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# Loss or Strategy? The Crispr/Cas System and Its Genomic Trade-Offs in *Staphylococcus aureus* and *S. epidermidis*

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## Abstract

*Staphylococcus aureus* and *Staphylococcus epidermidis* are major agents of nosocomial infections and exhibit high levels of antimicrobial resistance. The CRISPR/Cas system functions as a bacterial defense mechanism against mobile genetic elements, such as phages and plasmids, and may influence the acquisition of resistance genes. This study aimed to investigate the presence, diversity, and functional implications of the CRISPR/Cas system in complete genomes of *S. aureus* and *S. epidermidis*, including its relationship with integrated bacteriophages, anti-CRISPR genes, and antimicrobial resistance genes. A total of 30 complete genomes from each species were analyzed, all retrieved from the NCBI database. The tools CRISPR-CasFinder, CRISPRTarget, PHASTEST, ACRFinder, and Abricate were employed for the identification of the CRISPR/Cas system, spacer analysis, detection of prophages, anti-CRISPR genes, and antimicrobial resistance genes. Only two isolates (one from each species) exhibited a complete type III-A CRISPR/Cas system. The identified spacers were predominantly associated with phages. One of the CRISPR-positive genomes harbored intact prophages and anti-CRISPR genes. The resistance analysis revealed multiple genes in nearly all genomes, with a predominance of genes related to efflux pumps and beta-lactam resistance. The low prevalence of CRISPR/Cas was accompanied by a high burden of resistance genes. The scarce occurrence of the CRISPR/Cas system in *Staphylococcus* spp. may represent an adaptive advantage, allowing for greater acquisition of resistance genes via mobile genetic elements. These findings underscore the importance of understanding the interplay between bacterial immunity and antimicrobial resistance to inform the development of new therapeutic strategies.

**Keywords:** CRISPR/Cas system; Antimicrobial resistance; Prophages; Anti-CRISPR; Mobile genetic elements

## Introduction

*S. aureus* and *S. epidermidis* are Gram-positive microorganisms frequently associated with both hospital- and community-acquired infections, particularly noted for their resistance to beta-lactam antimicrobials, including methicillin and oxacillin [1–3]. In 2017, ANVISA reported coagulase-negative *Staphylococcus* spp. as the leading causative agents of primary bloodstream infections in adult, neonatal, and pediatric intensive care units, with resistance rates exceeding 70% to beta-lactams. *S. aureus* was identified as the third most frequent cause, also exhibiting high levels of resistance [4].

In light of this situation, the World Health Organization (WHO) has included *S. aureus* among the high-priority pathogens for the development of new therapies [5], while *S. epidermidis* has gained prominence due to its association with implant-related infections and the emergence of highly resistant strains [6].

Among the pathogens associated with antimicrobial resistance, *Staphylococcus* spp., particularly *S. aureus* and *S. epidermidis*, occupy a prominent position. *S. aureus* is one of the leading causes of invasive infections in humans, such as pneumonia, endocarditis, and sepsis, and is frequently associated with methicillin-resistant strains (MRSA), which compromise the efficacy of various therapeutic regimens [1, 3]. *S. epidermidis*, once considered an opportunistic commensal, has become an important agent of device-associated infections, with a high prevalence of resistance to multiple classes of antibiotics [6]. The persistence of these microorganisms in hospital environments and their capacity to acquire and disseminate resistance genes underscore the urgency of strategies that complement conventional therapies.

In this context, alternatives such as phage therapy have been explored for the control of multidrug-resistant bacterial infections [7]. However, its effectiveness depends on understanding bacterial defense mechanisms against mobile genetic elements (MGEs), such as the CRISPR/Cas system.

The CRISPR/Cas system is an adaptive immune mechanism found in bacteria and archaea, capable of destroying exogenous genetic material and generating memory of past encounters with mobile genetic elements (MGEs) [8-10]. It consists of a CRISPR locus (Clustered Regularly Interspaced Short Palindromic Repeats) and associated *cas* genes. The CRISPR array contains short direct repeats (DRs) interspersed with unique spacers, which are derived from foreign DNA sequences. The *cas* genes encode proteins with various roles in spacer acquisition, CRISPR RNA (crRNA) processing, and interference. CRISPR/Cas systems are classified into three main types (I, II, and III), with further subtypes based on their structural and functional components. In this study, the type III-A system was identified in two genomes, which is consistent with previous reports of diverse CRISPR/-Cas subtypes in *Staphylococcus* species.

