BIOPENDIX: Jurnal Biologi, Pendidikan dan Terapan
Volume 13, Issue 1, March 2026
Pages: 7-13
https://doi.org/10.30598/biopendixvol13issue1page7-13

P-ISSN:2407-4969
E-ISSN:2684-8341

Original Article

OPEN ACCESS

In Silico Detection of Antimicrobial Resistance and Virulence Genes in Methicillin Resistant *Staphylococcus aureus* Clinical Isolates: A Comparative Genomics Approach

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Submitted: July 31, 2025; Accepted: August 31, 2025; Available online: September 11, 2025; Published: March 31, 2026

Abstract. Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major public health threat in both community and healthcare settings due to its ability to evade β-lactam antibiotics and accumulate resistance to multiple drug classes. In this study, we sequenced and compared the genomes of 13 recent clinical MRSA isolates with two well-characterized reference strains (N315 and NCTC 8325). Using the ResFinder and Virulence Finder pipelines, we cataloged each strain's antibiotic resistance and virulence gene repertoire. All MRSA isolates carried the hallmark *mecA* gene, and most also harbored *blaZ*, encoding penicillinase. Additional resistance determinants were detected in various combinations, including aminoglycoside resistance genes (*aac(6')-Ie-aph(2")*, *aph(3')-III*), macrolide resistance genes (*erm(C)*, *mph(C)*), and chloramphenicol resistance (*cat* variants). On the virulence side, genes encoding α- and γ-hemolysins (*hla*, *hlgABC*) were universally present, and nearly all isolates carried phage-associated immune evasion factors (*sak*, *scn*). The total number of virulence genes ranged from 10 to 14 per genome, with two isolates harboring particularly gene-rich profiles. These findings highlight the genetic diversity of MRSA, where multidrug resistance coexists with a broad arsenal of virulence factors. Furthermore, this study demonstrates the efficiency of in silico screening tools for antimicrobial resistance surveillance and comparative genomics. Future research should integrate laboratory validation and clinical data to better link genomic profiles with patient outcomes.

Keywords: Antimicrobial Resistance; Comparative Genomics; Methicillin Resistant; *Staphylococcus aureus*; Virulence Factors; Whole-genome sequencing

How to cite this article:
Hidayat, M. T., Prayekti, E., Romdloni, M. A., & Syah, S. A. (2026). In Silico Detection of Antimicrobial Resistance and Virulence Genes in Methicillin Resistant Staphylococcus aureus Clinical Isolates: A Comparative Genomics Approach. *BIOPENDIX: Jurnal Biologi, Pendidikan dan Terapan, 13*(1), 7-13. https://doi.org/10.30598/biopendixvol13issue1page7-13

Journal homepage: https://ojs3.unpatti.ac.id/index.php/biopendix
Journal e-mail: jbiopendix@gmail.com

INTRODUCTION

Staphylococcus aureus is a formidable human pathogen, responsible for a wide spectrum of diseases ranging from skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, and sepsis (Bashir, 2025; Cheung et al., 2021). Among its variants, methicillin-resistant *S. aureus* (MRSA) has emerged as a major public health concern due to resistance not only to β-lactams but also to multiple antibiotic classes (Andrzejczuk et al., 2023; Bashir, 2025). This resistance primarily arises from the *mecA* gene, which encodes PBP2a, a penicillin-binding protein with reduced affinity for β-lactams (O'Neill, 2016). Frequently co-occurring is the *blaZ* gene, which confers penicillin resistance through penicillinase production (Andrzejczuk et al., 2023). Beyond antimicrobial resistance, MRSA strains harbor an extensive arsenal of virulence factors that enable colonization, immune evasion, and tissue destruction (Cheung et al., 2021; Touaitia et al., 2025).

Key virulence determinants include the phage-encoded immune evasion cluster (IEC) genes, such as *sak* (staphylokinase) and *scn* (staphylococcal complement inhibitor); which undermine host immune defenses (Bano et al., 2023). Cytolytic toxins such as α-hemolysin (*hla*) and γ-hemolysins (*hlgABC*) are nearly universal among clinical isolates, contributing to host cell lysis (Bashir, 2025; Larsen et al., 2012). Secreted enzymes, including aureolysin (*aur*) and the V8 protease family (*sspA/B/C*), further promote tissue invasion (Bano et al., 2023). Given this dual threat of antimicrobial resistance and virulence, dissecting the genomic architecture of MRSA is critical for epidemiological surveillance and the development of informed treatment strategies (Andrzejczuk et al., 2023; Laabei et al., 2015).

Advances in whole-genome sequencing (WGS) have revolutionized pathogen genomics, enabling high-throughput *in silico* detection of resistance and virulence determinants. User-friendly platforms such as ResFinder and VirulenceFinder automate BLAST-based screening against curated databases (Bortolaia et al., 2020; Touaitia et al., 2025). In this study, we employed ResFinder version 4.7.2 and VirulenceFinder version 2.0 to characterize antimicrobial resistance (AMR) and virulence genes in 13 clinical MRSA isolates alongside two well-characterized reference strains. Our objective was to map key resistance markers (e.g., *mecA*, *blaZ*, macrolide and aminoglycoside resistance genes) and virulence determinants (IEC genes, hemolysins, leukocidins, exoenzymes) across these genomes, thereby illustrating the utility of *in silico* screening approaches for AMR surveillance and comparative genomics.

