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Investigating the prevalence and function of the *mecA* gene and PBP2a protein among Staphylococcus and Mammaliicoccus species

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Abstract. The *mecA* gene, an acquired gene encoding an additional penicillin-binding protein (PBP2a) with low affinity to nearly all β-lactams, is associated with the epidemiologically most important mechanism of antibiotic resistance in Staphylococcus aureus. However, apart from *S. aureus*, the *mecA* gene and functional PBP2a protein have also been discovered in other staphylococcal or mammalicoccal species. This research uses the Basic Local Alignment Search Tool (BLAST) to gather *mecA* gene and PBP2a amino acid sequences from multiple bacterial species and analyse the topology, structure, and function of the aligned PBP2a proteins. BLAST analysis indicated that the *mecA* gene and PBP2a protein sequences are present in several staphylococcal and mammaliicoccal species, and both the structure and function of the PBP2a protein are highly conserved among the species. This research indicates that the *mecA* gene and PBP2a protein are present and functional in a wide range of staphylococcal and mammaliicoccal species and highlights the high conserveness of the PBP2a protein among those species.

Keywords: Staphylococcus, drug resistance, bioinformatics

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium within the Bacillales order [1]. As one of the most common pathogens for humans, *S. aureus* expresses an extensive range of virulence factors, including toxins (haemolysins and leucocidins), immune evasive surface factors (e.g., capsule and protein A), and tissue invasion-promoting factors (e.g., hyaluronidase). Among these, many are acquired by mobile genetic elements, such as the most studied arginine-catabolic mobile element (ACME) and Paton-Valentine leucocidin (PVL) [2]. These virulence factors contribute to the wide range of *S. aureus* clinical infections, making it the most common cause of infective endocarditis [3] and three major types of osteoarticular infections [4], as well as important causes of bacteremia [5,6], skin and soft tissue infections, pneumonia [7] as well as other pleuropulmonary infections, and device-related infections [4].

The extensive and dynamic host range of *S. aureus* is also a widely researched aspect since its discovery, as the bacterium's colonisation is observed in humans, livestock (e.g., cattle and lamb), companion animals (e.g., cats and dogs), and wild species [8,9].

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However, perhaps the topic that gained *S. aureus* the most notice is a particular strain of the bacteria, namely, the methicillin-resistant staphylococcus aureus (MRSA). Bacteria gain antimicrobial resistance through simple modes of natural selection. Different levels of resistance to certain antibiotics constantly and spontaneously occur as the bacteria undergo mutations or horizontal gene transfers with other strains. After being administered to patients, the antibiotic places selective pressure on the bacterial strain. With sufficient time and prolonged antimicrobial treatment, selection eventually favours the strain that has acquired or accumulated the highest antibiotic minimum inhibitory concentrations (MICs) [2].

In the case of MRSA, *S. aureus* first demonstrated penicillin resistance in the 1940s [10] through the β-lactamase encoded by the *blaZ* gene. This gene was previously shown as a gene acquired by the transposon Tn*552* or Tn*552*-like elements [11] and located on either a large plasmid or inserted into the bacterial chromatin [12]. After methicillin was introduced into clinical usage in 1959, methicillin-resistant *S. aureus* strains were discovered within two years in 1961 [13], the resistance rendered by the *mecA* gene acquired through the staphylococcus chromosome cassette *mec* (SCC*mec*) [14]. The SCC*mec* cassette contains the *mecA* gene complex and site-specific recombinase genes (*ccrA* and *ccrB*) responsible for methicillin resistance and mobility. It is integrated within the *orfX* gene in the *S. aureus* genome [15]. The *mecA* gene encodes for penicillin-binding protein 2a (PBP2a), a bacterial cell wall crossing peptidoglycan that demonstrates resistance to all β-lactam antibiotics due to its low affinity for β-lactams [16]. Since its first occurrence, MRSA has presented itself as a non-negligible problem for the medical system.

