Research Article

BASJVETRES

*BASRAH JOURNAL OF VETERINARY RESEARCH, 2024, 23(4):87-106.*
*https://bjvr.uobasrah.edu.iq/*

# Genetic Variations in *Staphylococcus aureus* Two-Component Systems and Antimicrobial Stress Responses

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**DOI: https://doi.org/10.23975/bjvr.2024.154912.1152**

**Received: 2 November 2024 Accepted: 6 December 2024.**

## Abstract

*Staphylococcus aureus* utilizes two-component signal transduction systems (TCSs) for sensing and responding to stressors such as antimicrobials. Variations in the sequence of TCSs involved in antimicrobial resistance, such as WalRK, VraSR, LytSR, GraSR, NsaRS, HptSR, and AirRS, may influence the resistance profile of *Staphylococcus aureus* in clinical isolates. This study analyzed the genomes of five local *S. aureus* isolates from humans, dogs, cats, cows, and sheep, recently deposited and annotated in the NCBI database. The genomes analysis showed that the total number of variants (Alteration in the DNA nucleotide sequence) was 19,176, 19,924, 20,168, 20,499, and 42,248 for MHC, MHB, MHH, MHF, and MHO, respectively. Compared to the other isolates, the MHO isolate from a sheep clinical case exhibited the highest number of variants. The synonymous and missense mutations were the highest in the MHO strain compared to other isolates. The MHH human strain showed resistance to seven antibiotics compared to other isolates of animals' origin, which showed resistance to antibiotics for two types of antibiotics according to the results of VITEK. In addition, the analysis of the genome sequence of the genes of the two-component system revealed differences between the local isolates, which may affect their antibiotic resistance profile.

**Keywords:** *Staphylococcus aureus*, antibiotics resistance, polymorphism.

## Introduction

*Staphylococcus aureus* is a major public health concern due to its ability to cause a variety of severe infections and diseases in both humans and animals. The ability of bacteria to adapt and resist environmental stresses and factors makes it a serious pathogen, and these diseases have resulted from the emergence of antibiotic-resistant strains of *Staphylococcus aureus* (1). Identifying and understanding the genetic variations within *Staphylococcus aureus* strains such as single nucleotide polymorphisms (SNPs) and insertions/deletions in genomic DNA is crucial for developing and studying new strategies to combat antibiotic resistant and virulent *Staphylococcus aureus* clones (2). Among the various genetic variations of *S. aureus*, SNPs and insertions/deletions (InDels) are particularly important for shaping the evolutionary and future host tropism dynamics and pathogenic potential of these bacteria (3). *Staphylococcus aureus* senses a variety of signals and markers depending on the stressor and uses two-component signal transduction systems (TCSs) as effective mechanisms to transmit them to control and control metabolism and other vital processes and in response to changes in environmental factors (4). Researchers have linked several TCSs to antibiotic resistance and demonstrated that changes in the sequences of these TCSs may have an effect on the antibiotic stress responses, (Table 1). This study utilized the genomes of five local *S. aureus* isolates from humans, dogs, cats, cows, and sheep, recently deposited and annotated in the NCBI database under the names and accession numbers MHH (GCA_040196155.1), MHB (GCA_040196135.1), MHO (GCA_040195495.1), MHF (GCA_040195555.1), and MHC (GCA_040195445.1), to investigate the sequences of TCSs in these isolates. Additionally, SNP analysis was used to further understand the genomic variations among these isolates. The objective of this work is to examine polymorphism in local isolates of *S. aureus* MHC, MHB, MHH, MHF, and MHO, with particular emphasis on TCSs associated with antibiotic resistance in these isolates.

## Materials and Methods

### Bioinformatics

Two approaches have been used for analysis to pin down the nucleotide variation in these isolates. The first approach was comparing the five local genomes to the closest reference genome based on the *16S RNA* analysis. The second approach was to compare the sequences of the TCSs of the five isolates to the TCS of the closest genome based on the whole genome similarity analysis. The genomes of MHH (GCA_040196155.1), MHB (GCA_040196135.1), MHO (GCA_040195495.1), MHF (GCA_040195555.1), and MHC (GCA_040195445.1) were downloaded from the NCBI database. The *16S RNA* sequence analysis was conducted using the NCBI blast analysis tool.

The PATRIC's comprehensive genome analysis tool: This software was utilized to examine the sequencing reads (12). This helped to detect both conserved sequence features and compared the genomes to determine the closest known genomes in the genomes' database.

The Haplotype Caller: The Haplotype Caller (13). was used for SNPs and InDels simultaneously via local reassembly of haplotypes in an active region (14).

SnpEff (4.3t): This software provides annotations for the possible impacts on genes resulting from variations detected during the mapping process. The tool not only determines whether the variant is synonymous or nonsynonymous using information from the reference sequence, but it also predicts amino acid changes resulting from the variant (15). In this case, SnpEff sorts the effects in putative order, considering the impact of variants.

Manta tool (1.6.0). : Structural variations and InDels from short, paired end sequence reads, was analyzed using the Manta tool (1.6.0) was used. To enhance efficiency, this software combined paired-end and split read guides while detecting and evaluating these structural variations (SVs) (16).

Clustal Omega Multiple Sequence Alignment program: The TCS sequences of the five isolates were aligned to the TCS of the reference genome using the Clustal Omega Multiple Sequence Alignment program(17).

The Proksee server: used to annotate and display the locations of each TCSs gene in the genomes of Staphylococcus aureus isolates. In addition, it was used to create a circular representation of the genome where the target proteins were displayed. (18).

RASTtk server (12) and Seed Viewer sequence-based comparison tool: These were used to compare the five isolates' genomes to the reference genome.

**Antibiotics Resistance Profile**

The Vitek 2 Biomerieux – France was used for the detection of antibiotic resistance profile and minimum inhibition concentration (MIC). *Staphylococcus aureus* isolates were cultured on nutrient agar then single colonies were taken from each nutrient plate and placed in tubes containing normal saline where the sample was adjusted according to the McFarland standard (0.5) using a turbidimeter (19). Where the antibiotic sensitivity test card for gram positive bacteria is attached and then inserted into the device where it is inoculated with the previously prepared suspension (20).