MATERIALS AND METHODS

Isolate Selection

Thirteen clinical *Staphylococcus aureus* isolates representing diverse MRSA lineages were selected from a recent Malaysian collection (Che Hamzah et al., 2024). For comparison, two reference genomes were included: MRSA strain N315 and methicillin-susceptible *S. aureus* NCTC 8325.

Genome Retrieval and Preparation

Draft genome assemblies in FASTA format were retrieved from NCBI and uniformly formatted for downstream analyses.

Antimicrobial Resistance Gene Detection

Antimicrobial resistance (AMR) genes were identified using ResFinder v4.7.2 (Center for Genomic Epidemiology) with default parameters (Bortolaia et al., 2020). Genes meeting identity and length thresholds were recorded, with particular attention to *mecA*, *blaZ*, *erm* genes, *mph(C)*, aminoglycoside-modifying enzymes (*aac(6')-Ie-aph(2")*, *aph(3')-III*), and chloramphenicol acetyltransferases (*cat* variants).

Virulence Gene Detection

Virulence genes were screened using VirulenceFinder v2.0, focusing on host immune evasion factors (*sak, scn, chp, spa*), toxins (*hla, hlgABC*; PVL: *lukS-PV, lukF-PV*; enterotoxins *sea, sec, seg, sei*), and extracellular enzymes (*aur, sspA/B/C, lip*) (Touaitia et al., 2025). Only full-length or high-confidence matches were considered present.

Data Compilation and Analysis

Presence/absence matrices and gene counts were generated in Microsoft Excel. Comparative analyses were performed to highlight both shared and distinct AMR and virulence gene profiles across isolates.

RESULTS AND DISCUSSION

Antimicrobial Resistance Gene Profiles

All MRSA isolates carried *mecA*—absent in *S. aureus* NCTC 8325—confirming their β-lactam resistance phenotype (O'Neill, 2016). The *blaZ* gene was nearly ubiquitous, consistent with penicillinase-mediated resistance (Andrzejczuk et al., 2023). Macrolide resistance determinants (*erm(C)*, *mph(C)*) were present in several clinical strains, while aminoglycoside-modifying enzymes (*aac(6')-Ie-aph(2")*, *aph(3')-III*) appeared in select isolates (e.g., M5, M20, M27). Chloramphenicol acetyltransferases were rare, in line with previous reports of their low prevalence among MRSA collections (Bano et al., 2023).

Two isolates (GCA_018967105.1 and GCA_018603845.1) carried up to seven distinct AMR genes. Of particular note, an ST398 livestock-associated strain harbored *mecA, blaZ, ant(9)-Ia, erm(C), cat(pC221),* and *tet(K)*, exemplifying the cumulative acquisition of resistance determinants (Afzal et al., 2022; Alkuraythi et al., 2023). This accumulation across chromosomes and plasmids mirrors observations in ocular MRSA, where plasmid-borne cadmium resistance genes (*cadC, cadD, cadA*) and multiple *blaZ*-bearing Tn552 elements were reported, underscoring the role of mobile genetic elements in amplifying resistance (Kathirvel et al., 2021).

Beyond antibiotic resistance, surveillance studies reveal expansion into non-antibiotic domains. Whole-genome sequencing in Tanzania, for instance, detected antiseptic and disinfectant resistance genes frequently co-occurring with PVL-positive MRSA, raising further concerns for infection control (Juma et al., 2025).

Taken together, these findings reaffirm *mecA* and *blaZ* as the core of MRSA resistance (Andrzejczuk et al., 2023; O'Neill, 2016), while additional determinants shape diverse multidrug-resistant profiles. The recurrence of such patterns across clinical, livestock, and environmental contexts highlights the importance of genome-wide surveillance to uncover hidden resistance reservoirs and anticipate therapeutic challenges, AMR Genes data can be seen in Table 1.

Table 1. AMR Genes

Isolate	mecA	blaZ	erm(A)	erm(C)	aadD	cat	aac(6')-aph(2'')	aph(3')-III	dfrG	msr(A)	mph(C)	cat(pC233)	tet(K)	Total AMR Genes
MRSA S. aureus N315	v	v	v		v									4
S. aureus NCTC 8325														0
GCA_018967385.1	v	v		v										3
GCA_018967345.1	v	v		v										3
GCA_018967295.1	v	v		v		v								4
GCA_018967265.1	v	v		v										3
GCA_018967185.1	v	v		v										3
GCA_018967165.1	v	v		v										3
GCA_018967145.1	v	v		v										3
GCA_018967105.1	v	v					v	v	v	v	v			7
GCA_018860225.1	v	v		v										3
GCA_018678095.1	v	v		v			v					v		5
GCA_018967425.1	v	v		v										3
GCA_018967465.1	v	v		v										3
GCA_018603845.1	v	v	v				v	v	v				v	7

Virulence Gene Profiles

Core hemolysin genes (*hla, hlgA/B/C*) were detected in all isolates, consistent with their near ubiquity in *S. aureus* populations (Larsen et al., 2012). Panton–Valentine leukocidin (PVL) genes (*lukS-PV, lukF-PV*) were present in approximately half of the isolates, mirroring reports of ~50–60% PVL prevalence among clinical MRSA (Afzal et al., 2022). Notably, recent epidemiological studies suggest that PVL-positive MRSA frequently harbor antiseptic resistance determinants, complicating both treatment and infection control strategies (Juma et al., 2025).

