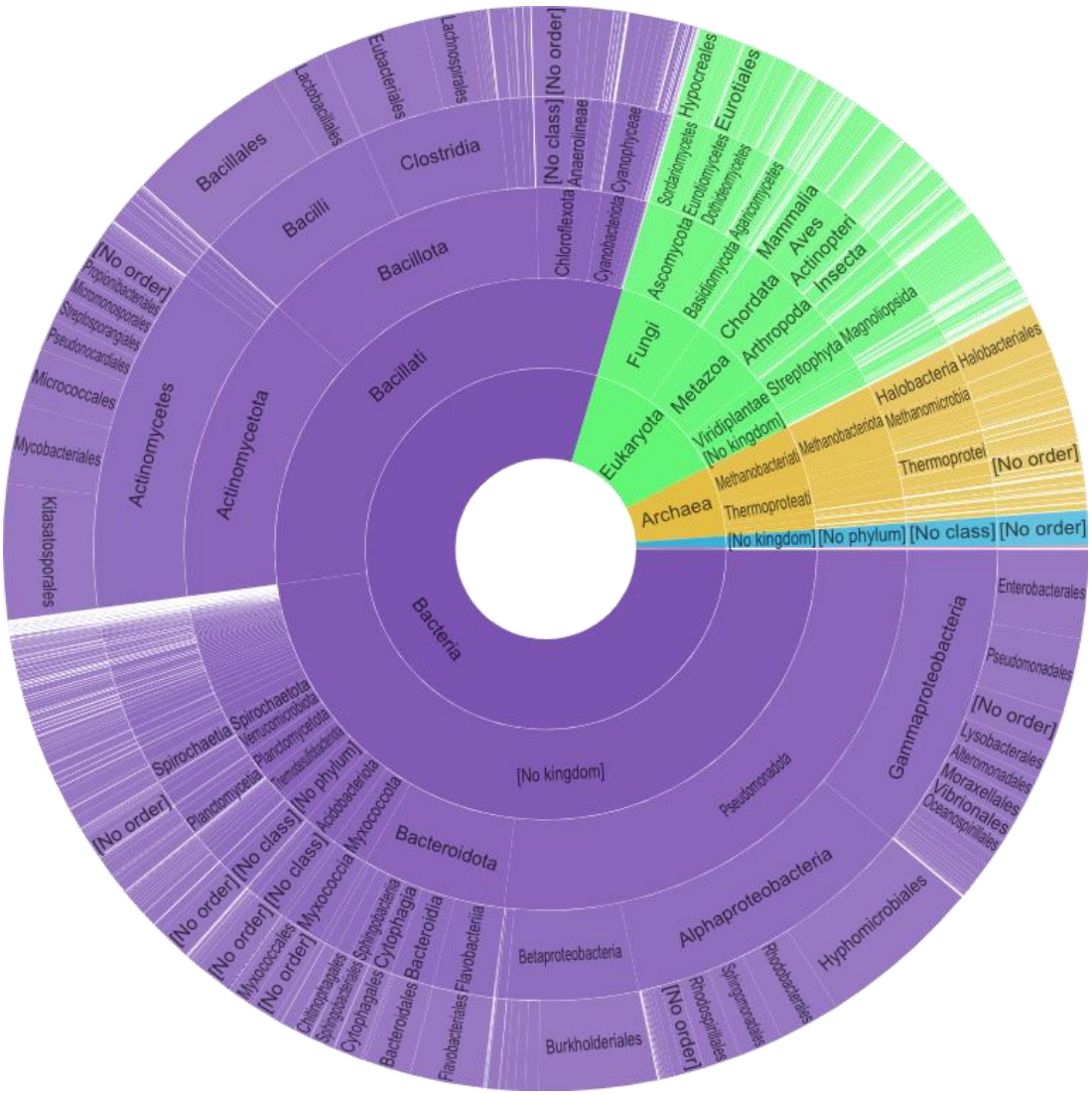
Following BLAST analysis of the nucleotide sequences, characteristics of the associated proteins were investigated using additional tools, including Interpro, the Worldwide Protein Data Bank (wwPDB), AlphaFold, and Phobius.

Analysis of the BlaVIM Protein in P. aeruginosa, Bacillus, and Colletotrichum sublineolum

Interpro's taxonomy analysis (**Figure 1**) generally supported BLAST results for bacterial species, but also indicated significant homologies in Eukaryota and Archaea. BLASTn produced only one result from Eukaryota (*Ancylostoma duodenale*, which was unsuitable for investigation due to its Query Coverage score of only 12%) and none from Archaea, even on the "More dissimilar" and "Somewhat similar" settings, and when all bacteria were excluded from results. BLASTp did not produce any results when all bacteria were excluded. Because BLAST does not account for 3D/shape homologies, but rather only homologies in nucleotide and amino acid sequences, BLAST results may not represent all species possessing proteins with significant homologies to the query protein. This is likely the reason for the disparity between BLAST and Interpro's results for Eukaryota and Archaea.

Figure 1.

Interpro analysis results showing species possessing proteins with significant homologies to P. aeruginosa's BlaVIM protein



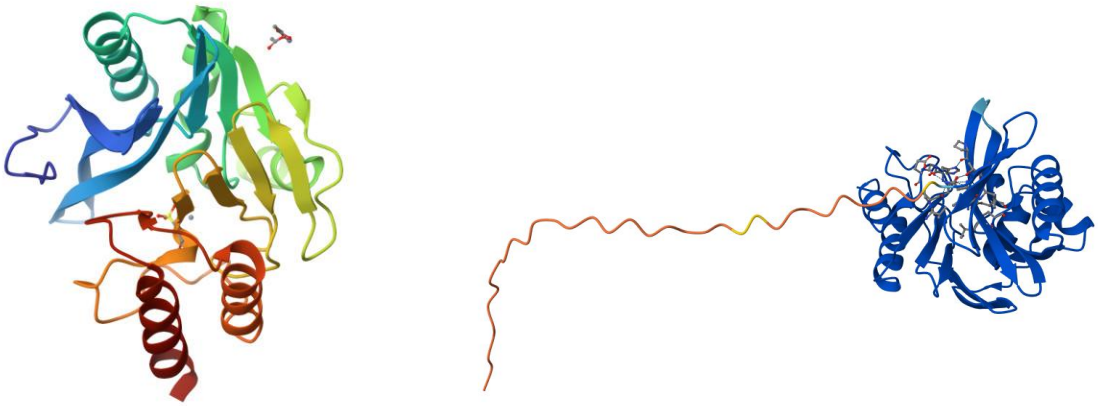
An entry of the BlaVIM protein in *P. aeruginosa* was retrieved from wwPDB, while the BlaVIM protein in *Bacillus* was predicted by AlphaFold (**Figure 2a**). The two proteins share one domain in common, a mixed alpha/beta structure. The *Bacillus* protein has a second domain consisting of only a long, thin "tail". However, model confidence for this domain is rated "Very low" by AlphaFold, whereas the confidence for the shared domain is "Very high" or "High", suggesting that the "tail" domain may not actually exist in the *Bacillus* protein. Despite the