**Table 1: Two-Component Systems (TCS) associated with resistance in *S. aureus*.**

| No. | Gene ID | Function |
|---|---|---|
| **1** | WalRK | The WalRK TCS is essential for maintaining cell wall integrity, which is a primary target of many antibiotics, such as beta-lactams. By regulating genes involved in cell wall metabolism, WalRK contributes to the bacterial ability to resist these antibiotics. Mutations or regulatory changes in the WalRK system can lead to alterations in gene expression patterns, potentially leading to reduced susceptibility to antibiotics. The WalRK system is, therefore, a key player in the development of antibiotic resistance in *S. aureus* (5). |
| **2** | VraSR | VraSR TCS is activated in response to cell wall stress, which often occurs when *S. aureus* is exposed to antibiotics like vancomycin, beta-lactams, and daptomycin. The VraS sensor kinase detects cell wall damage, and upon activation, phosphorylates the VraR response regulator. Beyond vancomycin, the VraSR system also contributes to resistance against other antibiotics that target the cell wall. Its activation leads to a generalized stress response that enhances the bacterial ability to withstand multiple antibiotics (6). |
| **3** | LytSR | LytSR TCS primarily controls the expression of the *lytA* gene, which encodes for an autolysin enzyme responsible for breaking down the bacterial cell wall. The system regulates autolysis, helping the bacteria maintain cell integrity under stress, including antibiotic exposure Given its role in controlling autolysis LytSR represents a potential target for new therapeutic strategies aimed at enhancing the effectiveness of existing antibiotics by promoting bacterial cell death or preventing biofilm-associated resistance (7). |
| **4** | GraSR | GraRS TCS is closely associated with resistance to daptomycin, a last-resort antibiotic that targets the bacterial membrane. GraRS regulates genes that modify the cell membrane and cell wall, reducing the binding and efficacy of daptomycin. This modification is a key mechanism behind the development of daptomycin-resistant *S. aureus* (8)**.** |
| **5** | NsaRS | The NsaRS TCS regulated efflux pump plays a significant role in multidrug resistance (MDR) in *S. aureus* By actively pumping out a variety of antimicrobial agents, the NsaRS system allows *S. aureus* to survive in the presence of different antibiotics, contributing to the MDR phenotype often seen in clinical isolates Although NsaRS is not the primary regulator of vancomycin resistance, it contributes to the overall stress response that can influence resistance mechanisms. By enhancing the cell's ability to expel harmful compounds and respond to cell envelope damage, NsaRS indirectly supports resistance to cell wall-targeting antibiotics (9). |
| **6** | HptSR | The HptSR TCS primarily regulates genes involved in phosphate uptake and metabolism. Phosphate is crucial for various cellular processes, including energy production, nucleotide synthesis, and cell wall biosynthesis. By managing phosphate homeostasis, HptSR helps ensure that *S. aureus* maintains vital cellular functions even under nutrient-limited conditions. efficient phosphate acquisition and utilization are essential for maintaining cell wall integrity, which can influence the effectiveness of cell wall-targeting antibiotics like beta-lactams and vancomycin (10). |
| **7** | AirRS | The AirSR TCS (also known as AirS/AirR) is primarily involved in detecting and responding to oxidative stress. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the bacteria to detoxify these harmful compounds. AirSR helps *S. aureus* manage this stress by regulating genes involved in oxidative stress defense mechanisms. AirSR is known to regulate the expression of various virulence factors in response to environmental signals. By controlling these factors, *S. aureus* can adapt to hostile conditions, such as those encountered in the human host or during antibiotic treatment. This adaptive capability can contribute to the bacterial persistence and survival, even in the presence of antibiotics (11). The AirSR two-component signaling pathway is closely associated with *S. aureus* resistance towards vancomycin. |

## Results

### TCSs related to antimicrobial stress response

Based on the literature and publications, WalRK, VraSR, LytSR, GraSR, NsaRS, HptSR, AirRS were investigated. A circular representation of the location of the studied TCSs on the genome are listed in (Figure 1).

### *16S RNA* blast analysis

The closest genome to the studied isolates was identified as NZ_AP014921.1 based in the *16S RNA* sequence identity. Therefore, this genome was used as a reference for the SNPs and InDels analysis.

The *16S RNA* blast analysis showed that *S. aureus* NZ_AP014921.1 was the most comparable reference to the five *S. aureus* MHC, MHB, MHH, MHF, and MHO local isolates (Data not shown).

### Genome analysis

The PATRIC's comprehensive genome analysis tool determined that the closest known genome to the *S. aureus* MHC, MHB, MHH, MHF, and MHO local isolates in the genomes' database was *S. aureus* CP011526. Thus, the sequences of each TCS of the five isolates were aligned against the TCS of the reference genome, *S. aureus* CP011526. In addition, the genomes of MHB, MHC, MHF, MHH, and MHO were compared against the reference genome *S. aureus* CP011526 using the RASTtk server (12) and Seed Viewer sequence-based comparison tool (Figure 3). The MHO strain had the least genome sequence similarity to all other isolates including the reference genome.

### Variant Annotation

A comparison between the variations in nucleotides (SNPs) and the count of insertions and deletions (InDels) in relation to the reference genome, *S. aureus* NZ_AP014921.1, was conducted. This reference genome was chosen because it had the greatest similarity to our isolates when analyzed using the *16S RNA* sequence analysis method.

The total number of Variants was 19,176, 19,924, 20,168, 20,499, 42,248 for MHC, MHB, MHH, MHF, and MHO respectively. The MHO isolate, which was isolated from sheep clinical case showed the highest number of variants compared to the other isolates. The Synonymous and missense mutations were the highest in MHO strain compared to other isolates. (Table 2) and (Figure 2,4) summarize the variations among the genomes of the targeted isolates in comparison to the reference genome. The interpretation involved the effect of these SNPs on amino acids sequences. (Table 2) lists the predicted changes and the possible effect on the codons and on amino acids sequences.