In *Staphylococcus* spp., the distribution of the CRISPR/Cas system is heterogeneous. In *S. aureus*, there is a predominance of orphan CRISPR loci, that is, loci lacking functional cas genes [2], and the absence or inactivity of the system is observed in various lineages. The reasons for this uneven distribution are not yet fully understood but may be related to selective pressures imposed by phages, anti-CRISPR genes, and the necessity to acquire resistance and virulence genes through horizontal gene transfer.

This study aimed to investigate the genomic and functional diversity of the CRISPR/Cas system in complete genomes of *S. aureus* and *S. epidermidis*, analyzing its relationship with the presence of integrated bacteriophages, anti-CRISPR genes, and antimicrobial resistance determinants, in order to understand the factors influencing its uneven distribution in these species and their evolutionary implications.

## Methodology

### Samples of *S. aureus* and *S. epidermidis*

Sequences of *S. aureus* and *S. epidermidis* were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/) (accessed on April 25, 2025). Only genomes classified as complete by the database were selected. A total of 30 genomes of *S. aureus* and 30 genomes of *S. epidermidis* were included in the analysis.

### CRISPR/Cas System Search

To investigate the presence and type of the CRISPR/Cas system, the selected genomes were analyzed using the CRISPRCasFinder tool [11], and only genomes containing both *cas* genes and a functional CRISPR locus were classified as complete systems. Genomes with only one of these components were classified as negative.

### Spacer Content Analysis in CRISPR/Cas-Positive Isolates

The identified spacers were analyzed to determine their possible origins using the CRISPRTarget tool [12], as well as by searching databases such as UniProt and Pfam for confirmation.

### Investigation of Integrated Bacteriophages and Anti-CRISPR Genes

The search for potential integrated bacteriophages in the genomes of CRISPR/Cas-positive *S. aureus* and *S. epidermidis* was performed using the online tool PHASTEST (https://phastest.ca/). Only sequences with over 70% similarity to bacteriophages integrated within the studied genomes were analyzed. Prophages were considered present if 1) PHASTEST results indicated phage region integrity as intact, or 2) PHASTEST results suggested questionable integrity but BLAST results showed an alignment coverage of 50% or more. To investigate anti-CRISPR (*acr*) genes in genomes harboring integrated bacteriophages, the ACRFINDER platform was employed [13].

### Investigation of Resistance Genes

Detection of antimicrobial resistance genes was performed using the Abricate tool [14] via the Galaxy Europe platform (https://usegalaxy.eu). Analyses were conducted based on the CARD – Comprehensive Antibiotic Resistance Database [15], employing the tool's default parameters for the identification of resistance determinants.

## Results

### *Presence of the CRISPR/Cas System*

Among the 60 complete genomes analyzed (30 of *S. aureus* and 30 of *S. epidermidis*), only two presented complete CRISPR/-Cas systems of the type III-A: one isolate of *S. aureus* (AR_0470) and one of *S. epidermidis* (FDAARGOS_153), representing 3.5% of the total (Table 1). An additional genome harbored *cas* genes (*cas1, cas2*, and *cas9*), but was considered negative due to the absence of a CRISPR locus.

**Table 1:** Genomes Positive for the CRISPR/Cas System

| Specie | Strain | Type and Subtype | ST |
|---|---|---|---|
| *Staphylococcus aureus* | AR_0470 | Type III A | ST 30 |
| *Staphylococcus epidermidis* | FDAARGOS_153 | Type IIIA | ST 5 |

## Spacer Analysis

Fifteen spacers were identified in each of the two CRISPR-positive isolates. Most (63.3%) matched phage sequences, while a smaller proportion matched bacterial (6.7%) or plasmid (3.3%) sequences. Six spacers (20%) showed no similarity to any known sequences and were classified as unmatched (Figure 1).