Apart from resistance to penicillin, methicillin, and other β-lactam antibiotics, MRSA also exhibits resistance to trimethoprim (acquired by gene *dfrA* and *dfrK*), erythromycin (developed by gene *ermC*), clindamycin (constitutively expressed *ermC*), tetracyclines (acquired by gene *tetK* and *tetL*) [2], and most recently, resistance to vancomycin, which was widely used as a treatment for MRSA before vancomycin-resistant *S. aureus* was observed in 1997 [17]. Thus, due to increased resistance to multiple antimicrobial drugs, MRSA is attributed to over 100,000 deaths and 3.5 million disability-adjusted life-years worldwide in 2019 [18], and 3.8% of the global population still carries the deadly pathogen [19].

Since the *mecA* gene is acquired and transmitted through a mobile genetic element that inserts itself into the host genome, the cassette must have originated from a separate organism other than *S. aureus*. The presence of the *mecA* gene and functional PBP2a protein has already determined in other bacterial species such as *S. pseudintermedius* and *S. epidermidis* (20,21). Apart from staphylococcal species, almost identical homologues of the *mecA* gene and PBP2a protein are also determined to be present in *Mammaliicoccus sciuri* (ex. *Staphylococcus sciuri*) and *Mammaliicoccus fleurettii* with high similarities, even able to fully replace the function of the *mecA* gene when inserted into an *S. aureus* bacterium [22,23]. The prevalence of the *mecA* gene and its corresponding PBP2a protein in multiple bacterial species could indicate an expansive range of methicillin and other β-lactam antibiotic resistance within the staphylococcal and mammalicoccal genus [24]. This research will focus on investigating the presence of the *mecA* gene and PBP2a protein among determined bacterial sequences, with a critical focus on analysing the structure and function of the PBP2a protein in multiple bacterial species.

2. Methods

To retrieve the *mecA* sequences, a search was carried out using the keywords "*mecA*" and "*S. aureus*" and the reference sequence of *mecA* (accession number MW682923). The corresponding amino acid sequence (accession number WP_000721310.1) Several data analyses using the Basic Local Alignment Search Tool (BLAST) were performed using different algorithms and exclusions. BLAST trails using BLASTn, tBLASTn, BLASTp, and BLASTx were performed using a query of either the *mecA* sequence or the PBP2a sequence. Within each database, four different trails adding different exclusions were simultaneously carried out, with the exclusion of none, *Staphylococcus aureus* (taxid 1280), Staphylococcus (taxid 1279), and Bacillus/Staphylococcus group (taxid 1385), respectively. To

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prevent counting in results generated by chance, bearing little biological significance, and to have poor similarity with the query sequence, at the end of each BLAST search, a filter is applied with percent identity 90%-100%, E-value 0-1e-6, and percent identity of 85%-100% was applied.

From BLASTn and BLASTx results, one PBP2a amino acid sequence from every Staphylococcal or Mammaliicoccal species is retrieved. Topological prediction using Phobius, annotation analysis and PDB structural file retrieving using Uniprot [25], conserved domain analysis using INTERPRO, and pairwise structural comparison using RCSB were carried out respectively using the amino acid sequences or the PDB structural files they correspond to.

3. Results

3.1. The mecA gene and PBP2a protein exist in multiple staphylococcus and mammaliicoccus species. In the first BLASTn and tBLASTn search with no specific exclusions, aligned sequences came mainly from the *S. aureus* species. Apart from *S. aureus*, *S. epidermidis* also occupied a sizable portion of results under *S. aureus*, though *S. epidermidis* results coming from tBLASTn were significantly less than that coming from BLASTn. Both searches, excluding *S. aureus* (taxid 1280), yielded similar results, with aligned sequences mainly coming from *S. epidermidis*. Still, tBLASTn results included alignments from 4 species that were not included in BLASTn results. In the BLASTn and tBLASTn search with the exclusion of Staphylococcus (taxid 1279), both BLASTn and tBLASTn had the most results from M. sciuri. In BLASTn, there also existed results from four enterococcus species. In the search with the exclusion of Bacillus/Staphylococcus, valid results from BLASTn cover only the four enterococcus species, while tBLASTn yielded no significant results.

