Journal of Wasit for Science and Medicine 2025: 18, (4), 24-31

Prevalence of Scimeca-Encoded Resistance Gens in Oral *Staphylococcus Aures* from Gingivitis Cases in Wasit Province, Iraq

Ayat Ayid Mahmood Alewi, and Khairi Jameel Al-Ruaby

Department of Biology, College of Science, Wasit University, Iraq.

Abstract

The current study investigates the prevalence and antibiotic resistance of *Staphylococcus aureus* in gingivitis patients at Aziziyah General Hospital from November 2024 to February 2025. A total of 139 clinical samples were collected and analysed using selective media, including mannitol salt agar, and blood agar, alongside gram staining and polymerase chain reaction (PCR) techniques. Results indicated that 63 isolates (45.3 %) were positive for *S. aureus*, with the highest prevalence found in plaque-induced gingivitis (39.6 %). Moreover, Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in 29 % of cases, with varying SCCmec types detected among isolates. Findings underscore the significance of *S. aureus* as a major pathogen in periodontal disease and highlight the need for effective monitoring and treatment strategies to combat antibiotic resistance.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), gingivitis, and SCCmec.

1. Introduction

Human oral cavity is conducive to microbial growth due to its warm temperature, moisture, and available nutrients [1]. This environment can lead to various oral health issues, including periodontal disease, tooth loss, dental abscesses, plaque buildup, and more. *Staphylococcus aureus* is a key pathogen in these infections, such as mucositis and dental caries [2].

This gram-positive *Staphylococcus aureus* is non-motile and non-spore-forming, often found in grape-like clusters. It is known for its virulence, toxin production, and antibiotic resistance [3]. Some strains have developed resistance to methicillin, resulting in MRSA [4-5]. Gingivitis is characterized by gum inflammation, leading to symptoms like

bleeding, swelling, and redness. Plaque serves as a habitat for pathogens, potentially triggering gingivitis, which can mineralize into tartar. The causes can be classified into three categories: caries, non-plaque-related factors, and bacterial accumulation within plaque.

Often, gingivitis is painless, and many patients are unaware of their condition [6-7]. While dental issues are rarely life threatening due to advancements in dentistry. They can lead to conditions such as periodontal disease, tooth loss, and abscess formation. Calculus contributes to inflammation in surrounding tissues. The clinical manifestation and prognosis of periodontitis are influenced by inflammatory responses, modifying factors, and microorganisms, along with genetic and environmental influences on each patient [8].

Staphylococcal Cassette Chromosome (SCC) mec is a mobile genetic element that harbours the mecA gene, responsible for methicillin resistance, along with other functional genes, consisting of two critical components, mec complex and ccr complex [9]. The mec gene complex comprises mecA, regulatory genes, and related insertion sequences, classified into six categories: A, B, C1, C2, D, and E. The ccr genes (ccrC or ccrA and ccrB) encode recombinases that enable the integration and excision of SCCmec [10]. The mecA gene encodes a low-affinity penicillin-binding protein, PBP2a, which imparts resistance to methicillin and all β-lactam antibiotics.

This protein is missing in susceptible staphylococci. The mecA gene is the principal factor conferring penicillin resistance in staphylococci, with SCCmec identified as the only carrier for mecA The SCCmec element includes site-specific cassette chromosome recombinase genes (ccrAB or ccrC), which integrate the SCCmec cassette into the core genome [11].

2. Materials and Methods

2.1 Samples collection

The study was conducted from November 2024 to February 2025. One hundred thirty-nine clinical samples were collected from patients, and visitors at the dental unit of Aziziyah General Hospital. Additionally, bacterial samples were collected from the health centre and Dr. Ziad Tariq's clinic in Aziziyah. Sterile gel swabs were used for bacterial sample collection, and swabs were transported to the bacteriology laboratory within two hours.

Samples were cultured on media selective (MSA) for *Staphylococcus aureus*, and isolates were stained and subjected to biochemical tests for identification.

2.3 Blood Agar

One gram of the medium (in powder form) was dissolve in distilled water to prepare one litre of solution to make blood agar. The mixture was then heated to a boil with continuous agitation to ensure complete dissolution of the components. After sterilizing, cooling the medium to 45 °C and adding 5 % human blood and pouring into Petri.

2.4 Mannitol Salt Agar

Clinical samples were swabbed and subsequently cultured on a selective medium mannitol salt agar (MSA). This medium serves to differentiate *Staphylococcus aureus* based on its capacity to ferment mannitol. Resulting the production of acidic by products that lower the pH of the medium. Consequently, phenol red indicator in the agar turns yellow [12]. Then, samples were subculture onto blood agar and subsequently onto mannitol salt agar [13].

2.5 Gram Stain Method

The Gram staining process was initiated by preparing a smear, which involved suspending a small bacterial colony in normal saline on a glass slide. The smear was heat-fixed and the primary stain, crystal violet, was applied. That was followed by the application of iodine as a mordant, decolorization with alcohol. Finally, counterstaining with safranin, where the slide was subsequently viewed under a 100 × oil immersion objective [14].

