Insilico Comparative analysis of inter and intra generic diversity of PbpA encoded by MecA gene of Staphylococcus aureus and their annotations

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Abstract : Methicillin resistant Staphylococcus aureus (MRSA) is an utmost concern by many researchers. MecA gene codes for the class of penicillin binding proteins (PBP) which are responsible for antibiotic methicillin resistance. It is a genetic cassette of 52 kb with two recombinase genes. In the present study, Penicillin binding protein sequences of ten intra generic Staphylococci were compared with five selected pathogens of other genera. pBLAST with PSI (Position Specific Iterated) algorithm was used to compare and analyse penicillin binding protein sequences from existing protein sequence databases. The percentage of identity with total query coverage and the relatedness were studied by constructing a dendrogram using MEGA 0.6 software. Further, the functional and structural annotations of ten intra and inter generic PBPs were compiled from Pfam and Uniprot databases for understanding the functional and structural domains.

IndexTerms **- mecA gene, comparative analysis, resistance, pBLAST, PSI BLAST and Protein Phylogeny.**

I. INTRODUCTION

Methicillin resistant S. aureus (MRSA) becoming a primary public health apprehension all over the world. Comparatively much incremented with its morbidity and mortality among other pathogenic bacteria [1]. Resistance of staphylococci to methicillin and all β-lactam antibiotics is associated with the low affinity of a penicillin-binding protein, PBP2a, which is not present in susceptible staphylococci and is a foreign DNA origin encoded by mecA gene [2]. mecA gene of staphylococcal chromosome also contains the genetic structures such as Tn554, pUB110, and pT181 that has resistance to non-β-lactam antibiotics. In staphylococcal genera, the prevalence of MRSA is predicted to be increased due to horizontal transfer of SCCmec genetic cassette [3].

Staphylococcus species exists in skin microflora of various animals and responsible for skin infections, commonly found in domestic animals [4]. Staphylococcus aureus causes threatening invasive infections on mild skin and rapidly develop resistance to different antibiotics which are in medical use [5]. Staphylococcus becoming more survival in the presence of semi-synthetic penicillins like methicillin and oxacillin are said to be methicillin resistance. Methicillin resistance and their treatment is a disquieting condition, because of its resistance to beta-lactam antibiotics and also to a wide variety of antibiotics [6]. The first report of MRSA was in England in 1961 [7].

Bacterial genetic evolutionary variation typically ascends the resistance over diverse antibiotics as the result of target proteins against the direct antibiotic attack. Based on the defensive bacterial gene mutations continually strike the same cellular sites remains to be one of the major cause of bacterial resistance over broad range of antibiotics. Henceforth, the novel antibiotic development, with the preliminary investigations may lead to design the specific constituents that anchor innovative binding sites are promising among the enzymes of core bacterial metabolism through constructing the novel targets [8].

In the present study, the genetic variations of mecA gene coding penicillin binding protein among the clinical pathogens such as intra species Staphylococcus aureus and other genus such as inter generic and inter species pathogens were studied through Insilico comparative analysis, structural and functional predictions, domain analysis and evolutionary relationships among these proteins by phylogenetic tree analysis.

II. MATERIAL AND METHODS

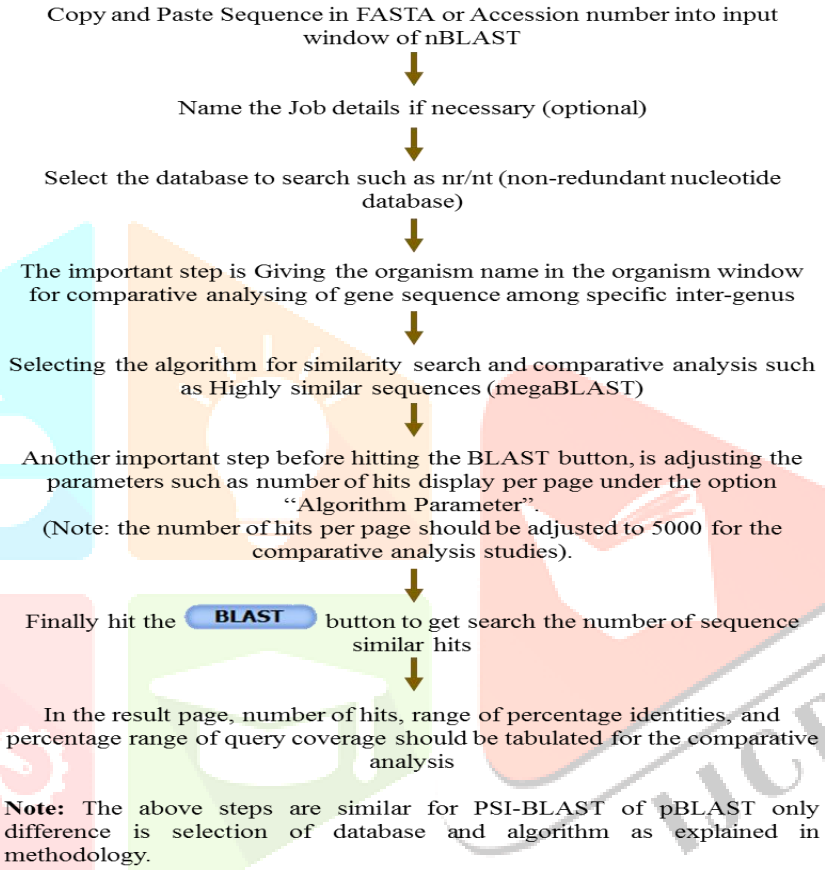
2.1 Retrieval of Nucleotide and Protein sequences