### Tow component systems sequence alignment

Due to the fact that 16S RNA similarity isn't very good at describing whole genome traits and TCSs play a role in controlling many genes, another analysis approach was needed.

This involved comparing the TCSs sequences to the closest reference genome to the local isolates. To make sure the variant analysis results were correct, PATRIC's full genome analysis tool was used. This showed that the *S. aureus* CP011526 genome is the

most similar to the five local isolates. Therefore, the TCSs sequences were aligned and analyzed against this reference to identify any changes in the nucleotide sequence using Clustal Omega Multiple Sequence Alignment program (17). (Table 3) shows the changes in the TCSs genes sequences compared to reference *S. aureus* CP011526. MHO strain showed the highest number of SNPs and polymorphism in the TCSs sequences.

**Antibiotics Resistance Profile:** To explore the antibiotics resistance profile, VITEK antibiotics sensitivity testing was conducted. MHH strain has shown resistance to seven antibiotics compared to other isolates which showed resistance to two antibiotics. (Table 4) shows the resistance profile among the *S. aureus* MHC, MHB, MHH, MHF, and MHO local isolates.

**Antibiotics Resistance Profile**

Some isolates were resistant to beta-lactam antibiotics and methicillin, and others were sensitive to these antibiotics. MHH strain which was isolated from humans had multiple antibiotics resistance profile whereas all other isolates showed resistance to two antibiotics (Table 4)

**Table 2 : Number and types of SNPs and indels in *S. aureus* MHC, MHB, MHH, MHF, and MHO**.

| SNP/InDel | MHC | MHB | MHH | MHF | MHO |
|---|---|---|---|---|---|
| Number of SNPs | 17,841 | 18,525 | 18,716 | 19,004 | 39,565 |
| Number of Insertions | 700 | 710 | 743 | 752 | 1,329 |
| Number of Deletions | 635 | 689 | 709 | 743 | 1,354 |
| **Total number of Variants** | **19,176** | **19,924** | **20,168** | **20,499** | **42,248** |
| **Variant Annotation** | | | | | |
| Upstream gene variant (Upstream of a gene - within 5K bases) | 4,409 | 4,788 | 4,786 | 4,899 | 9,553 |
| Downstream gene variant (Downstream of a gene -within 5K bases). | 236 | 208 | 268 | 210 | 592 |
| Frameshift variant (Insertion or deletion causes a frame shift) | 400 | 413 | 416 | 438 | 743 |
| Missense variant (Variant causes a codon that produces a different amino acid) | 4,118 | 4,228 | 4,280 | 4,360 | 8,030 |
| Synonymous variant (Variant causes a codon that produces the same amino acid) | 9,401 | 9,744 | 9,752 | 9,976 | 22,248 |
| Start lost (Variant causes start codon to be mutated into non-start codon | 7 | 4 | 5 | 4 | 12 |
| Stop gained (Variant causes a stop codon) | 37 | 39 | 39 | 42 | 78 |
| Others | 568 | 500 | 622 | 570 | 992 |