possibility of an additional domain in the *Bacillus* protein, the two proteins have very similar structures and likely serve the same function in bacterial cells.

It was notably difficult to find results on BLAST that were suitable for further investigation (those with relatively high Query Coverage scores and relatively low Percent Identity scores and E-values). This may be because the prevalence of the *bla*VIM gene and its corresponding protein across species has simply not yet been studied thoroughly enough to produce a greater variety of results in BLAST.

Figure 2a.

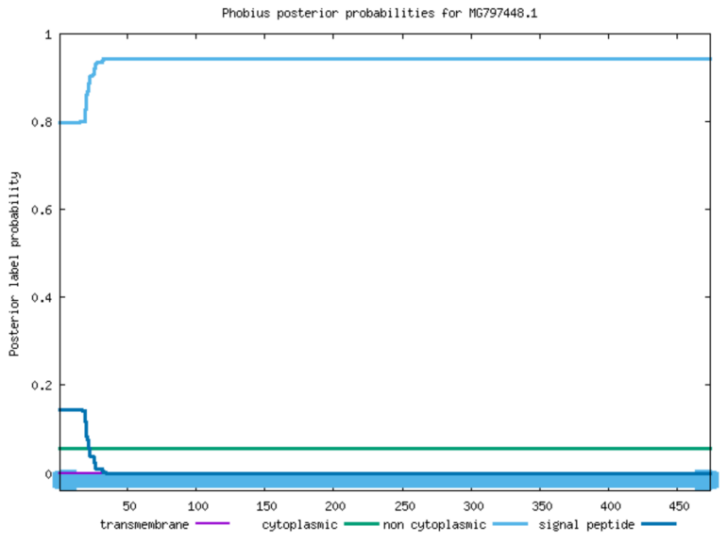
Entry of P. aeruginosa's BlaVIM protein retrieved from wwPDB (left) and structural model of Bacillus's BlaVIM protein generated by AlphaFold (right)

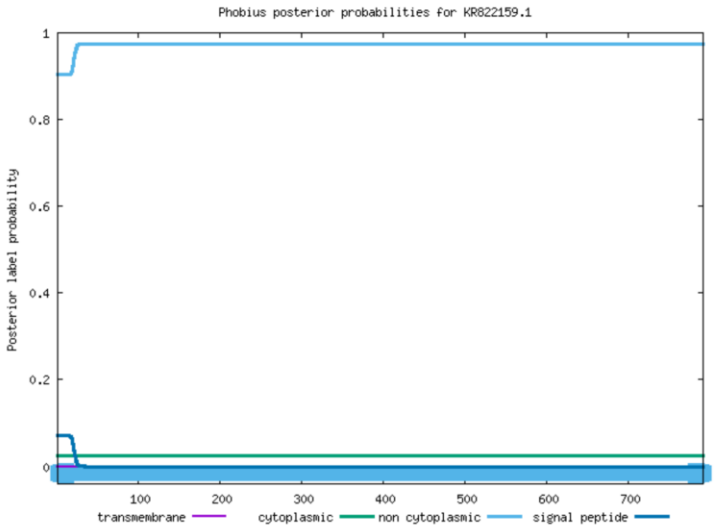


Phobius analyses of the BlaVIM protein in *P. aeruginosa* and *Bacillus* (**Figure 2b**) further indicate a shared function, as both charts show both a cytoplasmic and non-cytoplasmic component for the BlaVIM protein.

Figure 2b.

Phobius localization analyses of the BlaVIM protein in P. aeruginosa (top) and Bacillus (bottom), indicating both a cytoplasmic and non-cytoplasmic component for the protein in both species

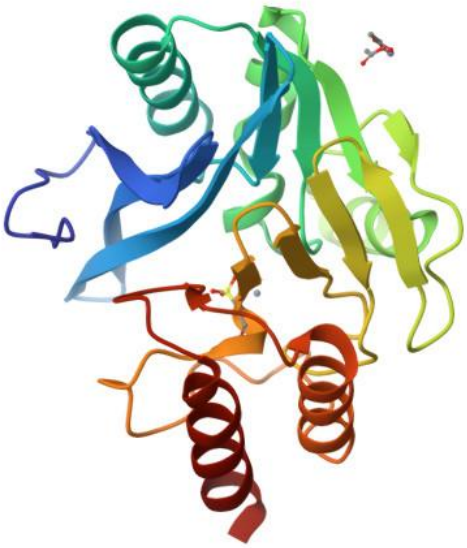


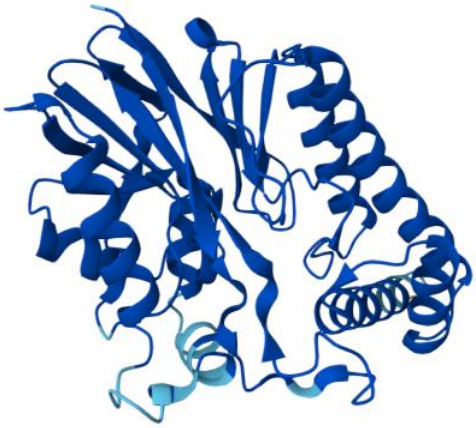


Colletotrichum sublineolum is one eukaryotic species included in Interpro's taxonomy report. The BlaVIM protein in *C. sublineolum* was predicted by AlphaFold (**Figure 3a**). The predicted protein is similar to the *P. aeruginosa* and *Bacillus* proteins, consisting of a single mixed alpha-beta domain. Except for a small section with a rating of "High", the entire domain has a confidence rating of "Very high". Based on structure, it seems possible that the BlaVIM proteins from *P. aeruginosa* and *C. sublineolum* share a common function.