The preliminary nucleotide sequence of mecA gene of Staphylococcus aureus has retrieved from European Nucleotide Archieve (ENA) for searching the NCBI (National Centre for Biotechnology Information) nucleotide database for the retrieval of ten intra-species gene sequences of the genus Staphylococcus. These sequences were used for megaBLAST [9] (Highly similar-Basic Local Alignment Search Tool) comparative analysis against the inter-generic five pathogenic micro-organisms. As the nucleotide megaBLAST has no hits found, further protein sequences were retrieved from trEMBL [10] protein subsets for analysing protein PSI-BLAST [11] for inter-generic comparative analysis. The UniProtKB (Universal Protein KnowledgeBase), penicillin binding protein and Adaptor protein sequences were retrieved for functional and structural domain analysis and their intra-species and inter-species phylogenetic studies.

2.2 Comparative analysis of mecA nucleotide and protein sequences by NCBI mega and PSI-BLAST

pBLAST (proteinBLAST) was performed for the trEMBL protein sequences using PSI BLAST as an algorithm for inter-genus comparative analysis, since there are no hits found for nBLAST. The procedure followed for pBLAST is same as explained in the above flowchart, the database selection and algorithm selection will be different such as non-redundant protein sequence database and PSI-BLAST as algorithm. After the BLAST hit, the results were tabulated.

The retrieved ten inter-species nucleotide sequences were subjected to megaBLAST using the nBLAST programme as shown in the flowchart 1.



Flowchart 1: Procedure for comparative analysis of mecA proteins both intra and inter specially.

2.3 Structural and Functional annotations of UniProt intra species and inter species mecA proteins

UniProt is a universal protein resource which is single protein annotation database. Generally annotations are studies into two ways such as computational annotations and literature based annotations. The present annotation work was carried out through computational annotations which are readily available on UniProt database. The ten intra species and six inter species protein annotation table has been generated as given in the UniProt resource.

2.4 Domain analysis

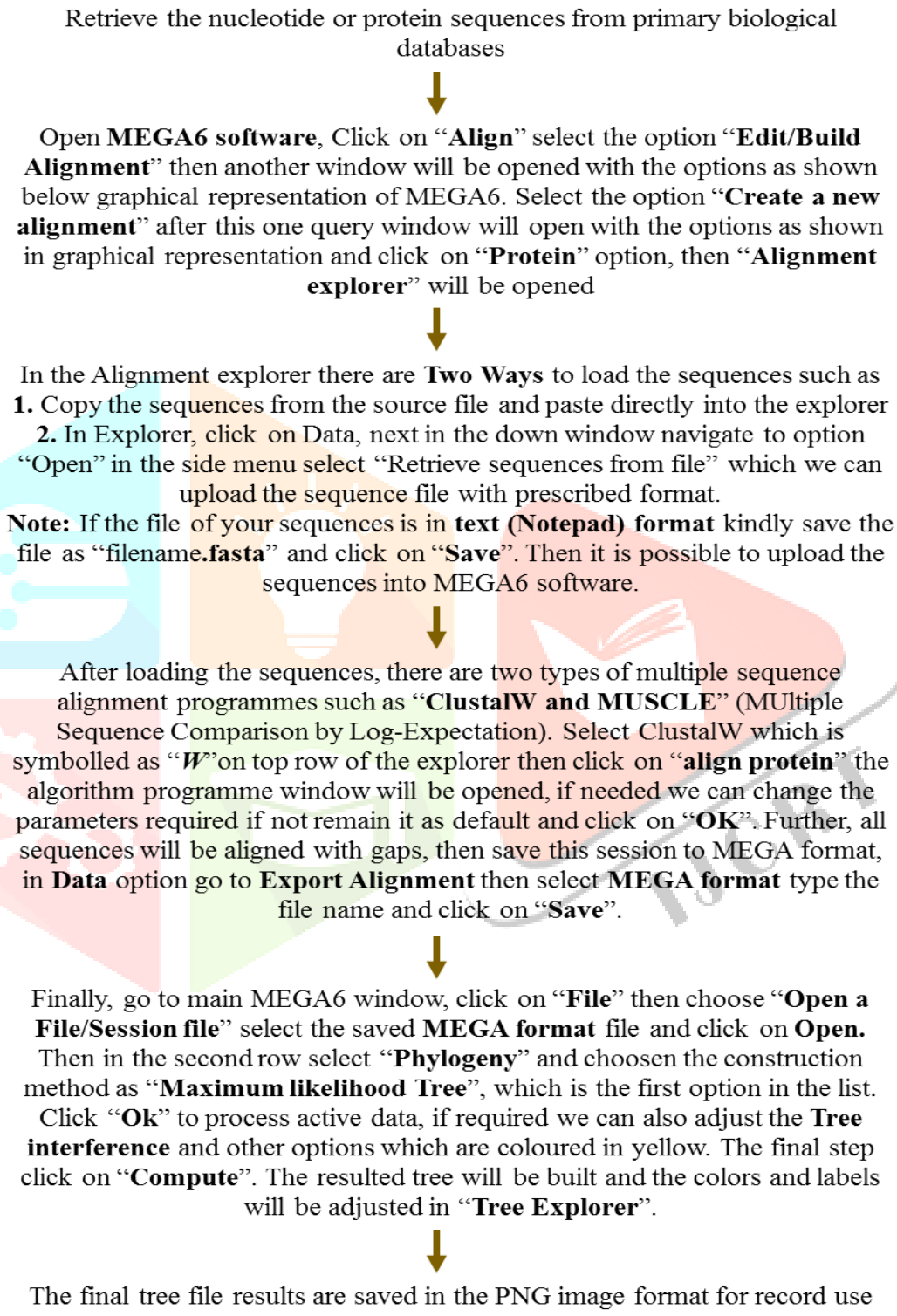
The functional regions in the protein sequences was enumerated using the UniProt domain datasets of Pfam [12] (Protein Families) and InterPro [13] (Integrated Protein signatures) domain databases. The amino acid position and active site determination was tabulated for all proteins of both selected intra and inter species penicillin binding proteins.

