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THE GENETIC AND EVOLUTIONARY BASIS OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS MRSA ST772: A COMPARATIVE AND EVOLUTIONARY BIOINFORMATICS STUDY

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Abstract

Staphylococcus aureus is a formidable opportunistic pathogen, with the emergence of **Methicillin-Resistant *Staphylococcus aureus*** (**MRSA**) posing a critical global health challenge. This study focuses on the **Bengal Bay Clone**, **MRSA ST772**, a virulent and multidrug-resistant Community-Acquired MRSA (CA-MRSA) strain increasingly linked to severe infections. Methicillin resistance in *S. aureus* is primarily mediated by the **mecA** gene, which encodes the penicillin-binding protein **PBP2a**. Despite the clinical significance of ST772, the genomic and evolutionary mechanisms underpinning its resistance phenotype remain poorly characterized.

To address this gap, we employed a comparative and evolutionary bioinformatics approach, analyzing whole-genome sequences of MRSA ST772 alongside Methicillin-Susceptible *S. aureus* (MSSA) and other MRSA strains. **Pan-genome analysis** revealed a conserved core genome but significant variations in the accessory genome, with ST772 uniquely harboring multiple antibiotic resistance determinants, including the **mecA** gene. **SCC*mec*Finder** confirmed the presence of *mec* class C2 and *ccr* class 5 components, suggesting a Type V (5C2) cassette and highlighting the role of **Horizontal Gene Transfer (HGT)** in its acquisition. Comparative genomic alignment identified structural differences and unique gene regions in ST772, potentially encoding resistance and virulence factors like the **Panton-Valentine Leukocidin (PVL)** toxin. **Phylogenetic analysis** of the mecA gene, corrected for recombination, provided strong evidence for the acquisition and spread of resistance via multiple HGT events. Finally, **protein domain analysis** confirmed the functional significance of the PBP2a protein, identifying the regulatory **MecA_N** domain and the β-lactam-resistant **FtsI** (PBP2a) domain.

These findings clarify the genetic architecture of resistance in MRSA ST772, underscoring the dynamic role of HGT and genomic flexibility in its evolutionary success and epidemiological spread. This research provides a foundation for developing targeted therapeutic and infection control strategies against this increasingly threatening multidrug-resistant pathogen.

1. Introduction

Staphylococcus aureus is a ubiquitous commensal organism of the human skin and mucosa that acts as a versatile opportunistic pathogen, causing a spectrum of diseases from localized skin infections to life-threatening conditions such as pneumonia, endocarditis, and septicemia (Lowy, 1998). The clinical management of *S. aureus* infections has been severely complicated by the widespread emergence of **antibiotic-resistant strains**, most notably **Methicillin-Resistant *Staphylococcus aureus*** (**MRSA**). MRSA has become a leading cause of both hospital- and community-acquired infections globally (David & Daum, 2010), presenting a major challenge to clinical therapeutics and infection control due to its resistance to β-lactam antibiotics (Chambers & DeLeo, 2009).

Methicillin resistance is primarily conferred by the **mecA** gene, which is located on a mobile genetic element called the **Staphylococcal Cassette Chromosome *mec*** (**SCC*mec***). The mecA gene encodes a modified transpeptidase, **Penicillin-Binding Protein 2a (PBP2a)**, which possesses a reduced affinity for β-lactam antibiotics, allowing the organism to maintain cell wall synthesis even in the presence of these drugs (Hartman & Tomasz, 1984).

Among the numerous MRSA lineages, the **Bengal Bay Clone**, **Sequence Type 772 (ST772)**, stands out for its virulence and multidrug resistance profile. Designated a Community-Acquired MRSA (CA-MRSA) strain, ST772 has been increasingly associated with severe clinical infections, including necrotizing pneumonia and serious skin and soft-tissue infections, affecting otherwise healthy individuals (Chen et al., 2014; Steinig et al., 2019). Its heightened virulence is partially attributed to the presence of the **Panton-Valentine Leukocidin (PVL)** toxin, which facilitates tissue necrosis and leukocyte destruction (Lina et al., 1999). Furthermore, the **multidrug-resistant** nature of ST772 complicates treatment with conventional antibiotics (Sharma et al., 2021).