Figure 3a.

Entry of P. aeruginosa's BlaVIM protein retrieved from wwPDB (top) and structural model of C. sublineolum's BlaVIM protein generated by AlphaFold (bottom)

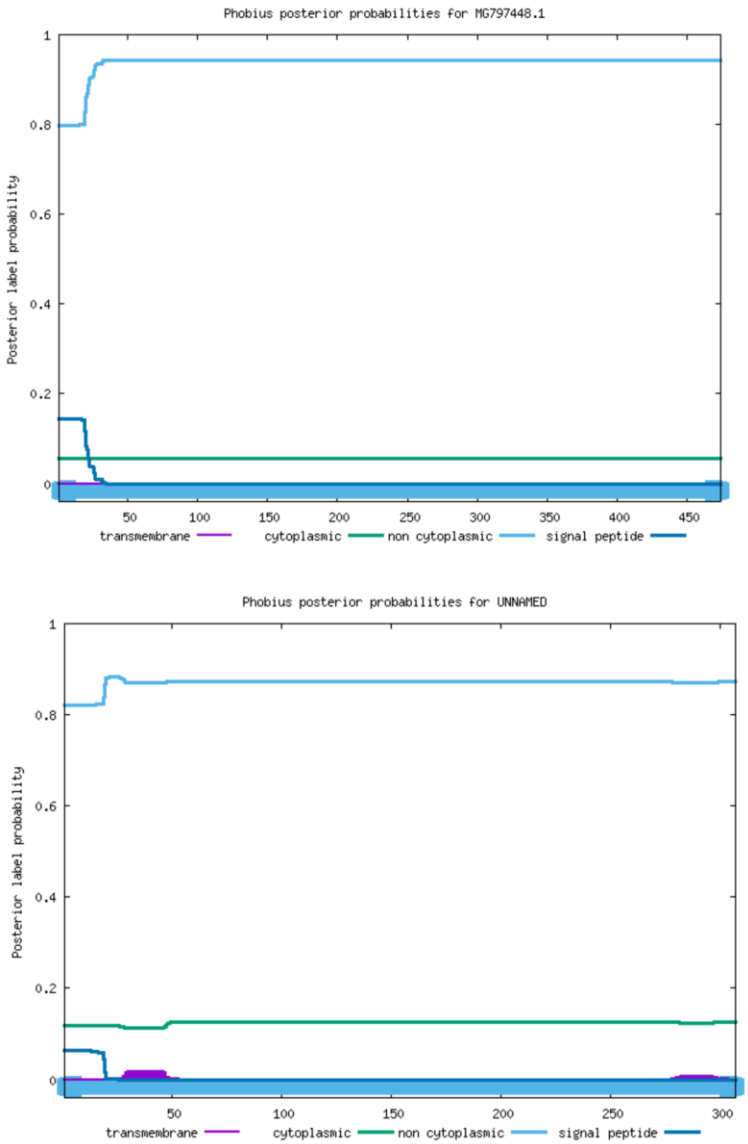




C. sublineolum's Phobius report (**Figure 3b**) matches *P. aeruginosa*'s less closely than *Bacillus*'s, but is similar enough to support the possibility that the *bla*VIM protein serves the same function in *P. aeruginosa* and *C. sublineolum*, especially considering their structures.

Figure 3b.

Phobius localization analyses of the BlaVIM protein in P. aeruginosa (top) and C. sublineolum (bottom), indicating both a cytoplasmic and non-cytoplasmic component for the protein in both species

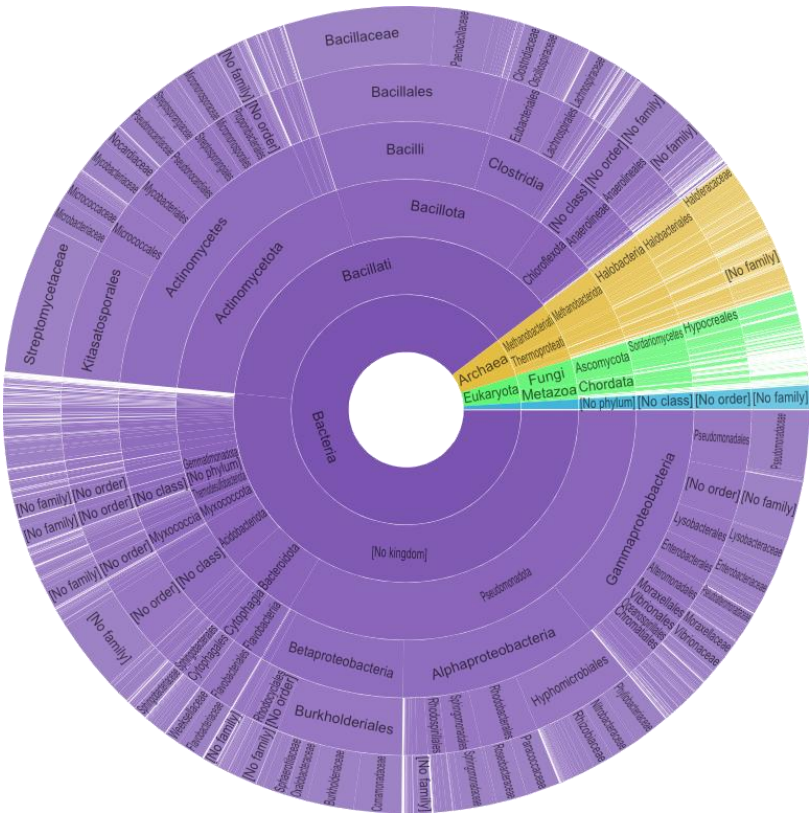


Analysis of the BlaSPM Protein in P. aeruginosa, Pseudobacteriovorax antillogorgiicola, and Ancylostoma ceylanicum

As with *bla*VIM, Interpro corroborated BLAST results for bacterial species but returned significant homologies in Eukaryota and Archaea (**Figure 4**), although no such results could be found through BLAST. The reason for this is likely the same as for *bla*VIM, namely that BLAST does not account for 3D homologies and therefore may not include results for all species carrying proteins with significant homologies to the query protein.