2.5 Phylogenetic relationship of intra and inter species mecA protein

Every organism has a definite life span on earth, only varies on nature of organism like the difference among visible and invisible one. The new trends in science and technology such as Insilico taxonomical identification of organisms made us to understand their evolutionary relationships. If the gene that encodes a protein get changed (mutation) during its multiplication from generation to generation, then the protein may get resistant to various molecules they interact. The retrieved intra and inter species penicillin binding proteins of Staphylococcus and other seven relevant human pathogens were evolutionarily compare and developed a dendrogram (Phylogenetic tree) using MEGA6 [14] software. MEGA (Molecular Evolutionary Genetics Analysis) is a

comprehensive tool for studying automatic and manual sequence alignment, constructing phylogenetic trees, mining web-based databases, reckoning rates of molecular evolution, and analysing evolutionary hypotheses.

The following flowchart 2 depicts the way of analysing the retrieved penicillin binding proteins of intra and inter species and constructing the phylogenetic tree for understanding the distantly relatedness among selected Methicillin and other class of β-Lactam antibiotic resistant pathogens.



Flowchart 2: Steps for operating MEGA6 software for the construction of phylogenetic tree

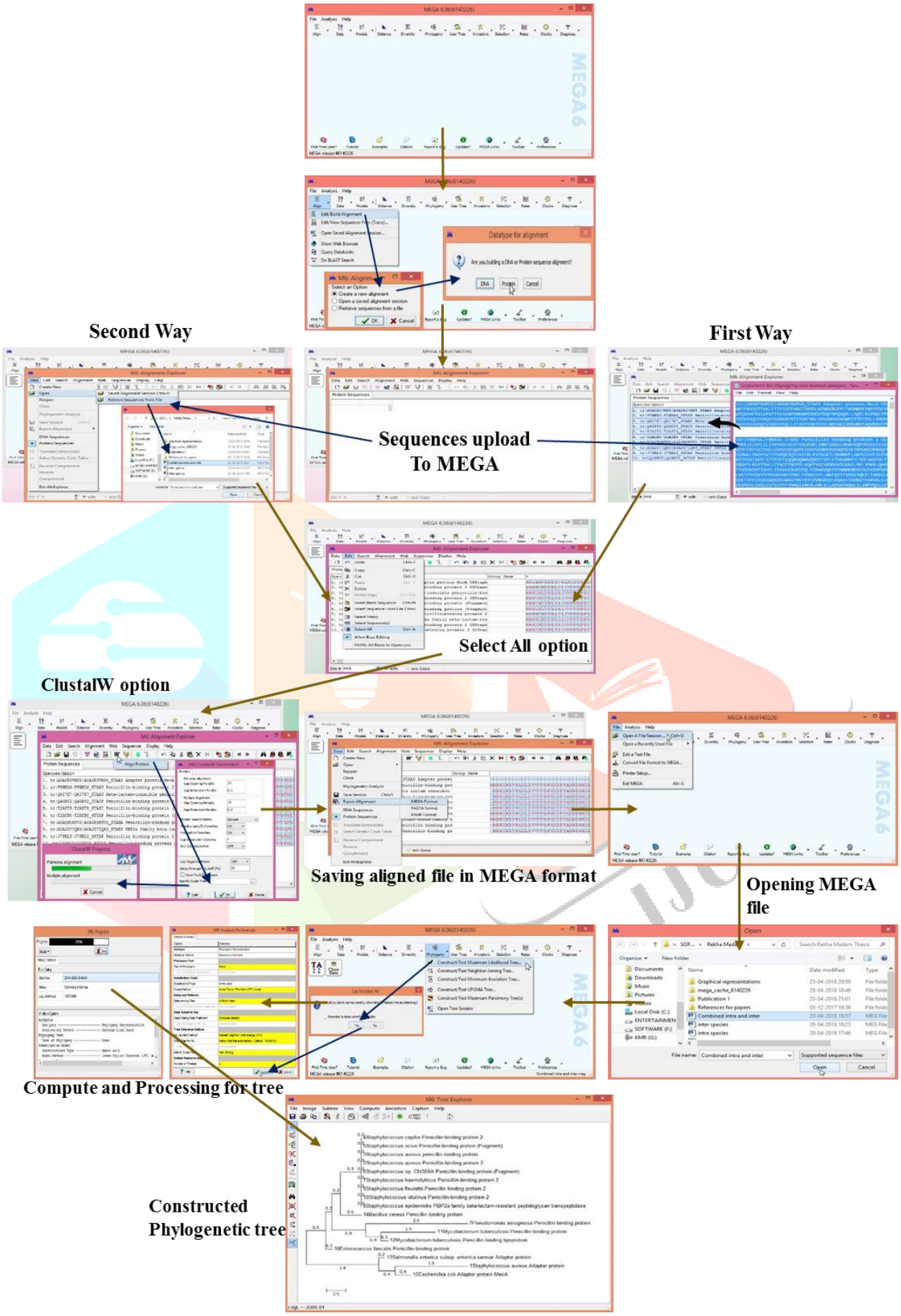