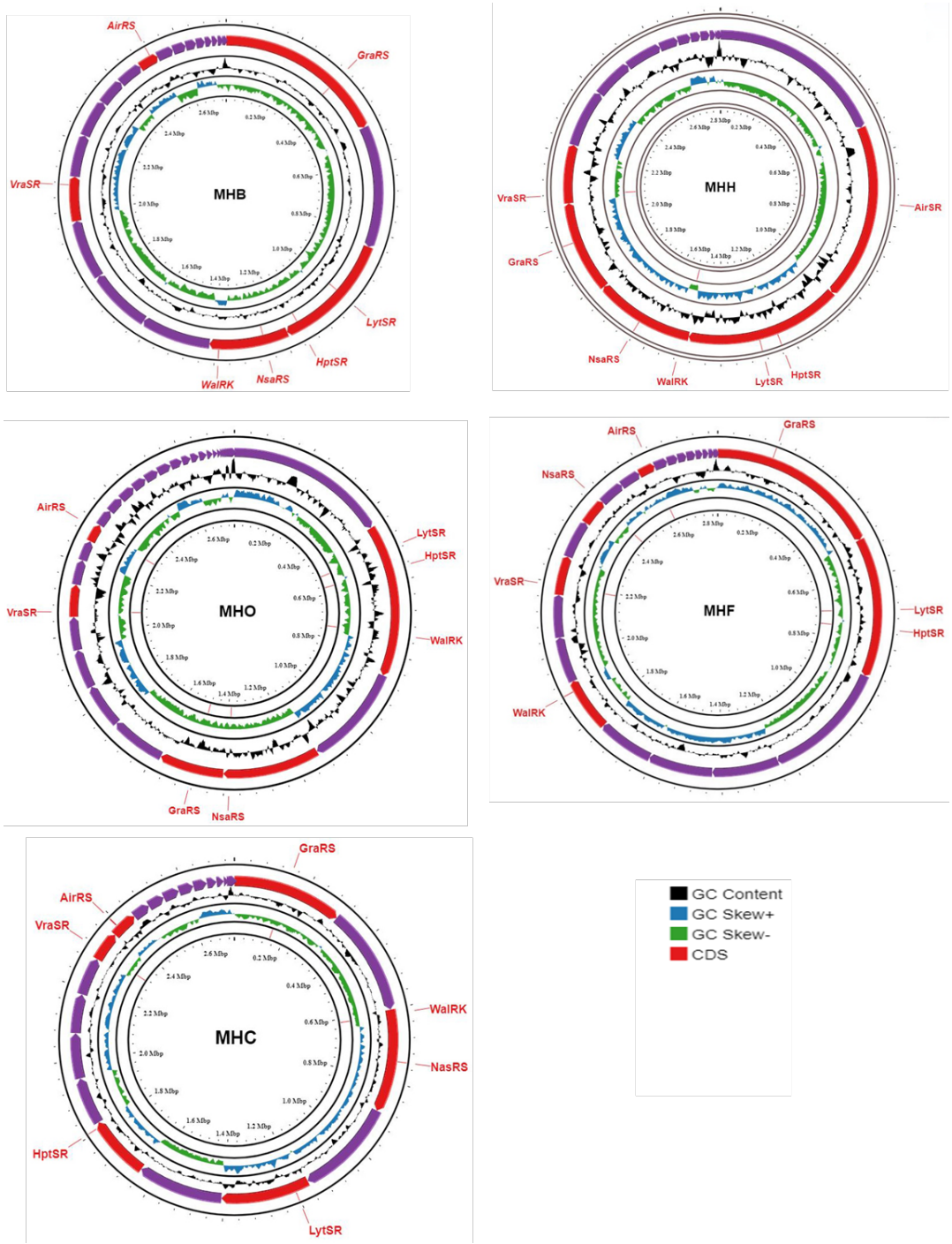


**Figure 1: Circular depiction of the genomes of *Staphylococcus aureus* isolates MHB, MHH, MHO, MHF, and MHC. The Proksee server was utilized to determine the precise positions of each TCS gene and to generate a circular depiction of the genomes (18). The information is arranged into feature rings (starting from outside to inside). In Ring 1, the protein-coding sequences (CDSs) are organized as genome contigs, and the TCSs locations are marked on the relevant contig (red contig). Ring 2 displays the GC content plot in black, while Ring 3 displays the G/C skew information in blue for the +strand and green for the -strand. The most inner circle ring 3 represents the genome scale in Mega base pairs (Mbp).**

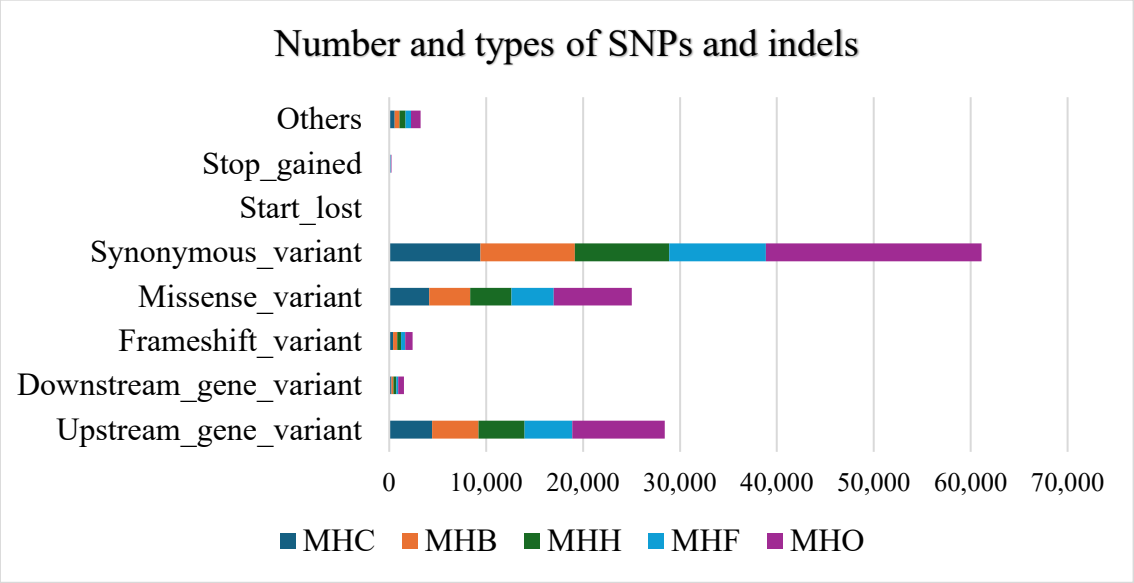


**Figure 2: Number and types of SNPs and indels in S. aureus MHC, MHB, MHH, MHF, and MHO.**

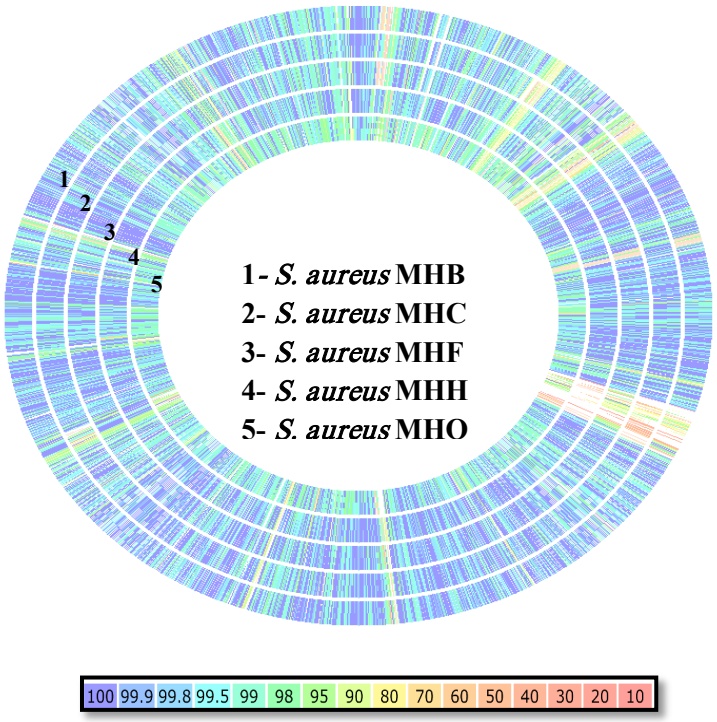


**Figure 3: Genome comparison map between the reference genome *Staphylococcus aureus* subsp. aureus DSM 20231 (CP011526) and five strains created using the Seed Viewer sequence-based comparison tool. The strains are (1) MHB, (2) MHC, (3) MHF, (4) MHH, and (5) MHO sorted from outer to inside rings. Colors range from 100% purple to 10% pale red, indicating the degree of amino acid similarity to the reference genome. The CP011526 reference strain's genome is not shown in the graphic.**