Figure 4.

Interpro analysis results showing species possessing proteins with significant homologies to P. aeruginosa's BlaSPM protein



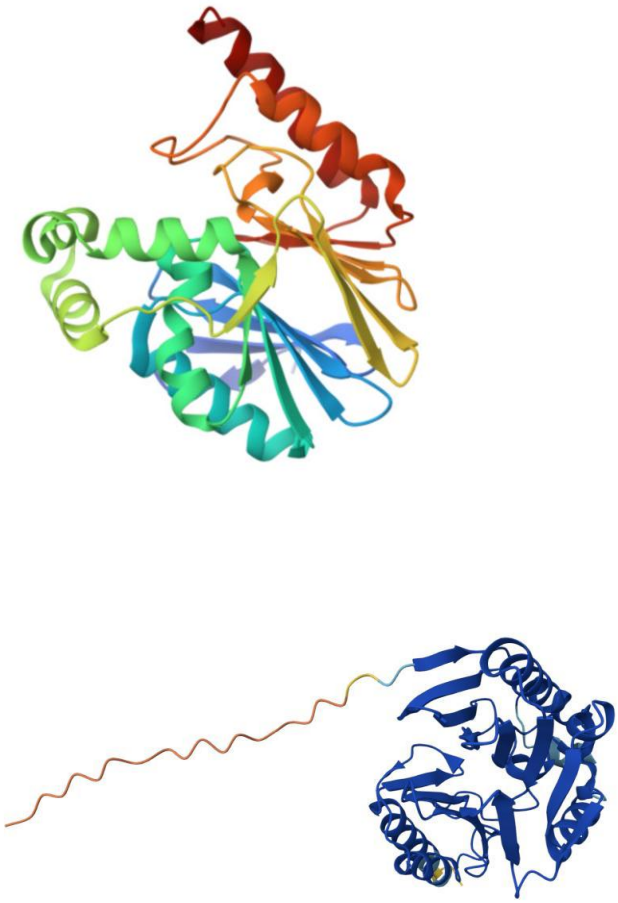
As with *bla*VIM, an entry of the BlaSPM protein in *P. aeruginosa* was retrieved from wwPDB, while the BlaSPM protein in *Pseudobacteriovorax antillogorgiicola* was predicted by AlphaFold (**Figure 5a**). The proteins are similar in structure, both containing a mixed alpha/beta domain. Like the predicted *Bacillus* protein, the predicted *P. antillogorgiicola* protein has an additional tail-like domain, but AlphaFold's confidence rating for this domain is "Very low".

Interestingly, AlphaFold's prediction of the *P. antillogorgiicola* protein matches the predicted BlaVIM protein from *Bacillus* almost exactly, except for *P. antillogorgiicola*'s slightly shorter "tail". This is not especially surprising, as the BlaVIM and BlaSPM proteins found in *P. aeruginosa* also look somewhat similar to each other, but it is notable because it suggests that the "tail" domain does not exist in *Bacillus* or *P. antillogorgiicola,* since model confidence for that unique domain is so low in both species. Discounting the "tail" domain, the structures of the

BlaVIM and BlaSPM proteins from *Bacillus* and *P. antillogorgiicola* are closer to the structures of their respective proteins in *P. aeruginosa* and thus more likely to serve the same function in bacterial cells.

Figure 5a.

Entry of P. aeruginosa's BlaSPM protein retrieved from wwPDB (top) and structural model of P. antillogorgiicola's BlaSPM protein generated by AlphaFold (bottom)



Phobius analyses of *P. aeruginosa* and *P. antillogorgiicola* (**Figure 5b**) are similar, both indicating that the BlaSPM protein has a mostly non-cytoplasmic localization, suggesting similarity between the BlaSPM proteins in *P. aeruginosa* and *P. antillogorgiicola*. However, Phobius analysis does not support the inference that the BlaVIM protein in *Bacillus* and the BlaSPM protein in *P. antillogorgiicola* share a common shape or function, as the reports for the BlaVIM and BlaSPM proteins (both in *P. aeruginosa* and in *Bacillus* and *P. antillogorgiicola*) are quite different.