Fig. 1. Graphical representation of constructing the Phylogenetic tree using MEGA6

III. RESULTS AND DISCUSION

3.1 Nucleotide and Protein sequences retrieval

Nucleotide sequences of mecA genes were retrieved from NCBI database were tabulated (table 1). As the PSI BLAST of nucleotide sequences were not resulted with best hits, hence the trEMBL protein sequences were retrieved and the accession numbers were tabulated as shown in table 1. The functional and structural annotations of ten trEMBL protein sequences were observed to be similar in both Pfam and InterPro database, thus, another relevant ten staphylococcal species penicillin binding proteins and its adapter proteins were retrieved from UniProt as shown in table 1 for domain analysis studies as shown in table 3.

Table 1. Accession numbers of retrieved nucleotide and protein sequences

Sl. No.	NCBI accession numbers	trEMBL sequences accessions	Phylogeny Sequences from UniProt (Intraspecies)	Other pathogen sequences from UniProt (Interspecies)
1	DQ227900 (ENA)	-Do-	A0A0E0VMS8	A0A0U0RC14
2	NG_047944.1	-Do-	F8WKG6	A0A0T9MF02
3	NG_047939.1	-Do-	Q6I7E7	A0A1S0Z9M5
4	NG_047941.1	-Do-	Q4GXY2	Q9LBL1
5	KF058905.1	T2AST5 (UniProt)	T2AST5	A0A209N2P9
6	KF058903.1	T2AUB5 (UniProt)	T2AUB5	Q47800
7	HE978799.1	J7SAX8 (UniProt)	A0A0B5KSV2	Q07806
8	HE978798.1	J7RT55 (UniProt)	A0A2G7IQH3	---
9	HE978794.1	J7SAX6 (UniProt)	J7SBL3	---
10	AM048802.2	Q4GXY5 (UniProt)	Q4GXY5	---