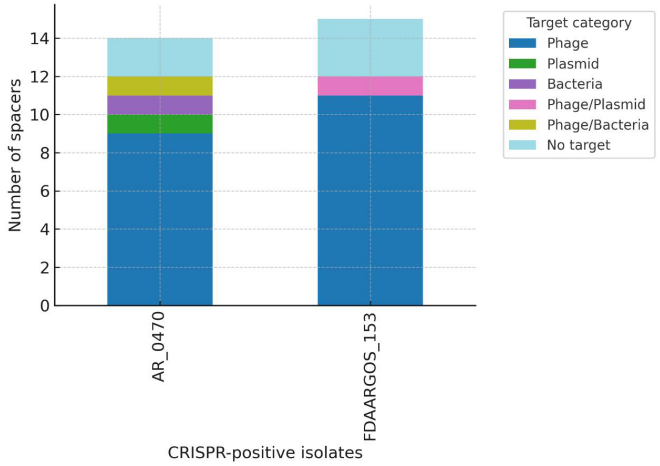


**Figure 1:** Target types of CRISPR spacers in CRISPR/Cas-positive isolates.Each bar shows the distribution of the 15 spacers identified in each isolate (*S. aureus* AR_0470 and *S. epidermidis* FDAARGOS_153), categorized by target type: phage, plasmid, bacterial, mixed targets (Phage/Bacteria or Phage/Plasmid), or no identifiable target. The presence of mixed categories indicates spacers with sequence similarity to more than one class of mobile genetic element.

## Presence of Prophages and Anti-CRISPR Genes

Only the *S. aureus* isolate AR_0470 presented integrated prophages in its genome, including two classified as intact and one as questionable (Figure 2). This same genome also contained anti-CRISPR genes detected by analysis with ACRFinder. No prophages or anti-CRISPR genes were identified in the *S. epidermidis* genome.

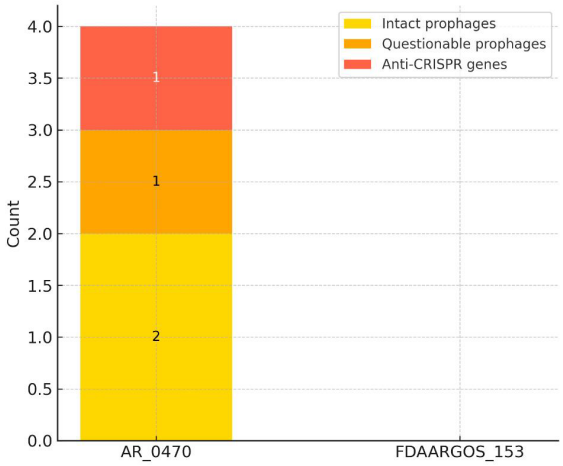


**Figure 2:** Presence and type of prophages, and anti-CRISPR genes, in CRISPR/Cas-positive isolates. Bars indicate the number of elements detected per isolate. The yellow segment represents intact prophages, the orange segment indicates questionable prophages, and the red segment corresponds to the presence of anti-CRISPR (*acr)* genes. Only the *S. aureus* isolate AR_0470 harbored both intact and questionable prophages as well as an *acr* gene, while *S. epidermidis* FDAARGOS_153 lacked all of these elements.

## Resistance Genes

Of the 30 *S. aureus* genomes, 29 presented antimicrobial resistance genes. All 30 *S. epidermidis* genomes also contained three or more resistance genes. Among the most frequent genes were *norA, mgrA, arlR, arlS, mepA, mepR, tet*(38), *lmrS, ermA, mecA, mecI, mecR1,* and *blaZ*. Genes related to resistance to fluoroquinolones, tetracyclines, and beta-lactams were widely distributed among the isolates (Figures 3 and 4) (Supplementary Material 1).