Despite the growing clinical significance of MRSA ST772, the comparative genomic and evolutionary pathways underlying its methicillin resistance and successful dissemination are underexplored. While the roles of mecA and SCC*mec* elements have been studied in *S. aureus* generally, the specific genomic variations that differentiate ST772 from other MRSA and Methicillin-Susceptible *S. aureus* (**MSSA**) strains remain unclear (Turner et al., 2019). Understanding the genetic components contributing to ST772's resistance and virulence is crucial for revealing its evolutionary adaptability and epidemiological success.

1.1 Research Aims and Questions

This study aims to elucidate the molecular mechanisms behind methicillin resistance in *S. aureus* ST772 through a comprehensive bioinformatics analysis. The following key questions are addressed:

1. What are the core genomic differences between MRSA ST772 and MSSA/other MRSA strains?
2. What structural characteristics of the SCC*mec* element contribute to the resistance variations in ST772?
3. What is the role of **Horizontal Gene Transfer (HGT)** in the acquisition and evolution of methicillin resistance genes in ST772?

1.2 Significance

This research is vital for advancing the understanding of the genetic and evolutionary mechanisms driving methicillin resistance in MRSA ST772. By integrating **comparative genomics**, **phylogenetics**, and **protein function studies**, the study uncovers critical genetic determinants that contribute to the survival and global spread of the ST772 clone. An enhanced understanding of resistance gene acquisition and adaptation mechanisms can inform the development of novel therapeutic strategies, enhance antibiotic stewardship initiatives, and ultimately guide future research and public health strategies to combat the rising global burden of antibiotic-resistant *S. aureus* infections (Baquero et al., 2009).

2. Materials and Methods

2.1 Data Collection

Whole-genome sequences (WGS) of selected *Staphylococcus aureus* strains were retrieved from the National Center for Biotechnology Information (NCBI) database. The strains chosen for comparative analysis included the primary focus, **MRSA ST772** (Accession ID: CP045324.1), along with the control strains **MSSA Newman** (Accession ID: AP009351.1; chosen as the primary methicillin-susceptible reference lacking mecA), **USA300** (MRSA; Accession ID: CP000255.1), and **MSSA476** (MSSA; Accession ID: BX571857.1). All retrieved sequences had undergone quality control evaluation using **FastQC** prior to NCBI submission (Andrews, 2010), ensuring the reliability of the genomic data.

2.2 Genome Annotation

Genome annotation for MRSA ST772 and MSSA Newman was performed using **Prokka** (Seemann, 2014) to accurately characterize protein-coding sequences, resistance elements, and virulence factors. The pipeline was executed on a Linux-based bioinformatics platform.

2.3 Pan-Genome Analysis

To investigate evolutionary relationships and genomic diversity, pan-genome analysis was conducted on all four selected strains (MRSA ST772, MSSA Newman, USA300, and MSSA476) using the **Roary** pipeline (Page et al., 2015). This analysis categorized genes into **core**, **shell**, and **accessory** genomes, providing a comparative assessment of conserved and variable genomic elements. Input for Roary consisted of the GFF3 files generated during the Prokka annotation step.

2.4 Bioinformatics Screening for Resistance and Mobile Elements

Specialized tools from the Center for Genomic Epidemiology (CGE) were used for targeted screening:

- **Antibiotic Resistance Gene Identification: ResFinder** (Zankari et al., 2012) was used to identify acquired antimicrobial resistance genes in MRSA ST772 and MSSA Newman.
- **Plasmid Detection: PlasmidFinder** (Carattoli et al., 2014) was used to screen for known plasmid sequences in both strains.
- **SCC*mec* Detection: SCC*mec*Finder** (Kaya et al., 2018) was employed to identify and classify SCC*mec* elements in MRSA ST772.

2.5 Comparative Genomic Analysis

A detailed comparative genome analysis of MRSA ST772 against the methicillin-susceptible reference MSSA Newman was performed and visualized using the **Artemis Comparison Tool (ACT)** (Carver et al., 2005). ACT was utilized to map whole-genome sequence alignments, allowing for the identification of synteny, structural rearrangements, and unique genomic regions between the two strains.

2.6 Phylogenetic and Evolutionary Analysis

To trace the evolutionary trajectory and spread of resistance, full homolog identification and phylogenetic analysis of the mecA gene were carried out.