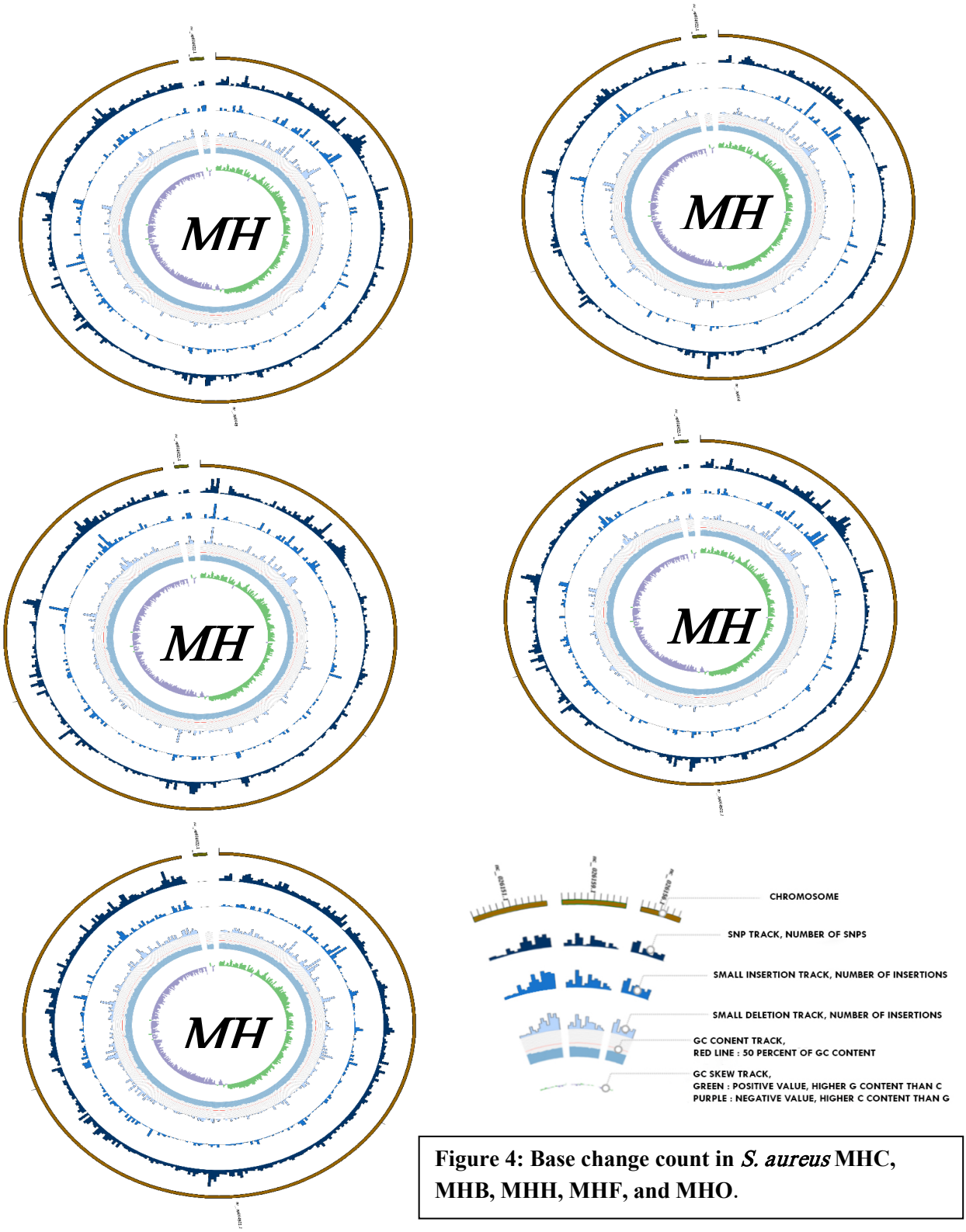


**Figure 4: Base change count in *S. aureus* MHC, MHB, MHH, MHF, and MHO.**

Tabel 3 Changes in the TCS gene sequences compared to the reference *S. aureus* CP011526. The sequence location and codon numbers are listed to denote the exact location of the variant. The nucleotide(s) change and the amino acids changes are indicated for each codon where the reference codon sequence contained * star symbol whereas the (-) indicates no change in the nucleotide or the codon sequence. In addition, the nt seq represents the exact nucleotide sequence location. The expected amino acid c**.**