Immune evasion cluster (IEC) genes (*sak, scn, chp*) were found in nearly all clinical isolates, highlighting their importance in human-adapted MRSA (Bano et al., 2023; Touaitia et al., 2025). Other virulence determinants, including leukocidins (*lukD/E*), enterotoxins (*sea, sec, seg, sei*), and exoenzymes (*aur, ssp* proteases, lipases); showed variable distributions across genomes. Such heterogeneity reflects sequence type–specific virulence repertoires. For instance, ocular MRSA lineages differ markedly: ST772 strains carried enterotoxin *sec* alongside multiple ARGs, while ST2066 strains encoded serine proteases (*splA–splF*) and exotoxins (*seb, set21*) but lacked enterotoxins (Kathirvel et al., 2021).

Virulence variation also extends beyond human infections. In livestock-associated *S. aureus*, such as bovine mastitis isolates of clonal complex 97 (CC97), strains carried the *sdrC* adhesin; linked to enhanced colonization; together with conserved resistance islands shaping pathogenicity (Rocha et al., 2024). Our findings are consistent with this broader pattern, in which virulence determinants are not uniformly distributed but cluster within specific lineages and host-adapted complexes.

Similar dynamics are observed in other staphylococci. *Staphylococcus epidermidis* strains from musculoskeletal infections often harbor IS256, a mobile element strongly associated with persistence and relapse in chronic disease (Santos et al., 2025). These parallels emphasize that persistence factors, whether in *S. aureus* or coagulase-negative staphylococci, are critical contributors to clinical outcomes.

Advances in sequencing technologies are expanding diagnostic capabilities. Shotgun metagenomic sequencing directly from clinical biopsies can detect *S. aureus* virulence and AMR genes with accuracy comparable to isolate-based WGS (Noone et al., 2021). This suggests that real-time genomic characterization could soon complement conventional diagnostics, particularly in implant- or biofilm-associated infections where rapid intervention is essential, Virulance genes data can be seen in Table 2.

Table 2. Virulance genes

Isolate	HostImm genes	#HostImm	Exoenzyme genes	#Exoenzyme	Toxin genes	#Toxin	Total Virulence Genes
MRSA S. aureus N315	sak, scn	2	aur, splA, splB, splE	4	hlgA, hlgB, hlgC, lukD, lukE	5	11
S. aureus NCTC 8325	-	0	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	10
GCA_018967465.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967425.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967385.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12

GCA_018967345.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967295.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967265.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967185.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967165.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967145.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, sen, seo, seu	9	12
GCA_018967105.1	scn	1	aur	1	hlgA, hlgB, hlgC, lukF-PV, lukS-PV, sea, sec, sec3, sei, sel, sem, seo	12	14
GCA_018860225.1	sak, scn	2	aur	1	hlgA, hlgB, hlgC, seg, sei, sem, seu	9	12
GCA_018678095.1	sak, scn	2	aur, splA, splB, splE	4	hlgA, hlgB, hlgC, lukD, lukE, sea,	8	14
GCA_018603845.1	sak, scn	2	aur, splA, splB, splE	4	hlgB, hlgC, lukD, lukE, sea, sek, seq	8	14

Overall, the co-occurrence of potent virulence determinants and multidrug resistance genes underscores the complex pathogenic potential of MRSA. Our findings align with growing evidence that resistance and virulence are frequently co-selected and shaped by plasmids, transposons, and clonal background (Kathirvel

et al., 2021; Rocha et al., 2024). Together with emerging features such as antiseptic resistance (Juma et al., 2025) and persistence-associated elements like IS256 (Santos et al., 2025), these results reinforce the importance of WGS-based surveillance (Noone et al., 2021) for guiding infection control and therapeutic strategies.

CONCLUSION

This comparative genomics analysis highlights the utility of ResFinder and VirulenceFinder for rapid and reliable profiling of MRSA isolates. We confirmed the universal presence of *mecA* and the near-ubiquity of *blaZ*, identified a range of additional resistance determinants, and mapped a diverse set of virulence factors; including core toxins and immune evasion genes; across clinical strains. These findings emphasize the genomic plasticity of MRSA and its implications for surveillance, infection control, and therapeutic decision-making. Future work should integrate genomic data with phenotypic assays and clinical outcomes to better define the impact of specific resistance–virulence gene combinations on disease severity and treatment response.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to all for their support and assistance in carrying out the research process

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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