- **Homolog Identification:** BLASTn (Altschul et al., 1990) was used to retrieve mecA homologs.
- **Phylogenetic Tree Construction:** Multiple sequence alignment was performed with **ClustalW** (Thompson et al., 1994).
- **Recombination Correction:** The **Gubbins** software (Croucher et al., 2015) was used to detect and account for recombination events, generating a recombination-free phylogeny to accurately reflect the evolutionary history of mecA and associated resistance determinants.

2.7 Protein Domain Analysis

To characterize the structural basis of β-lactam resistance, the mecA gene sequence was translated into its corresponding PBP2a protein sequence using **Transeq** (EMBOSS suite) (Rice et al., 2000). The resulting amino acid sequence was subjected to a similarity search using **BLASTp** (Altschul et al., 1997) against the NCBI non-redundant protein database. The functional domain architecture of the top homolog was then examined using the conserved domain search feature integrated within BLASTp (Marchler-Bauer et al., 2015).

3. Results and Discussion

3.1 Genome Annotation and Resistance Profile

Genome annotation via Prokka confirmed a slight difference in total gene counts between **MRSA ST772 (2,651 genes)** and **MSSA Newman (2,697 genes)** (Table I). Critically, **MRSA ST772** was confirmed to harbor the β-lactam resistance gene **mecA** (encoding PBP2a) and **blaZ** (β-lactamase), along with other aminoglycoside and macrolide resistance genes. In stark contrast, **MSSA Newman** was devoid of these acquired resistance determinants, aligning with its methicillin-susceptible phenotype. These findings establish the foundation for the observed resistance profile in ST772 and the need for further comparative analysis.

Table I: Genome Annotation Overview

Strain	Total Genes	Resistance Genes	Virulence Genes
MRSA ST772	2,651	Present (mecA, blaZ)	Present
MSSA Newman	2,697	Absent	Present

3.2 Pan-Genome and Phylogenetic Analysis

The pan-genome analysis of the four *S. aureus* strains (ST772, Newman, USA300, MSSA476) identified a large, conserved **core genome** of **2,214 genes**, indicating a shared genetic backbone essential for basal function. The **shell genome** (1,089 genes), however, revealed considerable variability, reflecting strain-specific genes often associated with adaptation, resistance, and virulence. The gene presence/absence matrix confirmed that MRSA ST772 possesses unique gene clusters, strongly suggesting the acquisition of antimicrobial resistance determinants via HGT (Figure 1, Matrix).

The phylogenetic tree generated from the core genome alignment positioned **MRSA ST772** and **MSSA476** as closely related, supporting the hypothesis that MSSA lineages can evolve into MRSA upon acquisition of the mecA element. While the highly virulent **USA300** clustered within the MRSA group, the **MSSA Newman** strain was the most distantly related, emphasizing the distinct evolutionary trajectories of these clinical isolates.