3.2 Intra and Inter species comparative analysis of PBPA protein

The Insilico comparative analysis of PBPA [15] proteins of intra Staphylococcal species and relevant inter generic pathogens for the evolutionary variations of this protein encoded by mecA gene family. Based on the percentage of similarity resulted in PSI-BLAST with both Better than Threshold (BT & B) and Worse than Threshold (WT & W) were analysed. The selected 10 Staphylococcal sp. were hit against the selected five pathogenic genera with taxonomic ID's such as Mycobacterium (taxid: 1773), Salmonella (taxid: 90370), Plasmodium (taxid: 5820), Escherichia (taxid: 561) and Pseudomonas (taxid: 286). The results with minimum and maximum percentages of identity of both BT and WT, minimum and maximum percentages of query coverage (QC) were studied and tabulated in table 2. Seq1 to Seq4 among Mycobacterium showed varied sequence similarities shows the diverse genetic changes from Staphylococcus sp. whereas from Seq5 to Seq10 showed similar. There are no much variations among sequences were found in Salmonella and Plasmodium sp. except among the first Seq1. Escherichia sp. showed varied result in different subsets such as Seq1 is different from Seq2, Seq3, but these two were varied comparatively among Seq4-6, the similar divergence was observed among Seq7-10 from the all Seq1-6. Pseudomonas sp. [16] displayed highest hits among all other genus shown from Seq4-10, whereas Seq2, 3 are displayed moderately and Seq1 exhibited huge difference among number of hits and their percentage of similarities. This study indicates the fledged divergence of Penicillin Binding Protein A of Staphylococcal sp. against the other dreadful pathogenic genus was found as tabulated in table 2.

3.3 Structural and Functional analysis of Penicillin bind proteins

Annotations are the predictions and hypotheses that are collected by research literature and computational entries. Structural and functional analysis is the major among other annotation features available in databases [17]. Preferably the protein structures are enumerated by NMR and X-ray crystallography today with the advent of the bioinformatics tools such as protein 3D modelling and storage databases. UniPort provides the universal data about structure and function of the proteins, thus the structural data and functional information along with the subcellular location of proteins were studied and the results were tabulated in table 3 for the structural and functional variable analysis. Seq1 is the Penicillin adapter protein and rest are Penicillin Binding Protein A. Seq2, 4 and 10 has modelled proteins available in modelling severs and databases, the rest of the Sequences of Staphylococcus sp. has found no structural data, but Seq8 has made redundant on 25th Apr. 2018. The functional studies showed that all are positive for penicillin binding and response to antibiotics except Seq3 has an extra function such as serine-type D-Ala-D-Ala carboxypeptidase activity. Seq1 for Subcellular location has shown no data, remaining all 9 sequences shown Transmembrane, integral component of membrane. Comparatively the seven interspecies pathogens of Seq11-13 showed no data and Seq14-17 has structural information, only Seq17 has an entry of RCSB-PDB [18]. The functions among these sequences are showing penicillin binding, response to antibiotic except Seq13 and 15 which has no data for all the three sections. Among functions Seq17 has many variable functions as shown in table 3 [19]. This study depicts the protein conformational variations with respect to their valuable functions among the bacterial cells.

Table 2. Percentage based comparative analysis of Intra and Inter *Staphylococcal* mecA gene encoding for Penicillin binding protein using PSI BLAST
B-Better than threshold (for No. hits); W-Worse than threshold (for No. hits); m-minimum percent; M-Maximum percent

Staphylococcus Sequence	Inter genus and species pathogenic microorganisms for comparative analysis of mecA protein based on the number of hits on PSI BLAST for trEMBL protein sequences (Note: Percentage of Identities found in Better than threshold [BT], Worse than threshold [WT] and the percentage of Query Coverage [QC] are covered as follows)																															
	Mycobacterium (taxid:1773)								Salmonella (taxid:90370)								Plasmodium (taxid:5820)								Escherichia (taxid:561)								Pseudomonas (taxid:286)							
	No. Hits		%BT		%WT		%QC		No. Hits		%BT		%WT		%QC		No. Hits		%BT		%WT		%QC		No. Hits		%BT		%WT		%QC		No. Hits		%BT		%WT		%QC	
	B	W	m	M	m	M	m	M	B	W	m	M	m	M	m	M	B	W	m	M	m	M	m	M	B	W	m	M	m	M	m	M	B	W	m	M	m	M	m	M
Seq-1	7	66	23	30	21	23	7	34	2	13	0	29	24	48	32	78	5	0	32	42	0	0	30	80	3	144	28	30	16	27	16	96	25	475	25	28	25	28	64	93
Seq-2	166	85	23	30	13	49	34	60	23	33	23	31	66	78	47	78	0	2	0	0	33	33	12	18	383	2	21	31	25	28	13	79	500	0	21	89	0	0	33	72
Seq-3	1040	469	18	38	17	34	8	75	23	33	47	78	21	56	34	78	0	2	0	0	25	33	12	18	383	2	21	31	25	28	13	79	500	0	21	89	0	0	33	72
Seq-4	168	74	20	29	13	21	7	90	23	9	23	31	28	48	4	78	0	2	0	0	30	33	12	15	405	4	20	31	25	28	13	80	2032	1923	48	92	11	38	30	94
Seq-5	170	87	20	31	20	36	6	91	23	33	23	32	28	48	4	79	0	3	0	0	25	33	11	18	426	4	20	31	25	28	13	80	2064	575	20	34	21	32	12	87
Seq-6	170	147	20	43	19	34	7	83	23	34	23	32	21	48	4	79	0	3	0	0	25	33	11	18	427	3	20	31	25	28	13	80	2449	671	20	34	21	29	8	87
Seq-7	170	84	21	43	19	36	6	77	30	178	23	45	19	64	2	78	0	2	0	0	28	33	11	12	580	6	19	31	21	29	13	80	2447	683	20	39	21	38	13	81
Seq-8	172	142	20	43	19	34	7	82	22	33	23	32	21	48	4	78	0	3	0	0	25	33	11	18	581	5	20	31	21	29	13	80	2449	671	20	34	21	29	8	87
Seq-9	172	71	20	43	19	34	7	82	22	9	23	31	28	48	4	78	0	3	0	0	28	33	11	14	580	6	19	31	20	29	13	80	2447	667	20	39	21	38	13	86
Seq-10	172	84	20	43	19	36	7	82	22	33	23	31	28	48	4	78	0	2	0	0	28	33	11	12	580	6	19	31	21	29	13	80	2447	683	20	39	21	38	8	86