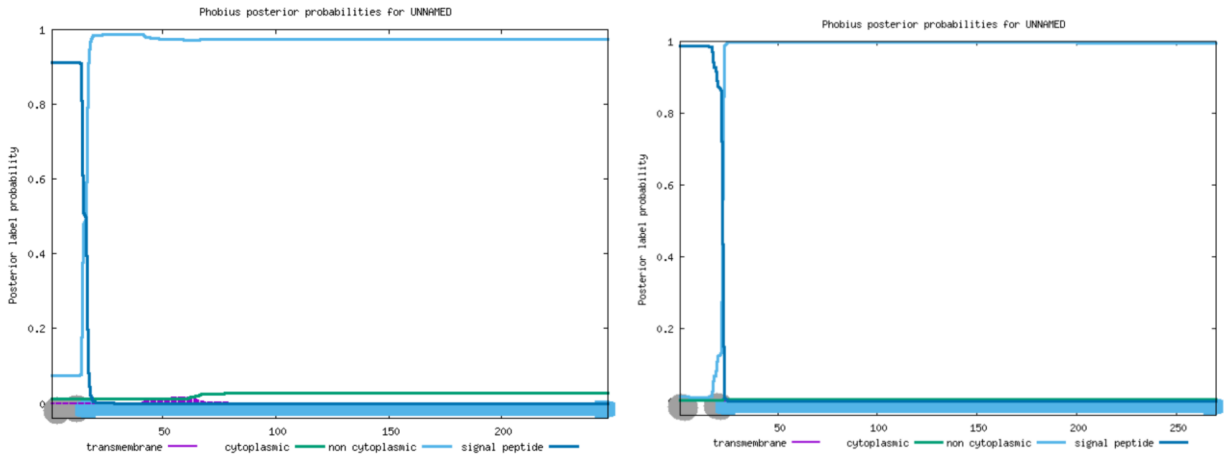


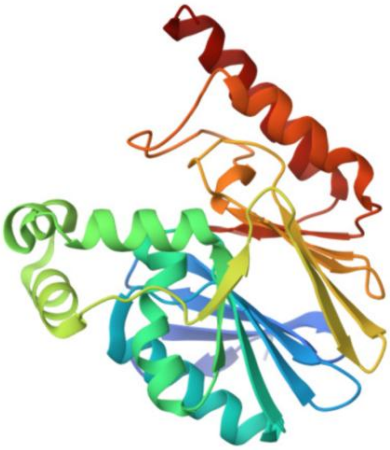
Figure 5b. Phobius localization analyses of the BlaSPM protein in *P. aeruginosa* (top) and *P. antillogorgiicola* (bottom), indicating a mostly non-cytoplasmic localization for the protein in both species

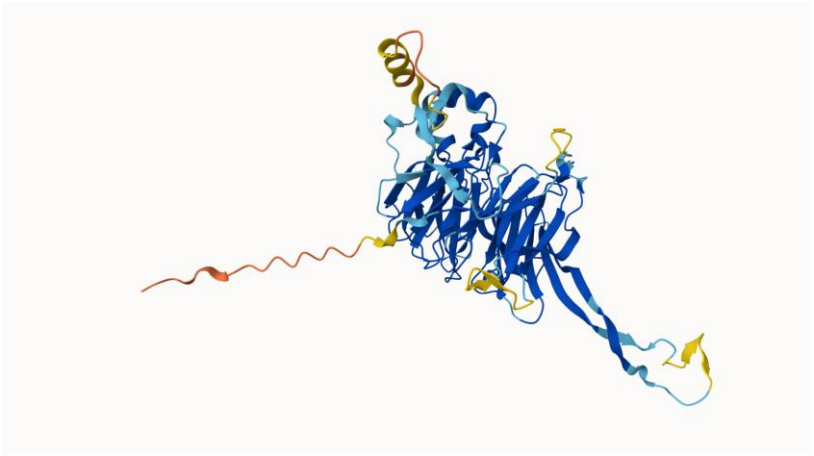
One eukaryotic species from Interpro's taxonomy analysis, *Ancylostoma ceylanicum*, was selected for investigation, and the BlaSPM protein in *A. ceylanicum* was predicted by AlphaFold (**Figure 6a**). The predicted protein's structure is somewhat similar to those of the predicted *P.*

antillogorgicola and *Bacillus* proteins, including a mixed alpha-beta domain and a thin tail-like domain, but it has two notable deviations: an alpha helix motif and a beta sheet motif that reach out from the mixed domain. As in the *Bacillus* and *P. antillogorgiicola* proteins, model confidence for the "tail" domain is rated "Very low". Model confidence for the rest of the protein ranges from "Very low" to "Very high"; most of the mixed domain is rated "High" or "Very high", while parts of the two unique domains are rated "Low" or "Very low". This suggests that the unique domains may be different in reality from the prediction, or possibly that they do not exist at all. In any case, the structure of the predicted BlaSPM protein in *A. ceylanicum* is not different enough from the *P. aeruginosa* protein to eliminate the possibility that they have similar functions.

Figure 6a.

Entry of P. aeruginosa's BlaSPM protein retrieved from wwPDB (top) and structural model of A. ceylanicum's BlaSPM protein generated by AlphaFold (bottom)