3. Results and Discussion

3.1 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a fundamental tool in molecular biology, used to rapidly and efficiently amplify small quantities of DNA. This technique relies on a series of thermal cycles. PCR technology in the study of oral *Staphylococcus aureus* isolates efficiently measures the prevalence of SCCmec genes responsible for methicillin resistance.

Table 1: PCR oligonucleotide primers and amplicon size.

Target gene	Primer sequence (5′→3′)	Amplicon size (bp)
SCCmec I	Fw:GCTTTAAAGAGTGTCGTTACAGG Rv:GTTCTCTCATAGTATGACGTCC	613
SCCmec II	Fw:CGTTGAAGATGATGAAGCG Rv:CGAAATCAATGGTTAATGGACC	398
SCCmec III	Fw:CCATATTGTGTACGATGCG Rv:CCTTAGTTGTCGTAACAGATCG	280

3.2 Prevalence of *S. aureus* among various type of gingivitis.

The prevalence of *S. aureus* among various type of gingivitis showed in (table 2). In the current study, the highest percentage of *S. aureus* infections was found in Plaque-induced gingivitis 25 (39 %). This bacterium can be considered one of the major agents of community- acquired

S. aureus infection in periodontal disease, followed by tooth decay and the frequency of *S. aureus* infections in periodontal disease. *S. aureus* was 17 (26 %), then ulcerative gingivitis 10 (16 %), systemic diseases 8 (13 %), and dentin hypersensitivity 3 (5 %).

Table 2: Prevalence of *S. aureus* among various oral infections.

Oral infections type	No.	%
Plaque-induced gingivitis	25	39.6
Specific infection-related gingivitis	17	26.6
Necrotizing ulcerative gingivitis	10	15.8
Systemic diseases gingivitis	8	12.6
Allergic gingivitis	3	4.7
Total	63	100
X2	39.6*	
P value	0	

* Highly significant difference (P < 0.01)

The present study's findings revealed that *S. aureus* isolated from gingivitis which may be caused by periodontal disease, could operate as a reservoir for opportunistic microorganisms. If antibiotics are used to treat periodontal disease or other infections, they can lead to an increase in *Staphylococcus spp*. in the oral cavity *S. aureus* strains can cause antibiotic resistance is widespread and can periodontitis develop because of antibiotic therapy.

The fact that *S. aureus* is more prevalent in the oral cavity might result in a more severe illness. The current percentages of isolated *S. aureus* are consistent with those reported by Al-Akwa [15]. That found Plaque-induced gingivitis was 36 (33.8 %) followed by tooth decay and dental plaque.

3.3 Prevalence MRSA among various type of gingivitis

The Prevalence of (MRSA) among various type of gingivitis was a variant rate were in Plaque-induced gingivitis 111/29 (37.9 %). Followed by specific infection-related gingivitis 8/29 (27.5 %) from patients most of them were already on antibiotics. Necrotizing ulcerative gingivitis was 5/29 (17.4 %), systemic diseases gingivitis 4/29 (13.9 %) while, allergic gingivitis was 1/29 (3.4 %). A study conducted in Mosul, Iraq revealed a significant prevalence of multidrug-resistant organisms (MDROs) at 86 %, 52.8 % of isolates most of these MRSA isolates were obtained from wound samples [16].

The high prevalence of pus is likely due to the exposure of wounds to microorganisms in the environment and the presence of *S. aureus* as a skin commensal, making wound prone to MRSA infection. Also, the prevalence of MRSA in urine was (14.8 %). This conclusion was not consistent with the findings of conducted in Ethiopia found that out of 422 gingivitis suspected patients, 53 (12.6 %) cultured *S. aureus* [17]. From these *S. aureus* isolates, 43.4 % (23/53) were MRSA. Another study

in a tertiary care hospital in Northern India, found out of 27 *S. aureus* isolates from urine samples, 13 (48.1 %) were MRSA [18].

3.4 Distribution of *Staphylococcal Cassette* chromosome (SCC) mec types among MRSA study isolates.

All Twentynine MRSA isolates were SCCmec type able, and the investigated SCCmec types from I to V were detected. Results of PCR assay revealed that the most predominant SCCmec types showed in (table 3).

Table 3: Distribution of SCCmec types among MRSA study isolates.

SCCmec types	Number	%
SCCmec I	3	10.3
SCCmec II	7	24.1
SCCmec III	5	17.2
SCCmec IV	8	27.4
SCCmec V	6	20.6
Total	29	100

SCCmec types I–VI are derived from mec and ccr complexes. Infections caused by health care-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are often linked to multidrug-resistant strains with SCCmec types I, II, or III, while type IV is rarely observed [19].