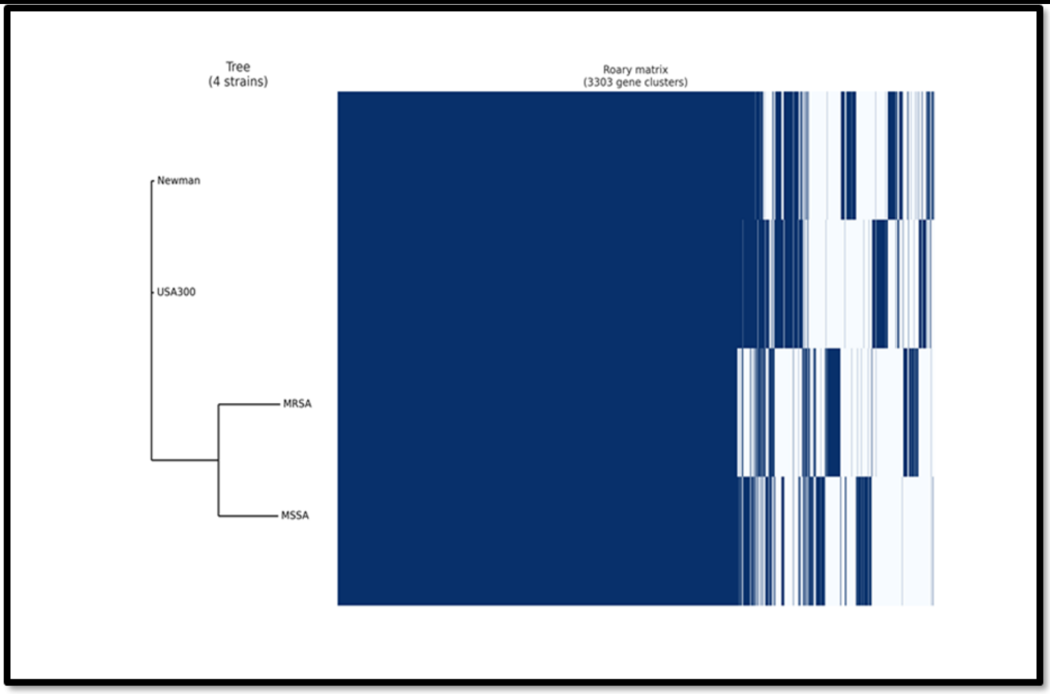
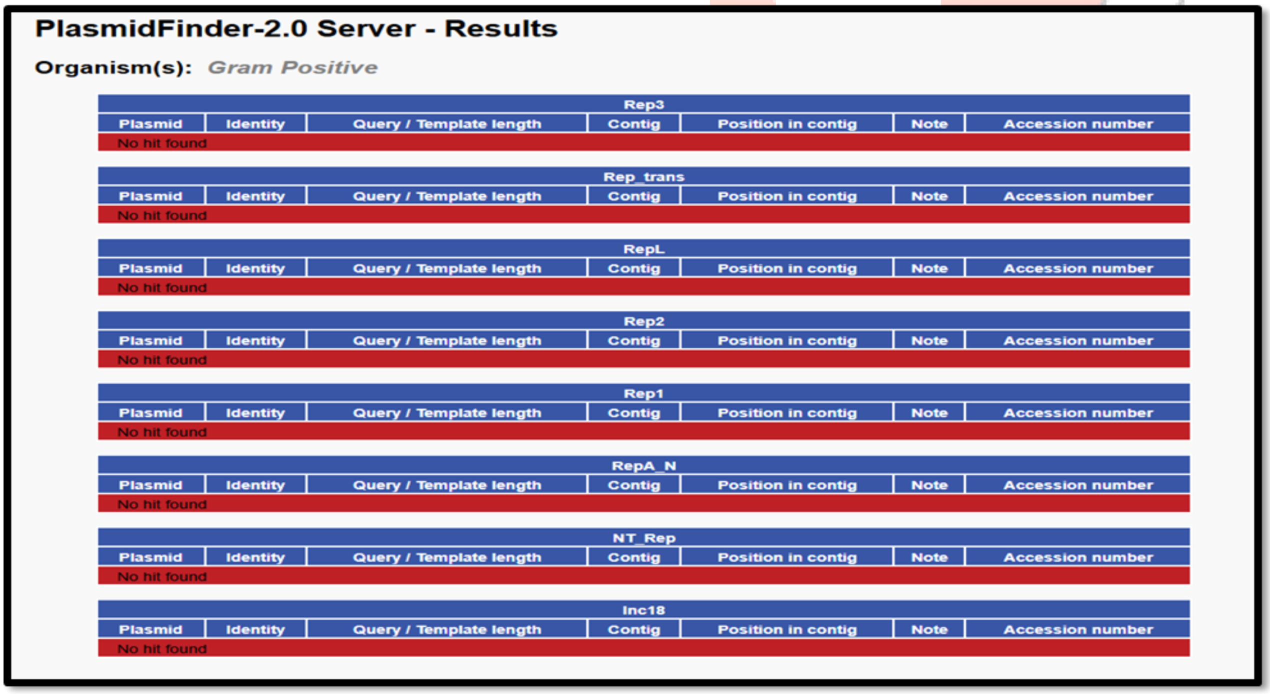


Figure 1: Comparative Pan-Genome Analysis: Core Gene Phylogeny and Variable Gene Presence/Absence Across S. aureus Strains.

3.3 SCC*mec* Detection and Mobile Elements

PlasmidFinder analysis found no known plasmid replicons in either MRSA ST772 or MSSA Newman (Figure 2), suggesting that the identified resistance genes, including mecA, are chromosomally encoded rather than plasmid-mediated.