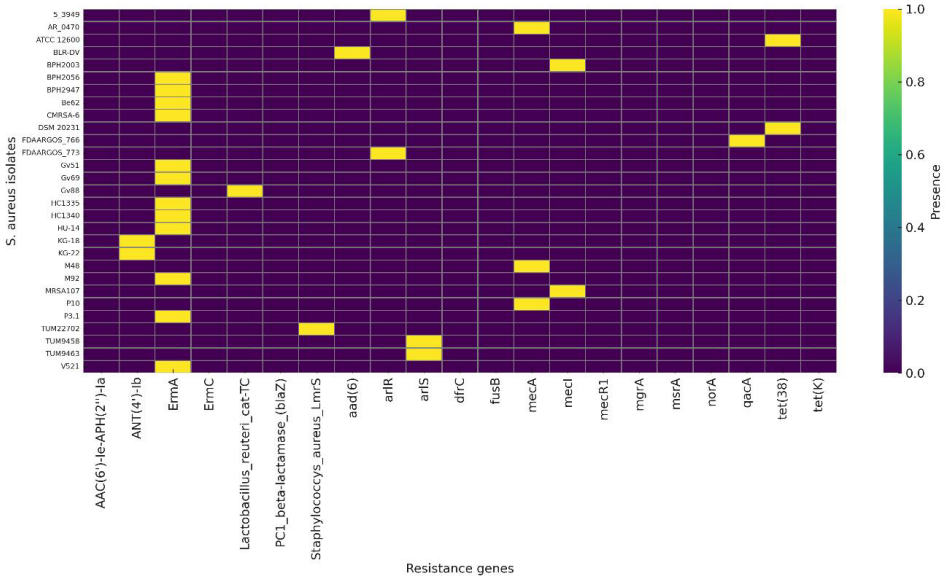


**Figure 3:** Antimicrobial resistance gene profiles among 29 *S. aureus* genomes. Each row represents an isolate, and each column corresponds to a resistance gene identified through genome analysis. Yellow squares indicate gene presence, while purple squares denote absence. The heatmap reveals variability in the presence of resistance genes across isolates, including genes associated with beta-lactams, macrolides, tetracyclines, and fluoroquinolones.

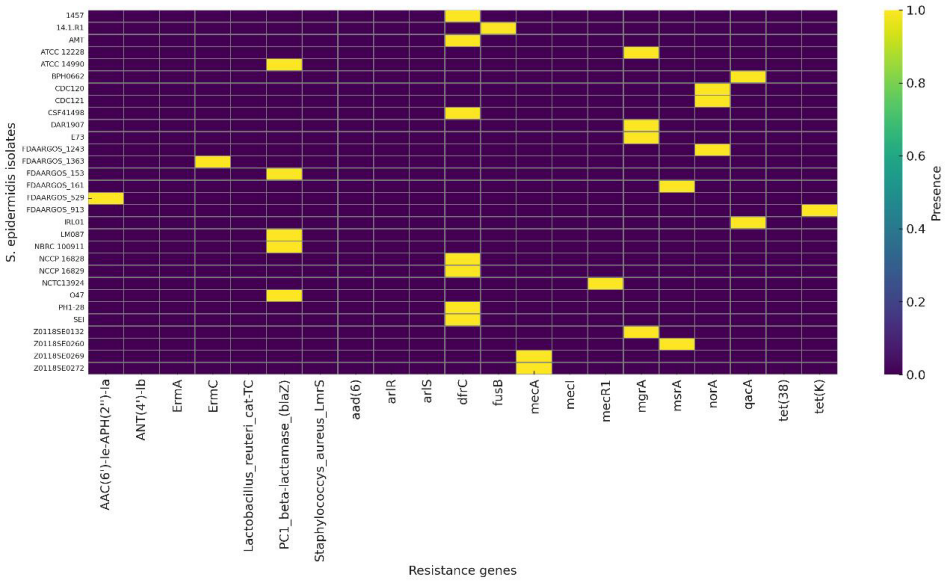


**Figure 4:** Antimicrobial resistance gene profiles among 30 *Staphylococcus epidermidis* genomes. Each row represents an isolate, and each column corresponds to a resistance gene identified through genome analysis. Yellow squares indicate gene presence, while purple squares denote absence. The heatmap reveals widespread distribution of resistance-associated genes, including *mecA, norA, tet*(38) and *qacA* across multiple isolates.

## Discussion

The low frequency of the CRISPR/Cas system in *Staphylococcus* spp. genomes observed in this study is consistent with previous findings reporting a rare or incomplete occurrence of this system in these species [2, 16]. This scarcity may reflect an evolutionary strategy favoring the acquisition of mobile genetic elements (MGEs), such as plasmids and phages, thereby allowing the incorporation of resistance genes and virulence factors. This hypothesis is supported by San Millan (2018) [17] and Rafiq et al. (2024) [18], who emphasize that the absence of defense systems like CRISPR/Cas can facilitate genomic plasticity and rapid adaptation in environments under intense selective pressure, such as hospitals.