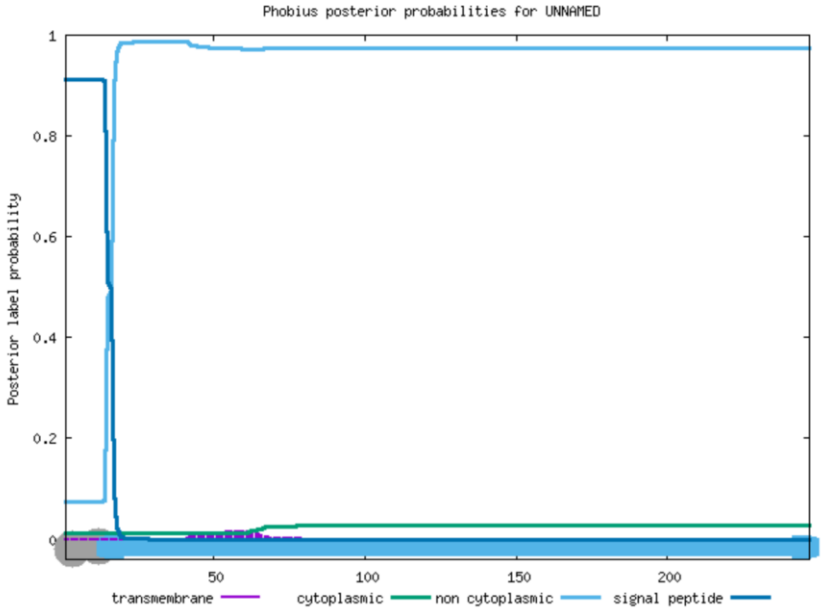


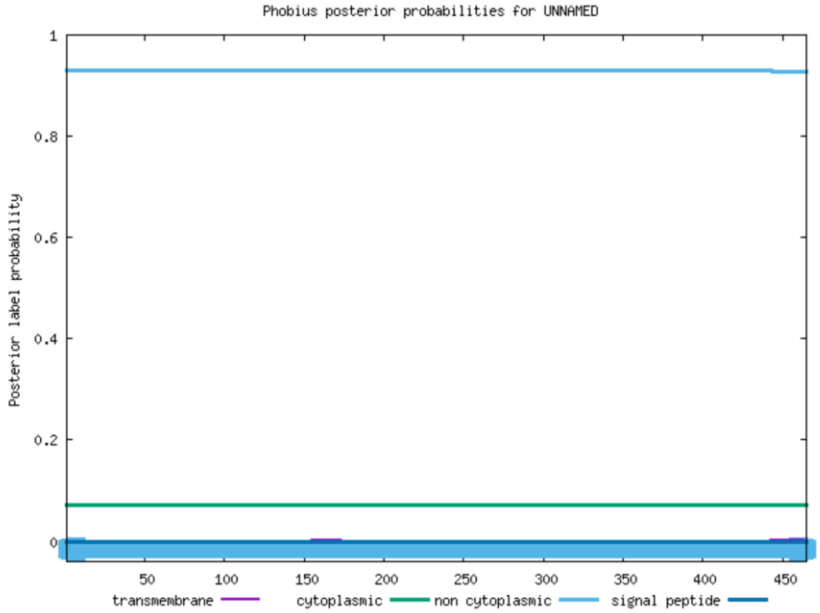


However, Phobius's analysis of the *A. ceylanicum* protein (**Figure 6b**) differs significantly from those of the *P. aeruginosa* and *P. antillogorgiicola* proteins, showing both cytoplasmic and non-cytoplasmic components, making it less likely that the BlaSPM protein in *A. ceylanicum* shares a common function with the BlaSPM proteins in *P. aeruginosa* and *P. antillogorgiicola*.

Figure 6b.

Phobius localization analyses of the BlaSPM protein in P. aeruginosa (top) and A. ceylanicum (bottom), indicating a mostly non-cytoplasmic localization for the protein in P. aeruginosa and both cytoplasmic and non-cytoplasmic components for the protein in A. ceylanicum



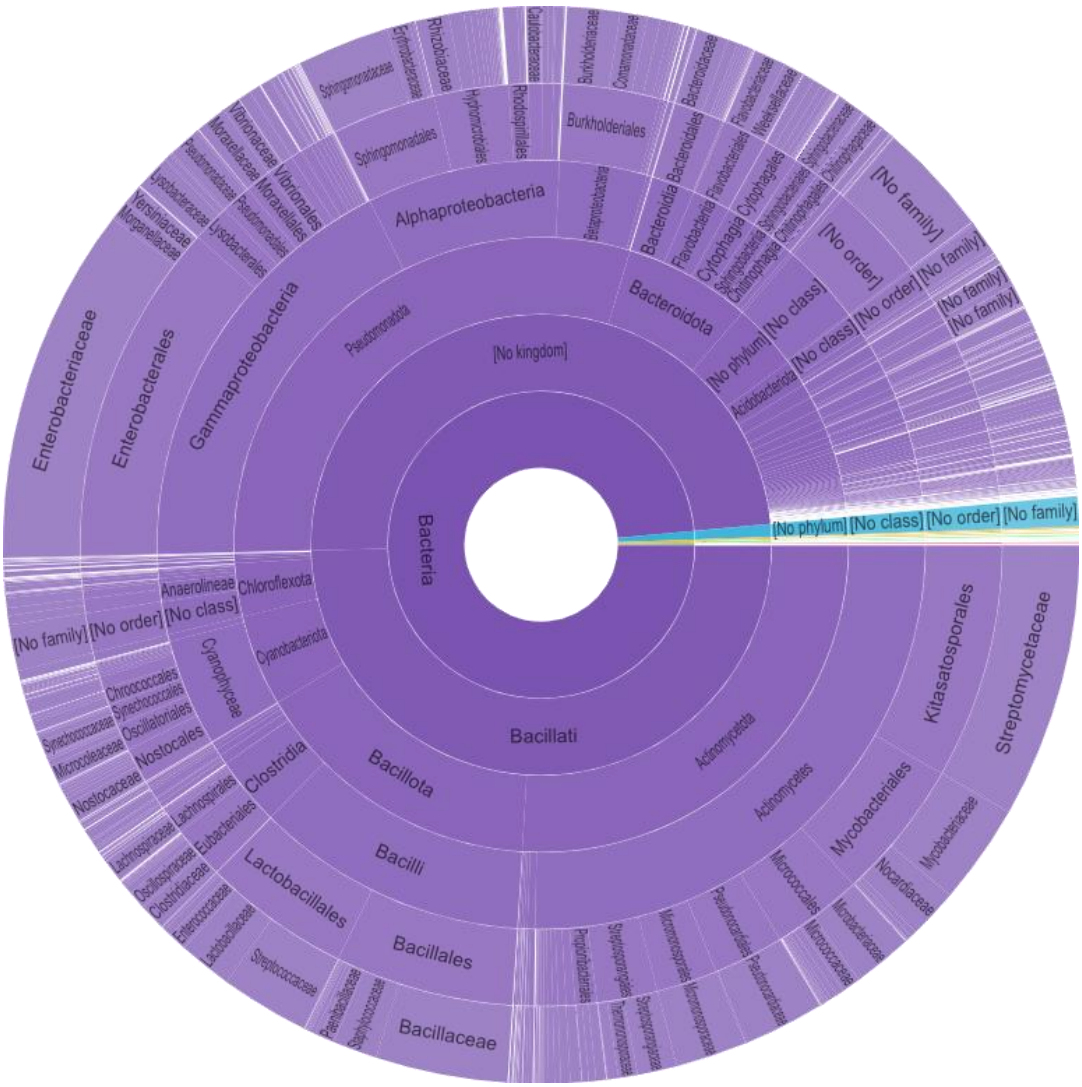


Analysis of the BlaKPC Protein in P. aeruginosa and Chromobacterium

Interpro's taxonomy analysis (**Figure 7**) corroborated BLAST results, demonstrating no notable deviations. Unlike for the BlaVIM and BlaSPM proteins, Interpro results seem to indicate that the BlaKPC protein does not exist beyond bacteria.

Figure 7.