PlasmidFinder-2.0 Server - Results

Organism(s): *Gram Positive*

Rep3						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

Rep_trans						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

RepL						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

Rep2						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

Rep1						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

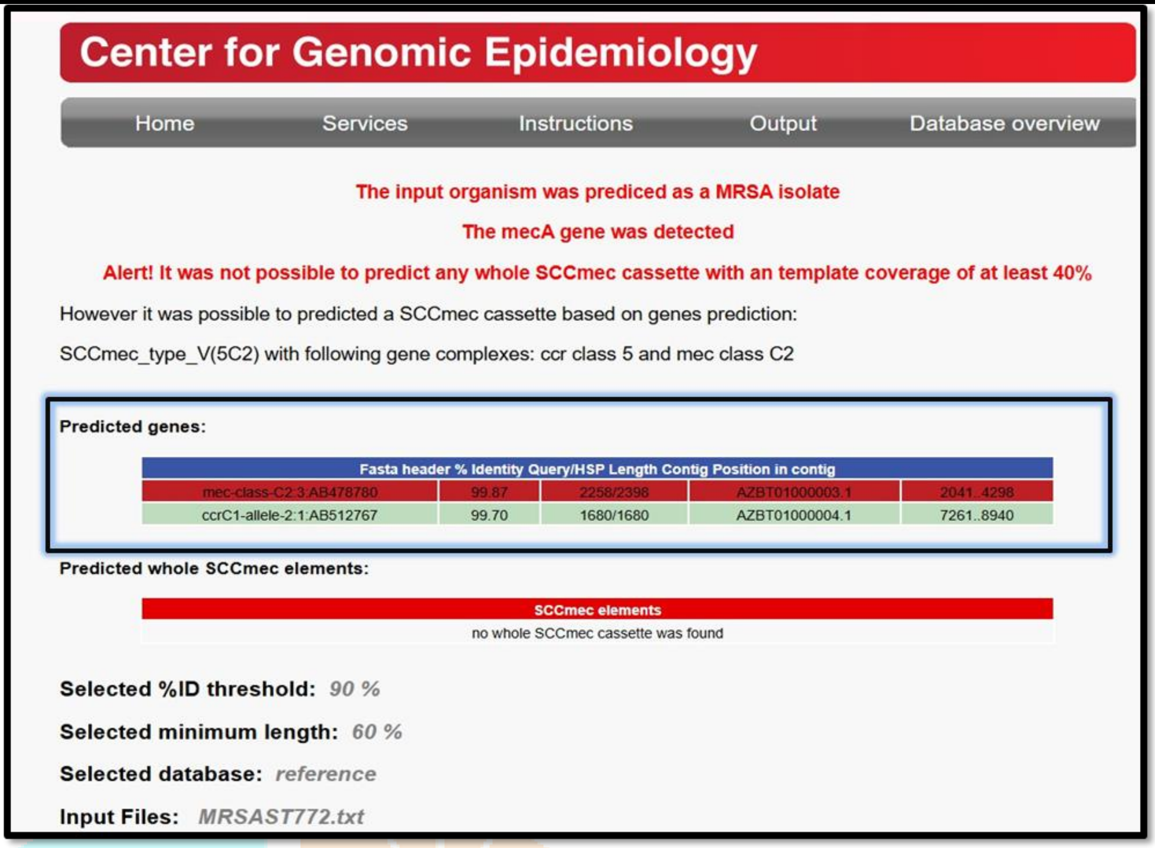
RepA_N						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

NT_Rep						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

Inc18						
Plasmid	Identity	Query / Template length	Contig	Position in contig	Note	Accession number
No hit found						

Figure 2: Absence of Known Plasmid Replicon Types in MRSA ST772 and MSSA Newman.

SCC*mec*Finder confirmed the presence of the **mecA** gene in MRSA ST772, thus validating its methicillin resistance (Figure 3). While an entire SCC*mec* cassette was not fully assembled or classified, the presence of **mec class C2** (99.87% identity) and **ccr class 5** (99.70% identity) components strongly suggests that MRSA ST772 carries a Type V (5C2) SCC*mec* element. The absence of any SCC*mec* components in the Newman strain underscores the acquisition of this mobile element as a pivotal event in the evolution of methicillin resistance in ST772.