| No. | Gene ID | MHB | | | MHH | | | MHO | | | MHF | | | MHC | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Accession No. | Codons | nt seq | Accession No. | Codons | Seq | Accession No. | Codons | nt seq | Accession No. | Codons | nt seq | Accession No. | Codons | nt seq |
| 1 | WalK (HK) | MEQ7660453 | 117 | 350 | MEQ7733553 | 117 | 350 | MEQ7740821 | 117 | 350 | MEQ7620490 | 117 | 350 | MEQ7670070 | 117 | 350 |
| | | | *TTC / - GA | Val | | --- / --- | | | --- / --- | | | *TTC / - GA | Val | | --- / --- | |
| | WalR (R) | MEQ7660452 | 125 | 373 | MEQ7733554 | 125 | 373 | MEQ7740822 | 125 | 373 | MEQ7620489 | 125 | 373 | MEQ7670071 | 125 | 373 |
| | | | --- / --- | | | --- / --- | | | *GCA / A - - | Thr | | --- / --- | | | --- / --- | |
| 2 | VraS (HK) | MEQ7661159 | --- | --- | MEQ7734074 | --- | --- | MEQ7742072 | --- | --- | MEQ7620782 | --- | --- | MEQ7671694 | --- | --- |
| | | | --- / --- | | | --- / --- | | | --- / --- | | | --- / --- | | | --- / --- | |
| | VraR (R) | MEQ7661159 | 121 | 363 | MEQ7734073 | 121 | 363 | MEQ7742071 | 121 | 363 | MEQ7620781 | 121 | 363 | MEQ7671693 | --- | --- |
| | | | *AGC / - - A | Arg | | *AGC / - - A | Arg | | *AGC / - - A | Arg | | *AGC / - - A | Arg | | --- / --- | |
| | | | --- | --- | | --- | --- | | 59 | 117 | | --- | --- | | --- | --- |
| | | | --- / --- | | | --- / --- | | | *GAA / - - T | Asp | | --- / --- | | | --- / --- | |
| 3 | LytS (HK) | MEQ7660127 | 65 | 198 | MEQ7733332 | --- | --- | MEQ7740644 | 615 | 1725 | MEQ7619381 | 65 | 198 | MEQ7670622 | --- | --- |
| | | | *CAT / T - - | Tyr | | --- / --- | | | *GTA / - C - | Ala | | *CAT / T - - | Tyr | | --- / --- | |
| | LytR (R) | MEQ7660126 | 118 | 354 | MEQ7733333 | 118 | 354 | MEQ7740643 | 118 | 354 | MEQ7619380 | 118 | 354 | MEQ7670623 | --- | --- |
| | | | *ATT / - A - | Asn | | *ATT / - C - | Thr | | *ATT / - C - | Thr | | *GTG / - - T | Asn | | --- / --- | |
| | | | 125 | 375 | | 125 | 375 | | 125 | 375 | | 125 | 375 | | --- | --- |
| | | | *AAC / - G - | Ser | | *AAC / - G - | Ser | | *AAC / - G - | Ser | | *AAC / - G - | Ser | | --- / --- | |
| | | | --- | --- | | 191 | 576 | | 127 | 381 | | --- | --- | | --- | --- |
| | | | --- / --- | | | *ACT / G - - | Ala | | *AGT / - A - | Asn | | --- / --- | | | --- / --- | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | GraS (HK) | MEQ7659510 | 224 | 672 | MEQ7733938 | 224 | 672 | MEQ7741498 | 224 | 672 | MEQ7618845 | 224 | 672 | MEQ7669621 | --- | --- |
| | | | *ACA / - T - | Ile | | *ACA / - T - | Ile | | *ACA / - T - | Ile | | *ACA / - T - | Ile | | --- / --- | |
| | | | --- | --- | | 25 | 78 | | 325 | 975 | | --- | --- | | --- | --- |
| | | | --- / --- | | | *TTC / - - G | Leu | | *AGA / - A - | Lys | | --- / --- | | | --- / --- | |
| | GraR (R) | MEQ7659511 | 148 | 444 | MEQ7733937 | 148 | 444 | MEQ7741499 | 148 | 444 | MEQ7618844 | 148 | 444 | MEQ7669622 | --- | --- |
| | | | *CAG / G - T | Asp | | *CAG / G - T | Asp | | *CAG / G - T | Asp | | *CAG / G - T | Asp | | --- / --- | |
| | | | --- | --- | | --- | --- | | 26 | 78 | | --- | --- | | --- | --- |
| | | | --- / --- | | | --- / --- | | | *AAT / - T - | Ile | | --- / --- | | | --- / --- | |
| | | | --- | --- | | --- | --- | | 135 | 405 | | --- | --- | | --- | --- |
| | | | --- / --- | | | --- / --- | | | *GTC / A - T | Ile | | --- / --- | | | --- / --- | |
| | | | --- | --- | | --- | --- | | 136 | 408 | | --- | --- | | --- | --- |
| | | | --- / --- | | | --- / --- | | | *GTT / A - A | Ile | | --- / --- | | | --- / --- | |
| 5 | NsaS (HK) | MEQ7660348 | 72 | 216 | MEQ7733660 | 196 | 588 | MEQ7741404 | 17 | 51 | MEQ7621050 | 72 | 216 | MEQ7670176 | --- | --- |
| | | | *TTT / - - C | Thr | | *ATG / G - - | Leu | | *ATT / G - - | Val | | *TTT / - - C | Thr | | --- / --- | |
| | NsaR (R) | MEQ7660349 | 148 | 444 | MEQ7733659 | 148 | 444 | MEQ7741405 | 148 | 444 | MEQ7621051 | 148 | 444 | MEQ7670175 | --- | --- |
| | | | *AGC / - A - | Asp | | *AGC / - A - | Asp | | *AGC / - A - | Asp | | *AGC / - A - | Asp | | --- / --- | |
| 6 | HptS (HK) | MEQ7660166 | 151 | 453 | MEQ7733293 | 92 | 276 | MEQ7740682 | 134 | 402 | MEQ7619420 | 151 | 453 | MEQ7671205 | --- | --- |
| | | | *ACC / - G - | Ser | | *GAA / - G - | Gly | | *CAG / - G - | Arg | | *ACC / - G - | Ser | | --- / --- | |
| | | | 161 | 483 | | --- | --- | | 248 | 744 | | 161 | 483 | | --- | --- |
| | | | *AGC / - A - | Asn | | --- / --- | | | *GCT / T - - | Ser | | *AGC / - A - | Asn | | --- / --- | |
| | | | 490 | 1470 | | --- | --- | | --- | --- | | 490 | 1470 | | --- | --- |
| | | | *CAT / A - - | Asn | | --- / --- | | | --- / --- | | | *CAT / A - - | Asn | | --- / --- | |
| | HptR (R) | MEQ7660167 | 124 | 372 | MEQ7733292 | --- | --- | MEQ7740683 | 189 | 567 | MEQ7619421 | 124 | 372 | MEQ7671204 | --- | --- |
| | | | *CAA / - G - | Arg | | --- / --- | | | *GAT / A - - | Asn | | *CAA / - G - | Arg | | --- / --- | |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | AirS (HK) | MEQ7661546 | 49 | 147 | MEQ7733118 | 260 | 780 | MEQ7742279 | 213 | 639 | MEQ7621196 | 49 | 147 | MEQ7671768 | --- | --- |
| | | | *AAT / - G - | Ser | | *ATG / - - C | Ile | | *TCC / G - - | Ala | | *AAT / - G - | Ser | | --- / --- | |
| | | | 211 | 633 | | --- | --- | | 350 | 1050 | | 211 | 633 | | --- | --- |
| | | | *AAC / - C - | Ser | | --- / --- | | | *AGA / - A - | Lys | | *AAC / - C - | Ser | | --- / --- | |
| | | | 350 | 1050 | | --- | --- | | 352 | 1056 | | 350 | 1050 | | --- | --- |
| | | | *AGA / - A - | Lys | | --- / --- | | | *ACC / - T - | Ile | | *AGA / - A - | Lys | | --- / --- | |
| | AirR (R) | MEQ7661547 | --- | --- | MEQ7733117 | --- | --- | MEQ7742280 | --- | --- | MEQ7621197 | --- | --- | MEQ7671769 | --- | --- |
| | | | --- / --- | | | --- / --- | | | --- / --- | | | --- / --- | | | --- / --- | |