Table 1. BLAST analysis results

	BLASTn				tBLASTn				BLASTp				BLASTx			
	N/A	1280	1279	1385	N/A	1280	1279	1385	N/A	1280	1279	1385	N/A	1280	1279	1385
S. aureus	58				67				72				72			
S. epidermidis	31	79			9	66			9	21			9	21		
S. pseudintermedius	1	8			1	7			8	56			8	56		
S. argenteus	6	7			6	6			1	1			1	1		
S. haemolyticus					2	6			1	5			1	5		
S. hominis						3			3	4			3	4		
S. hyicus		1			1	1										
S. capitis	2	2			2	3										
S. warneri	1	1			1	2										
S. lugdunensis		1				2										
S. taiwanensis	1	1			1	1										
S. schleiferi						1										
S. caprae						2										
S. cohnii										1				1		
M. sciuri			29				36				65				65	
M. vitulinus			9				9				6				6	
M. lentus			2				1									
M. fleurettii			9				9				8				8	
M. abscessus											1				1	
E. hirae			1	1												
E. faecium			1	1												
E. faecalis			1	1												
P. vulgaris			1	1												
Total	100	100	53	4	90	100	55	0	94	88	80	0	94	88	80	0

BLASTp and BLASTx search yielded identical results with any exclusion applied. In the investigation with no exclusion, most results came from *S. aureus*. From the search with the exclusion of *S. aureus* (taxid 1280), alignments came mainly from the species *S. epidermidis* and *S. pseudintermedius*. In the investigation with the exclusion of the staphylococcus group, results primarily originated from the species *M. sciuri*. Still, in both BLASTp and BLASTx, the search with

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the exclusion of the Bacillus/Staphylococcus group (taxid 1385) yielded no significant results (see table 1). The amino acid sequences of PBP2a protein from nine species with the most resemblance to the query were retrieved for further analysis (see table 2).

Table 2. Retrieved amino acid sequences of PBP2a protein from nine different bacterial species

Species	Accession number
S.aureus	WP_000721310.1
S.epidermidis	MCC3680226.1
S.pseudintermedius	WP_140240747.1
S.argenteus	WP_088811536.1
S.haemolyticus	WP_080400705.1
S.hominis	OAW33239.1
S,cohnii	ADM43473.1
M.sciuri	WP_204254155.1
M.fleurettii	WP_203154062.1

3.2. Protein topology prediction suggests high conserveness of PBP2a in nine different staphylococcal and mammaliicoccal species.

FASTA sequences of the PBP2a protein from nine different staphylococcus/mammaliicoccus species (*S. aureus, S. epidermidis, S. pseudintermedius, S. argenteus, S. haemolyticus, S. hominis, S. cohnii, M. sciuri, and M. fleurettii*) were put into trials of transmembrane topology and signal peptide prediction tests. Results (as shown in fig 1) suggest that despite its presence in multiple different Staphylococcus and Mammaliicoccus, PBP2a protein shared similar protein signatures, all nine proteins bearing a transmembrane topology order of a cytoplasmic region with the length of 6aa, a transmembrane region with the length of 18-19aa, and a non-cytoplasmic region with a length of 644aa. The only minor difference in the protein signature between species is that the PBP2a protein sequences of *S. epidermidis, S. pseudintermedius, S. argenteus, S. haemolyticus, M. sciuri, and M. fleurettii* bear a transmembrane region with the length of 19aa, differing from that of the others whose same region is of 18aa in length.