The input organism was prediced as a MRSA isolate

The mecA gene was detected

Alert! It was not possible to predict any whole SCCmec cassette with an template coverage of at least 40%

However it was possible to predicted a SCCmec cassette based on genes prediction:

SCCmec_type_V(5C2) with following gene complexes: ccr class 5 and mec class C2

Predicted genes:

Fasta header	% Identity	Query/HSP Length	Contig	Position in contig
mec-class-C2:3:AB478780	99.87	2258/2398	AZBT01000003.1	2041..4298
ccrC1-allele-2:1:AB512767	99.70	1680/1680	AZBT01000004.1	7261..8940

Predicted whole SCCmec elements:

SCCmec elements
no whole SCCmec cassette was found

Selected %ID threshold: *90 %*

Selected minimum length: *60 %*

Selected database: *reference*

Input Files: *MRSAST772.txt*

Figure 3: SSCmecFinder Analysis of MRSA ST772

3.4 Comparative Genomic Architecture

The **Artemis Comparison Tool (ACT)** visualization highlighted the genetic similarities (red blocks) and significant differences (white gaps) between MRSA ST772 and MSSA Newman (Figure 4). The white gaps in the ST772 genome likely correspond to strain-specific genetic material, including the antibiotic resistance genes (mecA, blaZ) and potential virulence factors (e.g., PVL toxin), that contribute to its distinctive resistance and pathogenic profile. The presence of blue lines indicated genomic rearrangements, such as inversions or translocations, which are often mediated by mobile genetic elements. These structural changes could potentially alter gene regulation and expression, further influencing the fitness and survival of ST772.

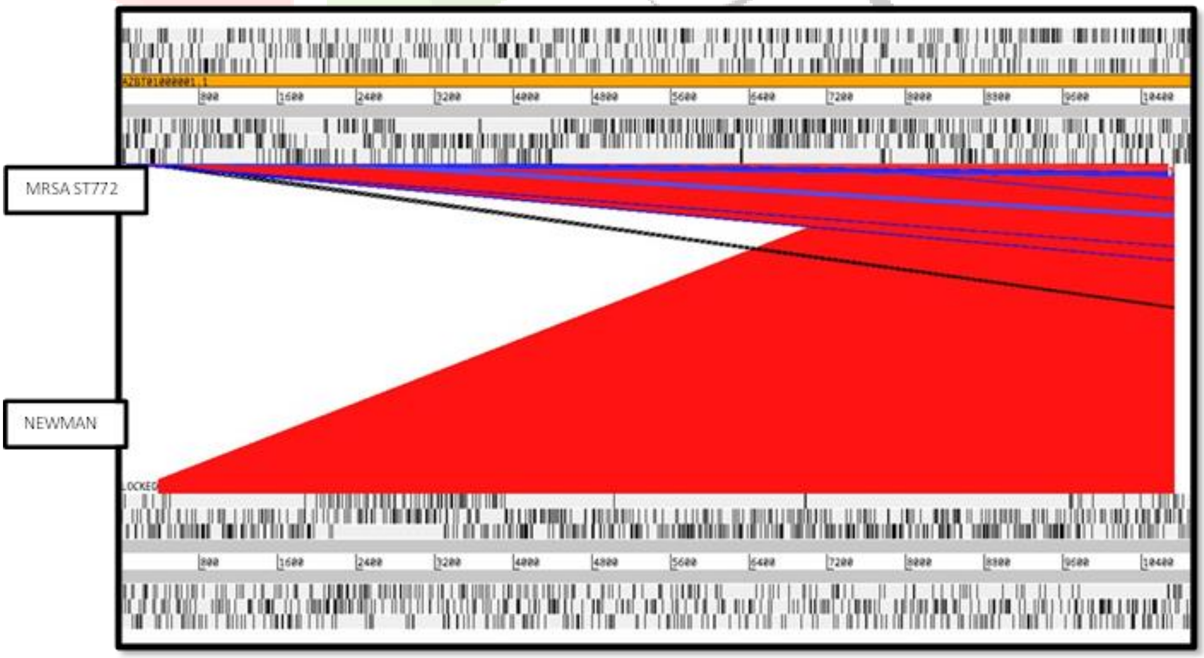


Figure 4: Comparative Genomic Map of MRSA ST772 and MSSA Newman (ACT).

3.5 Evolutionary Dynamics of mecA

Phylogenetic analysis of the mecA gene and its homologs demonstrated a high degree of sequence conservation, reflective of strong evolutionary pressure due to its essential role in resistance (Figure 5). The branching pattern strongly supported the notion that **HGT** has been a dominant force in the dissemination of mecA across different *Staphylococcus* strains.

The recombination-corrected phylogenetic tree generated by **Gubbins** (Figure 5) was essential for accurately resolving evolutionary relationships. The shorter branch lengths and the clustering of multiple independent lineages confirmed that methicillin resistance in *S. aureus* is not primarily a result of vertical inheritance but has been widely transmitted through frequent and distinct **HGT events**, leading to the rapid adaptation and global spread of resistance genes.