**Tabel 4: Vitek 2 system antibiotics sensitivity testing for in *S. aureus* MHC, MHB, MHH, MHF, and MHO. isolates.**

| Antimicrobial | Strains | | | | |
|---|---|---|---|---|---|
| | MHB | MHH | MHO | MHF | MHC |
| Benzylpenicillin | **R** | **R** | **R** | **R** | **S** |
| Oxacillin | **R** | **R** | **R** | **R** | **S** |
| Gentamicin | **S** | **R** | **S** | **S** | **S** |
| Ciprofloxacin | **S** | **R** | **S** | **S** | **R** |
| Moxifloxacin | **S** | **R** | **S** | **S** | **R** |
| Erythromycin | **S** | **R** | **S** | **S** | **S** |
| Clindamycin | **S** | **S** | **S** | **S** | **S** |
| Linezolid | **S** | **S** | **S** | **S** | **S** |
| Teicoplanin | **S** | **S** | **S** | **S** | **S** |
| Vancomycin | **S** | **S** | **S** | **S** | **S** |
| Tetracycline | **S** | **S** | **S** | **S** | **S** |
| Tigecycline | **S** | **S** | **S** | **S** | **S** |
| Fusidic Acid | **S** | **R** | **S** | **S** | **S** |
| Rifampicin | **S** | **S** | **S** | **S** | **S** |

## Discussion

In this study bioinformatics approach was employed to identify variation in TCSs that had been linked to antimicrobial resistance. Five local *S. aureus* isolates genomes were recently deposited in the NCBI database. These isolates were obtained from humans, dog, cat, cow, and sheep samples. Genomes in the NCBI database includes: MHH (GCA_040196155.1), MHB (GCA_040196135.1), MHO (GCA_040195495.1), MHF (GCA_040195555.1), and MHC (GCA_040195445.1). This study involved SNP analysis to elucidate the genomic differences among the isolates. This study aims to investigate polymorphism among local isolates of *S. aureus*, specifically MHC, MHB, MHH, MHF, and MHO, with a focus on antibiotic resistance-related TCSs in these isolates. We hypothesize that variation among TCSs from local isolates may explain their antimicrobial resistant profile. Therefore, the main focus of our study was about the antimicrobial related TCSs sequence variation among *S. aureus* MHC, MHB, MHH, MHF, and MHO local isolates. Therefore, the data of the variants were filtered and focused on the TCSs sequence differences. Variations among these sequences were identified. The effect of TCSs variation could be beneficial or detrimental to *S. aureus* and depends on the type and consequence of the mutation, the mode of action of antibiotics, and the role of TCSs in cellular activities (21-23). Various TCSs of *S. aureus* have been associated with multidrug resistance through alterations in the cell wall, efflux mechanisms, and the inhibition of drug uptake (23). The WalKR TCS locus plays an important role in modulating cell wall metabolism. Research demonstrates that mutations in WalRK can confer resistance to vancomycin, leading to co-resistance to both vancomycin and daptomycin. Furthermore, these mutations (triggered by single nucleotide alterations in either WalK or WalR) caused the characteristic cell wall thickening in resistant clinical isolates. These mutations result in co-resistance to vancomycin and daptomycin (24). The VraSR TCS plays a crucial role in antibiotic resistance particularly against cell wall active antibiotics such as vancomycin and methicillin. A significant decrease in resistance to vancomycin and beta-lactam antibiotics occurred when vraS was inactivated (25). A recent study showed that VraSR controls the susceptibility to complestatin (Cm) and corbomycin (Cb) which are glycopeptide antibiotics. Deletion of vraSR increased bacterial susceptibility to both antibiotics (26). Furthermore a study revealed that both ArlRS and VraSR TCS are determinants enabling *S. aureus* to endure sub MIC doses of ceftaroline The research further revealed that simultaneous disruption of both arlRS and vraSR produced an intense ceftaroline hypersensitivity phenotype (27).

NsaRS TCS (also known as BraRS) is a member of the intramembrane-sensing histidine kinase (IM-HK) family. The NsaRS two-component system has been investigated for its involvement in *Staphylococcus aureus* resistance to bacitracin and nisin. NsaS mutants exhibit a 200-fold reduction in their capacity to build

up resistance to the cell-wall-targeting antibiotic bacitracin (28). Bacitracin antibiotic interferes with peptidoglycan formation by binding to undecaprenyl pyrophosphate (UPP), the lipid carrier essential for the translocation of cell envelope precursors to the extracellular side of the cell membrane (29). demonstrated that the complete lack of the NsaRS two-component system renders staphylococci significantly susceptible to bacitracin, and this regulatory mechanism regulates the production of the BraDE and VraDE ABC transporters, which serve distinct functions in antibiotic resistance. Interestingly, whereas the BraDE transporter is essential for activating the phosphorylated conformational state of BraR and does not contribute to antibiotic resistance, VraDE is necessary to confer resistance to bacitracin and nisin in bacteria (29).

*Staphylococcus aureus utilizes* the hexose phosphate transport (HPT) system to acquire energy and synthesize cellular components. The HPT system in *S. aureus* includes the HptSR TCS, the *hptA* gene (a putative phosphate sensor), and the *uhpT* gene (a hexose phosphate transporter). Mutational analyses demonstrated that the inactivation of the *hptA*, *hptRS*, or *uhpT* genes hindered bacterial growth under conditions where glucose-6-phosphate was the sole carbon source, compromised survival and proliferation inside several host cell types, and enhanced resistance to Fosfomycin (30).