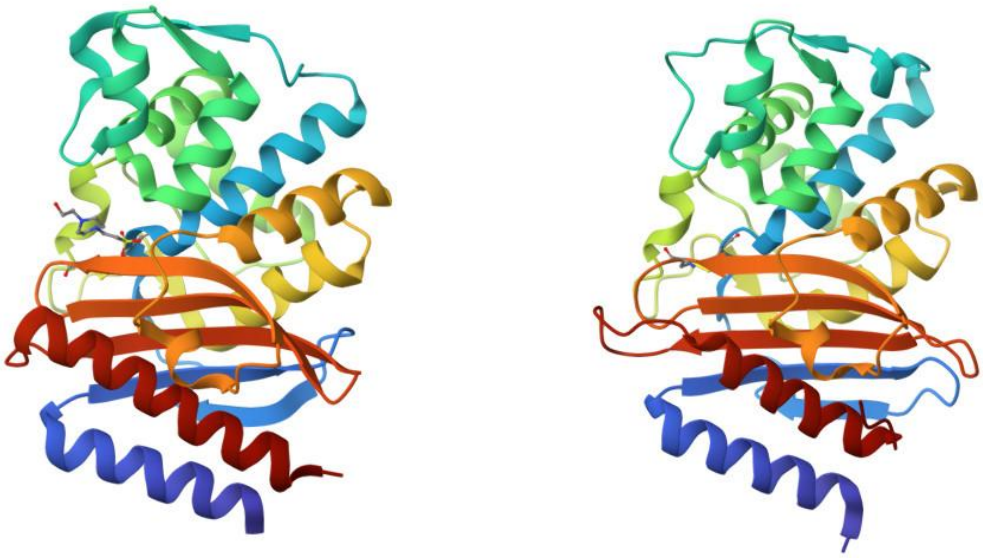
Interpro analysis results showing species possessing proteins with significant homologies to P. aeruginosa's BlaKPC protein



Entries of the BlaKPC protein from *P. aeruginosa* and *Chromobacterium* were retrieved from wwPDB (**Figure 8a**). The proteins are nearly identical in structure, each consisting of a single mixed alpha/beta domain, including top and bottom alpha helix motifs with a beta sheet motif between them. The similarity between the two proteins is especially remarkable given that BLAST searches indicated that the nucleotide sequence of *Chromobacterium*'s *bla*KPC gene deviated significantly from *P. aeruginosa*'s, with a Percent Identity score of only 74.65%.

Figure 8a.

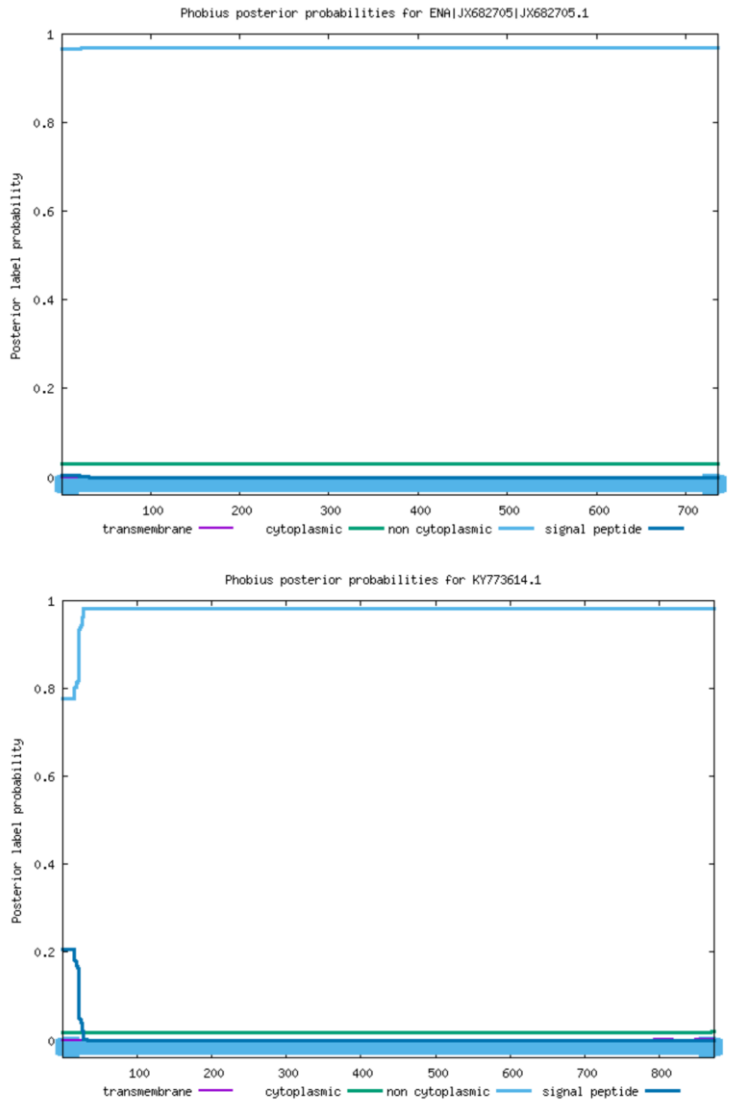
Entries of P. aeruginosa's BlaSPM protein (top) and Chromobacterium's BlaSPM protein (bottom) retrieved from wwPDB



Phobius analyses of the BlaKPC protein in *P. aeruginosa* and *Chromobacterium* (**Figure 8b**) are not quite as alike as their structures would suggest, but still indicate that the BlaKPC protein serves the same function in *P. aeruginosa* and *Chromobacterium*, as both show cytoplasmic and non-cytoplasmic components to the protein.

Figure 8b.

Phobius localization analyses of the BlaKPC protein in P. aeruginosa (top) and Chromobacterium (bottom), indicating both a cytoplasmic and non-cytoplasmic component for the protein in both species



Methodology

For each of the three genes, BLAST was used to find species with significant homologies. All BLAST settings (BLASTn, BLASTp, BLASTx, and tBLASTn) and all similarity settings for BLASTn (Highly similar, More dissimilar, and Somewhat similar) were used. The *P. aeruginosa* sequence was inputted, and for the first query, no species were excluded from the results. For each subsequent query, one or more species were excluded which came up frequently in the results. When no more individual species could be excluded, all bacteria were excluded. This process was recorded in charts which were later consolidated into **Tables 2a–f**. Those bacterial species which were selected for further comparison to *P. aeruginosa* in this study had relatively high Query Coverage scores and relatively low Percent Identity scores; that is, the sequences matched more than only a small segment of the query sequences from *P. aeruginosa*, but differed enough from the *P. aeruginosa* sequences that it was uncertain whether their structures were similar enough to suggest a common function.