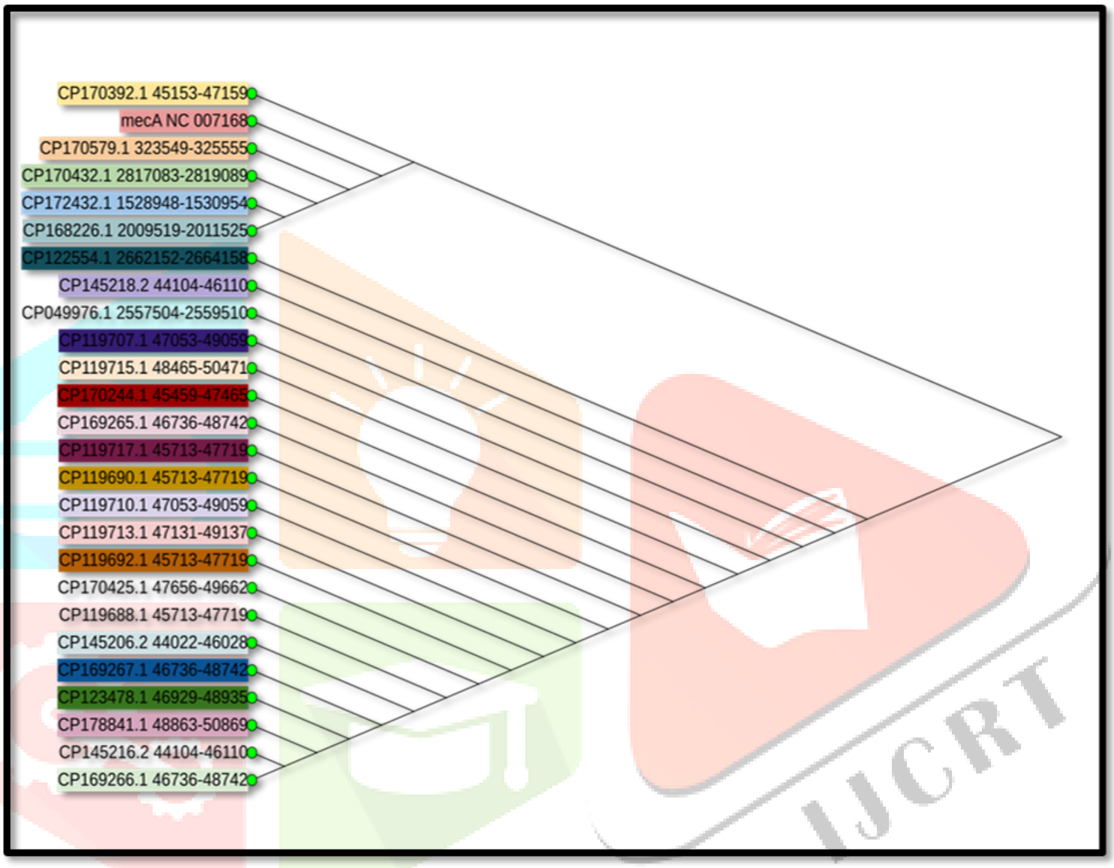
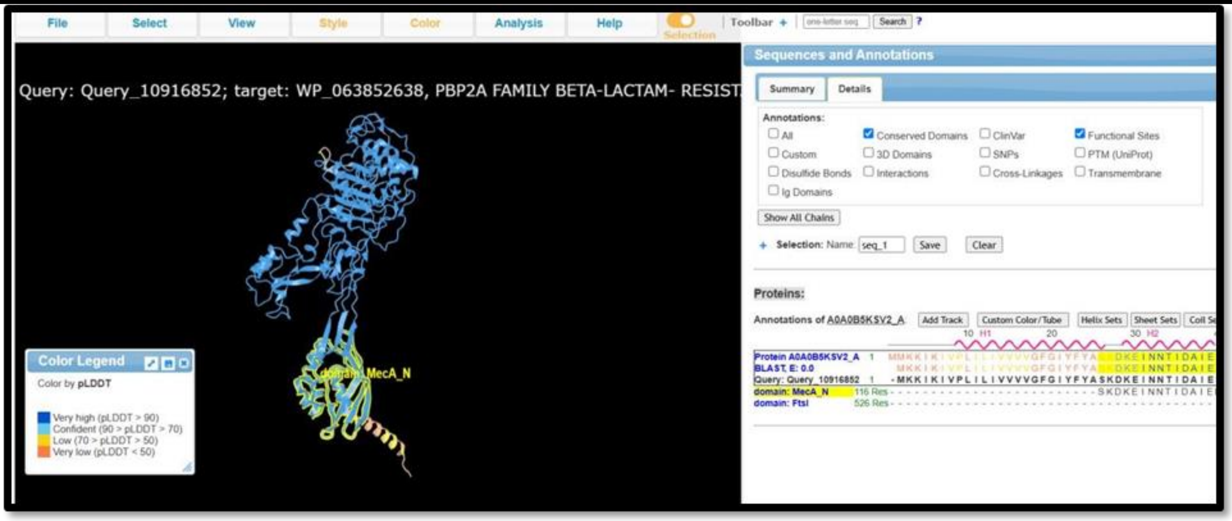