A study showed that deleting the AirSR TCS from *S. aureus* NCTC8325 decreased the activity of genes involved in cell wall metabolism, such as cap, ddl, and pbp1. It also showed a significant drop in the minimum concentration of this mutant that could stop vancomycin from working. Subsequent analysis revealed that the AirR protein directly interacts with the promoter regions of cell wall biosynthetic genes, including cap, ddl, and pbp1. The positive regulation of these genes improved the cell wall's anabolic process, thereby increasing the bacteria's susceptibility to vancomycin. (31).

*Staphylococcus aureus* LytSR TCS controls the lrgAB operon, which in turn controls programmed cell death and lysis, along with the related cidABC operon. The cid and lrg operons influence murein hydrolase activity, stationary-phase viability, antibiotic resistance, and biofilm development. LytSR may not directly alter antibiotic tolerance; nevertheless, it affects the variables that determine antibiotic response and efficacy. For instance, it impacts biofilm development, which can reduce antibiotic effectiveness. The LytRS TCS has a dual function by regulating extracellular DNA production during biofilm formation while protecting *S. aureus* against the effects of cationic antimicrobial polypeptides (CAPs). The LytRS TCS is triggered when CAPs attempt to damage the integrity of the cell membrane, leading to bacterial death through a series of lytic mechanisms (32). As CAPs damage the surface of the bacterial cell, the sensor LytS is auto phosphorylated. This then turns on the regulator LytR, which raises the expression of the *irgAB* gene. This stops the cell from dying normally death (33) (7).

*Staphylococcus aureus* employs the two-component regulatory system GraRS to sense and respond to host defense peptides

(HDPs) GraS (34). The genetic inactivation of GraRS results in heightened vulnerability to the cationic peptides nisin A and/or nukacin ISK I (22). Furthermore, another research indicates that the deletion of graRS in the MRSA USA500 strain led to heightened susceptibilities to ampicillin, oxacillin, vancomycin, and gentamicin (8).

The reason the SNPs analysis was employed in comparison to the closest genome *Staphylococcus aureus* subsp. aureus DSM 20231 (CP011526) and not NZ_AP014921 is that the response regulator's main function is to bind to the promoter of many genes across the genome. Therefore, genome-based selection for the reference may increase the resolution of the predicted variations.

The changes in amino acids of the TCSs compared to the reference TCSs sequence may have an influence on the function of sensing and responding to antimicrobials. Although not conclusive our results showed that the host background of the strain may have an influence on the TCSs variations and antibiotic resistance profile. For instance, the MHH strain that was isolated from a human host had multi-resistance to seven antibiotics, which is due to long exposure to several types of antibiotics compared to animals, which usually receive limited types of antibiotics and less frequently throughout their lives, which is consistent with the findings of other studies (35). In contrast to this notion, the number and types of SNPs variation were not attributed to the host as can be noted in (Table 3) which shows that some isolates had high degree of polymorphism regardless to the host of isolation. This may be explained by the zoonotic nature of the *S. aureus* which could have more tropism to specific hosts (24,36). The authors believe this was the case in the current study, and both hosts and history of antibiotic exposure are the main drivers for TCSs sequence variation, which cumulatively affected the antibiotic resistance profile of local *S. aureus* isolates. The authors also propose that SNPs in these TCSs listed in (Table 3) may have an influence on antibiotics resistance profile of the local isolates which in (Table 4). However, more in depth wet lab experiments are required to confirm the SNPs effects.

## Conclusions

In conclusion, our results show that there are sequence variations in the TCSs of *S. aureus* local isolates from humans, dogs, cats, cows, and sheep. There is significant variation among the genomes of the local isolates that may affect their biological responses. This sequence variation among local isolates may have an impact on the antibiotic resistance profile and could explain the differences in virulence between humans and animals' isolates.

## Conflicts of interest

The authors declare that there is no conflict of interest.

## Ethical Clearance

This work is approved by The Research Ethical Committee.

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# الاختلافات الجينية في بكتريا المكورات العنقوديه الذهبيه للنظام المكون من مكونين واجهاد استجابة المضادات الحيوية

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## الخلاصة

تستخدم *Staphylococcus aureus* أنظمة نقل الإشارة المكونة من مكونين (TCSs) لاستشعار والاستجابة للعوامل المسببة للتوتر مثل مضادات الميكروبات. قد تؤثر الاختلافات في تسلسل أنظمة نقل الإشارة المشاركة في مقاومة مضادات الميكروبات، مثل WalRK و VraSR و LytSR و GraSR و NsaRS و HptSR وAirRS، على ملف مقاومة *Staphylococcus aureus*في العزلات السريرية. حللت هذه الدراسة جينومات خمس عزلات محلية من S. aureus من البشر والكلاب والقطط والأبقار والأغنام، تم إيداعها مؤخرًا وشرحها في قاعدة بيانات .NCBI أظهر تحليل الجينومات أن العدد الإجمالي للمتغيرات (التغيير في تسلسل نوكليوتيدات الحمض النووي) كان 19176 و19924 و20168 و20499 و42248 لـ MHC و MHBو MHHو MHFو MHOعلى التوالي. وبالمقارنة مع العزلات الأخرى، أظهرت عزلة MHOمن حالة سريرية للأغنام أعلى عدد من المتغيرات. وكانت الطفرات المترادفة والمنسوخة هي الأعلى في سلالة MHOمقارنة بالعزلات الأخرى. وأظهرت سلالة MHH البشرية مقاومة لسبعة مضادات حيوية مقارنة بعزلات أخرى من أصل حيواني، والتي أظهرت مقاومة للمضادات الحيوية لنوعين من المضادات الحيوية وفقًا لنتائج .VITEK بالإضافة إلى ذلك، كشف تحليل تسلسل الجينوم لجينات النظام المكون من مكونين عن اختلافات بين العزلات المحلية، مما قد يؤثر على ملف مقاومة المضادات الحيوية لديها.

**الكلمات المفتاحية:** المكورات العنقودية الذهبية، مقاومة المضادات, تعدد الأشكال.