The higher relative proportion of CRISPR/Cas-positive genomes in *S. epidermidis* (considering the total number available in NCBI) may indicate that, in this species, the system still plays a functional role in certain lineages. Studies such as Li et al. (2016) [19] have also reported the presence of complete CRISPR/Cas systems in clinical strains of *S. epidermidis*, suggesting a possible correlation with the type of ecological niche or infection profile.

The predominance of spacers targeting phages in both positive genomes aligns with the classical role of the CRISPR/Cas system as an adaptive barrier against viral infection. However, spacers without identifiable targets raise relevant questions. Such sequences may represent interactions with yet uncharacterized MGEs, extinct viral elements, or highly divergent sequences. Furthermore, recent studies have proposed that some "orphan" spacers may be involved in non-canonical regulatory functions, such as silencing endogenous genes or participating in mechanisms modulating gene expression [20].

The presence of prophages and anti-CRISPR genes solely in the *S. aureus* AR_0470 genome indicates a complex evolutionary relationship between bacteriophages and the CRISPR system. Anti-CRISPR (*acr*) genes are known to enable lysogenic phages to evade CRISPR/Cas immunity, facilitating their integration into the bacterial genome [20, 21]. This may explain the coexistence of functional CRISPR systems and lysogenic elements in some bacterial genomes, especially in lineages constantly interacting with phages.

The results related to antimicrobial resistance are particularly concerning: all *S. epidermidis* genomes and nearly all *S. aureus* genomes harbored multiple resistance genes, including those associated with efflux pumps, resistance to beta-lactams, macrolides, and trimethoprim. The absence of the CRISPR/Cas system in most isolates may be facilitating this acquisition through horizontal gene transfer, as observed by Tsao et al. (2018) [22]. In hospital settings, this strategy may represent an adaptive advantage, allowing a rapid response to intensive antibiotic use.

From a translational perspective, understanding the balance between the presence of CRISPR/Cas systems, *acr* genes, and MGEs may provide new therapeutic strategies. For example, phage therapy selection can be optimized by considering whether the target bacterium possesses a functional CRISPR system. Additionally, analysis of CRISPR loci can be explored as a molecular typing tool, providing useful epidemiological markers for genomic surveillance of hospital outbreaks.

In summary, our results suggest that the scarcity of the CRISPR/Cas system in *Staphylococcus* spp. is not incidental but rather a reflection of an evolutionary adaptation prioritizing genetic flexibility and survival in hostile environments. Understanding this dynamic is essential for developing more precise interventions against antimicrobial resistance, especially those involving the use of phages or CRISPR-based systems for bacterial editing or control.

## Conclusion

This study highlighted the low prevalence of the CRISPR/Cas system in complete genomes of *Staphylococcus aureus* and *S. epidermidis*, associated with a high burden of antimicrobial resistance genes. The predominance of spacers targeting phages, the

presence of anti-CRISPR genes, and the identification of prophages point to a complex evolutionary dynamic shaped by interactions with mobile genetic elements. These findings suggest that the absence of the CRISPR/Cas system may represent an advantageous adaptation, allowing greater genomic flexibility and facilitating the horizontal acquisition of resistance genes, a critical trait for the survival of *Staphylococcus* spp. in hospital environments under intense selective pressure.

Beyond enhancing the understanding of resistance mechanisms, the results provide relevant insights for the application of alternative therapies such as phage therapy, which may benefit from prior analysis of the CRISPR/Cas status in clinical isolates. Likewise, characterization of CRISPR loci and their spacers can be explored as tools for molecular and epidemiological surveillance. The functional absence of CRISPR/Cas, far from indicating vulnerability, may reflect a refined evolutionary strategy. Future investigations should focus on the functional expression of these loci and their interaction with resistance genes, paving the way for more precise and effective interventions against antimicrobial resistance. Future studies should aim to validate these findings through functional assays, such as RT-qPCR to assess cas gene expression, plasmid clearance assays to evaluate interference activity, and induction of spacer acquisition under phage challenge, in order to confirm the operational status of the CRISPR/Cas system in *Staphylococcus* spp.

## Supplementary Information

https://www.annexpublishers.com/articles/JMC/9101-Supplementary-Material.pdf

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