Figure 5: Phylogenetic Tree of mecA And Its 24 Homologs

3.6 Structural Basis of Resistance

Translation and domain analysis of the mecA gene revealed the presence of two critical domains in the PBP2a protein (Figure 6):

1. **MecA_N Domain:** Positioned at the N-terminus (116 residues), this domain is involved in the regulatory function of mecA expression and activity.
2. **FtsI Domain (PBP2a):** This large domain (526 residues) is the core functional component, directly responsible for providing β-lactam resistance. The low affinity of the PBP2a transpeptidase for β-lactam antibiotics ensures the continuation of peptidoglycan cross-linking and cell wall synthesis, a key survival mechanism for MRSA strains.



Query: Query_10916852; target: WP_063852638, PBP2A FAMILY BETA-LACTAM- RESIST

Color by **pLDDT**

- 🟦 Very high (pLDDT > 90)
- 🟦 Confident (90 > pLDDT > 70)
- 🟨 Low (70 > pLDDT > 50)
- 🟧 Very low (pLDDT < 50)

Annotations:

☐ All ☑ Conserved Domains ☐ ClinVar ☑ Functional Sites
☐ Custom ☐ 3D Domains ☐ SNPs ☐ PTM (UniProt)
☐ Disulfide Bonds ☐ Interactions ☐ Cross-Linkages ☐ Transmembrane
☐ Ig Domains

+ **Selection:** Name: seq_1

Proteins:

Annotations of A0A0B5KSV2_A

```
Protein A0A0B5KSV2_A  1   MMKKIKIVPLILIVVVVGFGIYFYASKDKEINNTIDAIE
BLAST, E: 0.0              MKKIKIVPLILIVVVVGFGIYFYASKDKEINNTIDAIE
Query: Query_10916852  1  -MKKIKIVPLILIVVVVGFGIYFYASKDKEINNTIDAIE
domain: MecA_N   116 Res -------------------------SKDKEINNTIDAIE
domain: FtsI     526 Res --------------------------------------
```

Figure 6: Domain Architecture of PBP2a Protein Involved in Methicillin Resistance

4. Conclusion

This comparative and evolutionary bioinformatics study successfully elucidated key genetic and structural factors contributing to methicillin resistance in the globally significant pathogen, **MRSA ST772**.

We confirmed that the methicillin-resistant phenotype of ST772 is primarily driven by the acquisition of the **mecA** gene, which is absent in the reference MSSA strain Newman. The pan-genome analysis highlighted the importance of a variable **accessory genome** in conferring adaptive advantages, while **SCC*mec*Finder** data strongly suggested the presence of a mobile Type V cassette acquired via **Horizontal Gene Transfer (HGT)**. This mechanism of dissemination was further supported by the **recombination-corrected phylogenetic analysis** of mecA, which confirmed a history of multiple genetic exchange events. Finally, the **protein domain analysis** of PBP2a provided a molecular structural basis for resistance, confirming the presence of the critical **FtsI** domain responsible for β-lactam insensitivity.

Collectively, these findings underscore the genomic flexibility and continuous evolutionary adaptation of MRSA ST772. By clarifying the molecular mechanisms driving resistance gene acquisition and maintenance, this research lays an essential groundwork for the development of potent, targeted strategies to effectively treat and control infections caused by this multidrug-resistant *S. aureus* lineage.

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