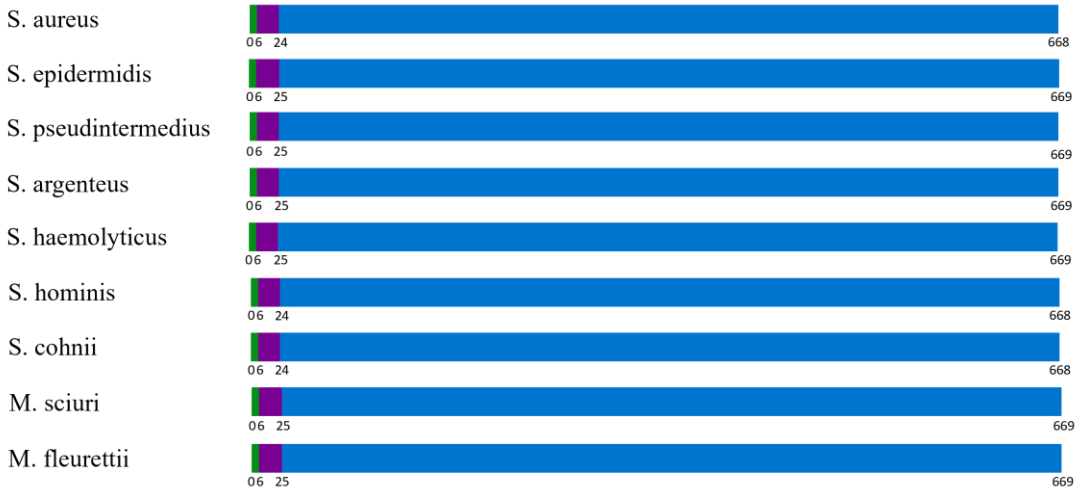

Figure 1. Phobius topology prediction of the cytoplasmic region (green), transmembrane region (purple), and non-cytoplasmic region (blue).

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3.3. High similarities of conserved domains in PBP2a indicate similar functions between bacterial species

The FASTA sequences of the PBP2a protein originated from nine different bacterial species and were put into INTERPRO trails to determine conserved domains within the nine sequences. Results (as shown in fig 2) indicated that all nine sequences share similar conserved domains. In the non-cytoplasmic regions of the nine proteins, the conserved domains *mecA*_N (115aa in length), PBP dimer (162aa in size), and PCN binding transpeptidase (312aa in length).

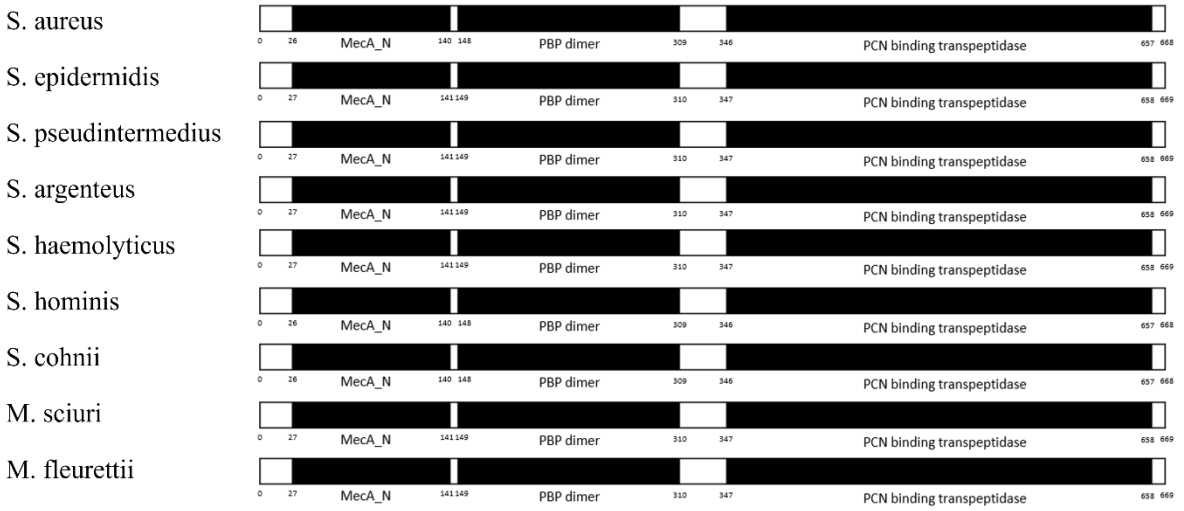

Figure 2. A comparison of the INTERPRO conserved domain analysis of PBP2a from nine different species.