Table 3. Structural and functional analysis of PBPA of Staphylococcus and other relevant pathogens.

Sl. No.	UniProt Accession numbers for structure and functional analysis based on available annotations			
	Intraspecies Accesssions	**Structural**	**Molecular Functional and Biological Process**	**Subcellular location**
1	A0A0E0VMS8	No data	protein binding, bridging	No data
2	F8WKG6	SMR, ModBase, MobiDB	penicillin binding	Transmembrane, integral component of membrane
3	Q6I7E7	No data	penicillin binding, serine-type D-Ala-D-Ala carboxypeptidase activity, response to antibiotic	Transmembrane, integral component of membrane
4	Q4GXY2	ProteinModelPortal ModBase MobiDB	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
5	T2AST5	No data	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
6	T2AUB5	No data	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
7	A0A0B5KSV2	No data	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
8	A0A2G7IQH3	On 25 April 2018 this entry made redundant		
9	J7SBL3	No data	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
10	Q4GXY5	ProteinModelPortali SMR ModBase MobiDB	penicillin binding, response to antibiotic	Transmembrane, integral component of membrane
	Interspecies Accessions			
11	A0A0U0RC14	No data	penicillin binding, response to antibiotic	No data
12	A0A0T9MF02	No data	penicillin binding, response to antibiotic	No data
13	A0A1S0Z9M5	No data	Not mentioned	No data
14	Q9LBL1	ProteinModelPortal ModBase MobiDB	penicillin binding, response to antibiotic	No data
15	A0A209N2P9	No data	Not mentioned	No data
16	Q47800	ProteinModelPortal ModBase MobiDB	penicillin binding, response to antibiotic	No data
17	Q07806	PDBe RCSB PDB PDB ProteinModelPortali SMR ModBase MobiDB	penicillin binding, response to antibiotic, peptidoglycan glycosyltransferase activity, serine-type D-Ala-D-Ala carboxypeptidase activity, cell wall organization, peptidoglycan biosynthetic process, regulation of cell shape	Transmembrane, integral component of membrane, Topological domain, Plasma membrane

3.4 Domain analysis by Pfam and InterPro

The structural and functional properties of every protein depends on the presence of respective domain. The domain analysis from UniProt has retrieved and analysed as shown in table 4. Among all selective PBPA proteins of Staphyloccus sp., except Seq1 an adapter protein has only one domain in Pfam and two on InterPro. Seq8 has been redundant, and all other entries showed three domains in Pfam and six on InterPro databases. Among interspecies domains Seq11 has two domains in Pfam which is from Mycobacterium which is a similar adapter protein compared with Seq1 and three on InterPro. Another adapter protein sequences Seq13 and 15 has similar domains that of Seq1. Seq12, 14 and 16 are similar to that of Seq2. Among all the interspecies domains Seq17 has diverse set of functional and structural domains as shown in table 4. The analysis depicts the average diverse among the presence of domain on both Pfam and InterPro databases.

Table 4. Domain analysis for variable regions among intra and inter pathogenic species.

Sl. No.	UniProt Accession numbers	Pfam domains		InterPro domains	
	Intra species	Domain ID	Name	Domain ID	Name
1	*A0A0E0VMS8	PF05389	MecA	IPR038471 IPR008681	MecA_C_sf Neg-reg_MecA
2	*F8WKG6	PF05223 PF03717 PF00905	MecA_N PBP_dimer Transpeptidase.	IPR012338 IPR007887 IPR032710 IPR005311 IPR036138 IPR001460	Beta-lactam/transpept-like MecA_N NTF2-like_dom_sf PBP_dimer PBP_dimer_sf PCN-bd_Tpept
3	Q6I7E7	Similar as *F8WKG6			
4	Q4GXY2	Similar as *F8WKG6			
5	T2AST5	Similar as *F8WKG6			
6	T2AUB5	Similar as *F8WKG6			
7	A0A0B5KSV2	Similar as *F8WKG6			
8	A0A2G7IQH3	On 25 April 2018 this entry made redundant			
9	J7SBL3	Similar as *F8WKG6			
10	Q4GXY5	Similar as *F8WKG6			
	Inter species				
11	A0A0U0RC14	PF05223 PF00905	MecA_N Transpeptidase	IPR012338 IPR007887 IPR001460	Beta-lactam/transpept-like MecA_N PCN-bd_Tpept
12	A0A0T9MF02	Similar as *F8WKG6			
13	A0A1S0Z9M5	Similar as *A0A0E0VMS8			
14	Q9LBL1	Similar as *F8WKG6			
15	A0A209N2P9	Similar as *A0A0E0VMS8			
16	Q47800	Similar as *F8WKG6			
17	Q07806	PF17092 PF00912 PF00905	PCB_OB Transgly Transpeptidase	IPR012338 IPR001264 IPR023346 IPR036950 IPR031376 IPR001460	Beta-lactam/transpept-like Glyco_trans_51 Lysozyme-like_dom_sf PBP_transglycosylase PCB_OB PCN-bd_Tpep