For Interpro analysis, the protein sequences from the selected species were inputted as queries, and subsequent investigation was based on the reports returned by Interpro for those specific sequences. It should be noted that Interpro results did not include species from Eukaryota or Archaea when the proteins were searched for by name only.

Discussion

BLAST analysis revealed that one bacterial species, *Acinetobacter baumannii*, possesses all three of the genes and expresses all three of the proteins investigated in this study, and that several species, including *Klebsiella pneumoniae*, *Serratia marcescens*, and *Escherichia coli*, possess all three genes and express the BlaVIM and BlaKPC proteins (BLASTp results returned only two species possessing the BlaSPM protein: *P. aeruginosa* and *A. baumannii*). For *A. baumannii* and *K. pneumoniae*, this was not surprising, as they are well understood to be extensively resistant to antibiotics. However, researchers may want to direct some attention to *E. coli* and *S. marcescens,* species which are not considered especially dangerous in most cases, since their advanced resistance to carbapenems may become problematic in the future. The results of this study suggest that it may be prudent to avoid using carbapenems to treat infections by species that possess and express all three genes and associated proteins, including *A. baumannii*, and consider using different antibiotics before resorting to carbapenems for infections by species that possess and express a combination of the genes and proteins, such as *Enterobacter cloacae*, *Citrobacter freundii*, and *Burkholderia cenocepacia*.

Interpro's results showing significant homologies with *P. aeruginosa* for the BlaVIM and BlaSPM proteins in eukaryotic and archaic species were surprising given their total absence from BLAST results and may indicate that the *bla*VIM and *bla*SPM genes and associated proteins have not yet been studied enough to produce consistent results across databases and analysis tools. Carbapenem resistance in eukaryotic and archaic species is unlikely to be very problematic in a clinical sense, since most of those species do not cause infections in humans, or, if they do,

are not ordinarily treated with antibiotics. However, it may still be worthwhile to investigate the presence and function of the genes and proteins in non-bacterial species.

If further research confirms the presence of the *bla*VIM and *bla*SPM genes and associated proteins in eukaryotic and archaic species, the question of why organisms that apparently have no need of them express them nonetheless remains. *C. sublineola*, for instance, is a plant pathogen which, if chemically treated at all, is treated with fungicides, while *A. ceylanicum* is a hookworm which is usually treated with antiparasitics, including ivermectin, pyrantel pamoate, and sometimes benzimidazoles (Ciofini et al, 2022; Aziz & Ramphul, 2025). Being a eukaryotic organism, *A. ceylanicum* does not have a cell wall, making the purpose of a carbapenemase still more unclear, as carbapenems work by inhibiting cell wall synthesis (Ciofini et al, 2022). The simplest explanation is that the proteins serve different functions in eukaryotic and archaic species than they do in bacteria. This may explain the presence of the BlaSPM protein in *A. ceylanicum*, as AlphaFold's prediction of the protein's structure is significantly different from the structure of the BlaSPM protein found in *P. aeruginosa*. However, it does not fully explain the presence of the BlaVIM protein in *C. sublineola*, as AlphaFold's prediction of the *C. sublineola* protein is remarkably similar to both the protein found in *P. aeruginosa* and the prediction of the *Bacillus* protein.

Several species were listed in BLASTn results but not BLASTp results, including, for *bla*VIM, *Stuzerimonas*, *Stenotrophomonas*, *Achromobacter*, *Sphingomonas*, *Aeromonas*, *Leclercia*, and *Salmonella*; for *bla*SPM, *Escherichia*, *Klebsiella*, *Pseudobacteriovorax*, *Raoultella*, *Serratia*, *Citrobacter*, *Ornithobacterium*, and *Riemerella*; and for *bla*KPC, *Raoultella*, *Proteus*, *Citrobacter*, *Morganella*, *Salmonella*, *Providencia*, *Achromobacter*,

Acinetobacter, *Pantoea*, *Hafnia*, *Chromobacterium*, *Brucella*, *Burkholderia*, *Rugamonas*, *Pseudoduganella*, *Bordetella*, *Duganella*, *Massilia*, *Janthinobacterium*, and *Variovorax*. It is unclear whether the prevalence of the *bla*VIM, *bla*SPM, and *bla*KPC genes and associated proteins in those species has simply not been investigated enough to yield consistent results, or the genes are indeed present, but not expressed. If experimentation confirms that the genes are present in such species, it raises the question of why they acquire the gene, but do not use it.

Peculiarly, some species were listed in BLASTp results but not BLASTn results, including, for *bla*VIM, *Shigella*, *Marinobacter*, *Kangiella*, and *Bowmanella*; and for *bla*KPC, *Luteolibacter*. Experimentation will be necessary to confirm that these species do not possess the genes; if they do not, it is unclear how they can express proteins with significant homologies to the BlaVIM and BlaKPC proteins found in *P. aeruginosa* without also possessing corresponding genes with significant homologies to the *bla*VIM and *bla*KPC genes found in *P. aeruginosa*. Such a finding may have interesting implications, including in a clinical context, as they may modify the current understanding of the avenues of acquisition for drug resistance proteins like carbapenemases.

Conclusion

The findings of this study raise three main questions, namely, why some species possess one or more of the genes investigated in this study but apparently do not express them; how researchers should manage their attention in light of the finding that there are some species which are not widely considered especially clinically relevant and yet possess and express potentially problematic combinations of the genes and proteins investigated in this study; and why eukaryotic and archaic species express two of the proteins investigated in this study despite having no clear use for them.

Experimentation will be necessary to determine whether the discrepancy between the possession and expression of the genes in some species suggested by BLAST results is real or due merely to a lack of sufficient empirical data available to BLAST, and it will take further investigation and collaboration by researchers to determine the implications of and the proper response to the possession and expression of all three genes and proteins or all three genes and two of the three proteins in some bacterial species, particularly those which are not currently recognized as seriously dangerous. The apparent existence of the BlaVIM and BlaSPM proteins in eukaryotic and archaic species is surprising and generates fascinating questions about the acquisition and use of those proteins outside of bacteria. It is possible that non-bacterial species use the proteins for different purposes which are yet unknown. Further exploration and experimentation can reveal more information about the relationship between non-bacterial species and genes and associated proteins such as those investigated in this study, which may prove valuable in a clinical context.

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