4. Discussion

From BLAST search analysis, one may conclude that the *mecA* gene and PBP2a protein is somewhat prevalent in staphylococcal and mammaliicoccal genus. Apart from *S. aureus*, the *mecA* gene was also observed in 12 different staphylococcal bacterial species and four different mammaliicoccal species. These results indicate that many species in the two groups have been observed to bear the *mecA* gene in their genome. An explanation for such high prevalence. However, despite the high prevalence of the *mecA* gene, the PBP2a protein it codes for is somewhat less common in those species; only in a mere seven staphylococcal species and two mammaliicoccal species were homologous proteins observed in this study. This might be explained by the lack of data for PBP2a protein except for the few species (such as *S. aureus, S. epidermidis, M. sciuri*, etc.) where the most significant research takes place.

Apart from traces of the existence of the *mecA* gene and PBP2a protein in staphylococcal and mammaliicoccal bacterial species, BLASTn also suggested a nucleotide sequence similar to that of *mecA* in *S. aureus* exists in three enterococcus species, one mycobacterium species, and one proteus species. Even so, homologous proteins to PBP2a were not found in corresponding BLASTp and BLASTx searches. This might be explained as horizontal gene transfer that occurred purely by chance, with six foreign genuses acquiring the *mecA* gene through mobile genetic elements but unable to express them.

From Phobius topology prediction, conserved domain analysis, and protein 3-D structure comparison, it is of great significance that the PBP2a protein is highly conserved among different staphylococcal and mammaliicoccal bacterial species. From the analysis of 7 staphylococcal species and two mammaliicoccal species, it is clear that structure and domain function is identical among these species. This phenomenon is of conservation among antimicrobial genes and proteins and is not without logic. From the Phobius topological and conserved domain analysis, it can be concluded that a

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majority of the PBP2a protein is outside of the cytomembrane, and all the conserved domains are located within the non-cytoplasmic region of the protein, presumably to fulfil its function of deactivating the antimicrobial agent before it enters the cell. As long as the ligand, in this case, methicillin and other β lactam antibiotics do not change its structure, so would not the structure of the protein.

In the results collecting section from BLAST, it is easily noticed that despite BLASTp and BLASTx yielding identical results, BLASTn and tBLASTn generated more dissimilar results. This could be partly attributed to the differences in the translation algorithm between nucleotide to amino acids and adversely, for it is commonly known that one specific amino acid most likely corresponds to multiple codons. At the same time, the several-for-one pattern does not necessarily apply inversely.

5. Conclusion

BLAST results from this study indicated that both the *mecA* gene and its corresponding PBP2a protein are present and even of noticeable prevalence beyond *S. aureus*, with the presence of *mecA* spreading into other species of Staphylococci: *S. epidermidis*, *S. pseudintermedius*, S. argenteus, S. haemolyticus, S. hominis, S. hyicus, S. capitis, S. warneri, S. lugdunensis, S. taiwanensis, S. schleiferi, S. caprae, and into the Mammaliicoccus genus: M. sciuri, M. vitulinus, M. lentus, and M. fleurettii. Similarly, the PBP2a protein is also present in both groups, including BLAST hits in *S. epidermidis, S. pseudintermedius, S. argenteus, S. haemolyticus, S. hominis, S. cohnii, M. sciuri, M. vitulinus, M. fleurettii, and M. massiliense.* Furthermore, of the species bearing the PBP2a protein, the topology, domains, functions, and structure are highly conserved and highly similar to that of *S. aureus*.

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