3.5 Evolutionary relationships of mecA coding penicillin binding proteins among Staphylococus and other pathogens

The major species diversity and their genetic diversity will be studied based on the nucleotide or protein sequences conservation from one generation to the other. The penicillin binding proteinA of ten Staphylococcal sp. and other intergenus and interspecies penicillin binding proteins were phylogenetically studied using MEGA6 software and their constructs were represented as Intraspecies phylogeny (Fig. 2), Interspecies phylogeny (Fig. 3) and combined phylogeny both target and test pathogens for studying the complete divergence among mecA gene [20] and its product PBPA. The interspecies phylogeny shows the adapter protein has much distant compare to other nine sequences of Staphylococcus sp. and the PBPA sequences are completely divergent with zero distance and indicates the most similarities among both functions and structures. There are much distant relationships were found among the interspecies pathogens with different scale values indicates the divergence among the power of antibiotic resistance. The combine phylogeny of both group of pathogens clearly shows that they have different scale values and not correlated with each other as shown in Fig. 4. This study clearly indicates the divergence of the mecA genes among the selected pathogens have different multi-drug resistance power [21]. The color representations in all the phylogenetic trees indicates the similar relatedness and divergence among both intraspecies and interspecies PBPA diversity. The proven studies of these are not correlated in literature hence much literature support with specified discussions were not included.

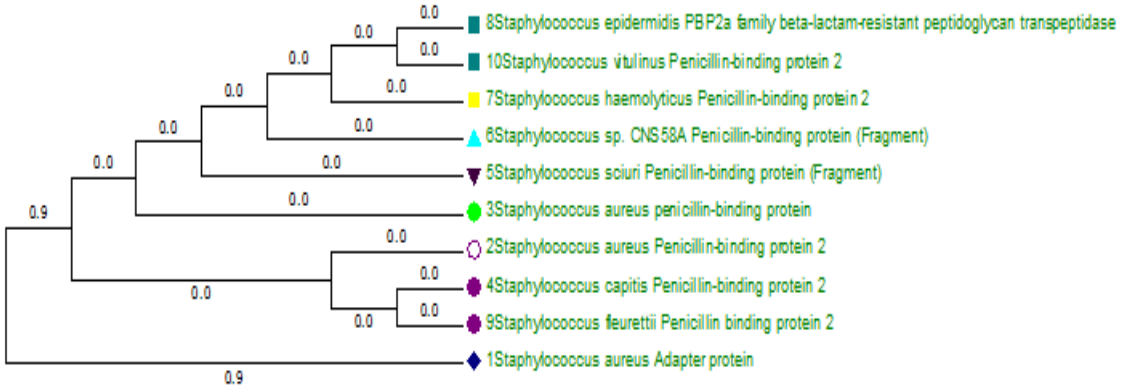


Fig. 2. Intra Staphylococcal distant relationship of penicillin binding protein

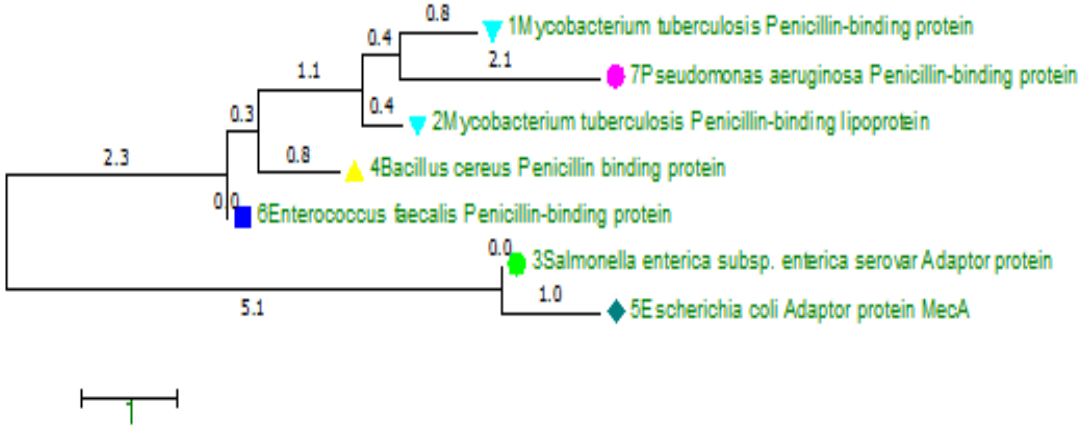


Fig. 3. Distant relationship of penicillin binding proteins of other interrelated (interspeices) pathogens

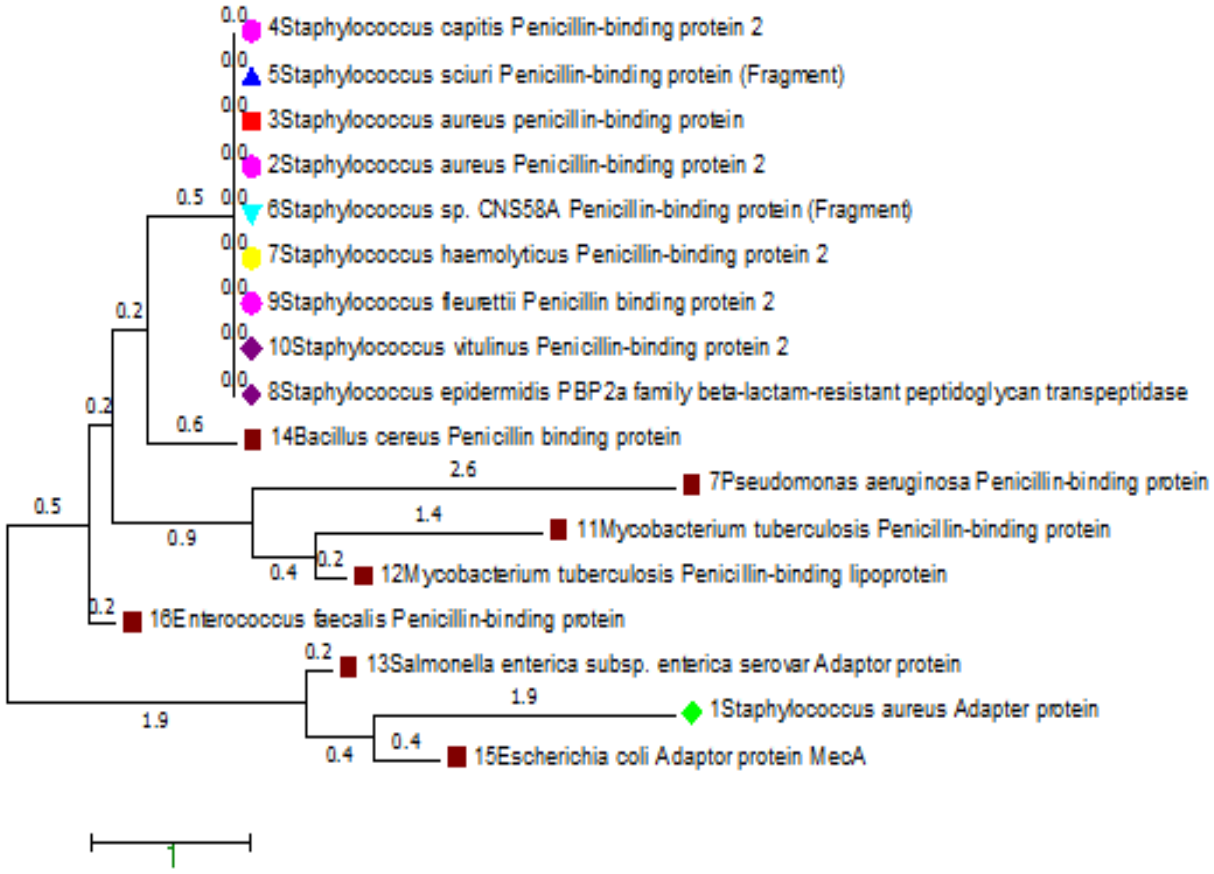


Fig. 4. Combined relatedness of penicillin binding protein of both intra and inter species of pathogenic microorganisms

IV. CONCLUSION

The computational analysis of MecA gene that codes for penicillin binding protein A of Staphylococcus sp. and other selected pathogens proven to be noteworthy data for clinical research. The comparative analysis and relevancy of PBPA protein for antibiotic resistance reveals the stage for designing the best analogues for the target proteins and reduce the multi-drug resistant phenomenon of diverse pathogenic bacteria. The target based redundancy of these genetic variations will give a better and healthier life to all human population around the world. With relevance of the generated data, further structure development of hypothetical PBPA protein and finding the drug sites and their molecules will be achieved.

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