Community-associated methicillin-resistant *Staphylococcus aureus* typically carries one of three SCCmec types, is sensitive to most non-β-lactam antibiotics, produces Panton-Valentine leukocidin (PVL), and exhibits distinct pulsed-field gel electrophoresis (PFGE) patterns. All SCCmec elements share four characteristics, contain the mec gene complex (mec), include the ccr gene complex (ccr), are flanked by characteristic nucleotide sequences and repeats, and integrated at the integration site sequence (ISS) for SCC [20].

The SCCmec gene is a major mobile genetic element responsible for methicillin resistance, including the mecA gene, which enables resistance to beta-lactam antibiotics. SCCmec moves using chromosomal cassette recombination (Ccr), facilitating its spread among S. aureus cells. Our study's findings on SCCmec prevalence align with a study from healthcare workers in Duhok, confirming the presence of the mecA gene and SCCmec type-encoding genes. MRSA strains tested positive for the mecA gene, with SCCmec types I, II, and III present at rates of 31 % (18 isolates), 20.1 % (12 isolates), and 20.1 % (12 isolates), respectively.

Additionally, 19.2 % of strains were type IV (10 strains) [21]. Similar rates (28 % to 40 %) were reported in Saudi Arabia and Iraq, indicating the commonality of this MRSA gene [22].

A recent study at Imam Reza Hospital in Birjand, Iran, revealed SCCmec I at 27.9 %, type III at 23.3 %, and the most

prevalent type, SCCmec IV, at 37.2 %. Types I and IV were most common in HA-MRSA isolates (32.4 % each), while SCCmec type IV (66.7 %) was prevalent in CA-MRSA isolates. The study emphasized the need for effective antibiotic management and microbiological surveillance in healthcare settings to control resistant bacteria [23].

4. Conclusion

The study reveals a significant relationship between gingivitis and genes associated with resistance to *Staphylococcus aureus*. The mecA gene, located within the SCCmec element, contributes to the bacteria's resistance to beta-lactam antibiotics, thereby increasing the likelihood of exacerbated gingival inflammation.

Individuals carrying specific genes may be more susceptible to infections due to a weakened immune response, allowing pathogenic bacteria to proliferate in the oral cavity. Additionally, the presence of these genes can interact with environmental factors, such as oral hygiene, which further intensifies the severity of gingival inflammation. Furthermore, genetic factors may determine individuals' responses to various treatments, influencing the effectiveness of therapeutic approaches.

Thus, understanding the interplay between genetics and gingivitis highlights the importance of genetic considerations in developing effective treatment strategies.

5. References

1. Zhu Y., Wang Y., Zhang S., Li J., Li X., Ying Y., Yuan J., Chen K., Deng S., and Wang Q., (2023). Association of polymicrobial interactions with dental caries development and prevention. Frontiers in microbiology. 14, 1162380.
2. Passariello C., Puttini M., Iebba V., Pera P., and Gigola P., (2012). Influence of oral conditions on colonization by highly toxigenic Staphylococcus aureus strains. Oral diseases. 18, 4, 402-409.
3. Congdon S. T., Guaglione J. A., Ricketts O. M. A., Murphy K. V., Anderson M. G., Trowbridge D. A., Al-Abduladheem Y., Phillips A. M., Beausoleil A. M., Stanley A. J., Becker T. J., and Silver A. C., (2023). Prevalence and antibiotic resistance of *Staphylococcus aureus* associated with a college-aged cohort: life-style factors that contribute to nasal carriage. Frontiers in cellular and infection microbiology. 13, 1195758.
4. Faden A., (2019). Methicillin-resistant Staphylococcus aureus (MRSA) screening of hospital dental clinic surfaces. Saudi journal of biological sciences. 26, 7, 1795-1798.
5. Rajaduraipandi K., Mani K. R., Panneerselvam K., Mani M., Bhaskar

M., and Manikandan P., (2006). Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study. Indian journal of medical microbiology. 24, 1, 34-38.

6. Leous P., Palianskaya L., and Leous L., (2009). Oral hygiene and gingival inflammation in 6–8-year-olds from a junior school in Minsk who participated in a supervised oral hygiene programme. Oral Health Dent Manag. 7, 27-30.
7. Pawlaczyk-Kamieńska T., Torlińska-Walkowiak N., and Borysewicz-Lewicka M., (2018). The relationship between oral hygiene level and gingivitis in children. Advances in clinical and experimental medicine: official organ Wroclaw Medical University. 27, 10, 1397-1401.
8. Bartold P. M., (2018). Lifestyle and periodontitis: The emergence of personalized periodontics. Periodontology 2000. 78, 1, 7-11.
9. Zong Z., Peng C., and Lü X., (2011). Diversity of SCCmec elements in methicillin-resistant coagulase-negative staphylococci clinical isolates. PloS one. 6, 5, e20191.
10. Saber H., Jasni A. S., Jamaluddin T. Z. M. T., and Ibrahim R. (2017). A Review of Staphylococcal Cassette Chromosome mec (SCCmec) Types in Coagulase-Negative Staphylococci (CoNS) Species. The Malaysian journal of medical sciences: MJMS. 24, 5, 7-18.
11. Wang Y., Zhao N., Jian Y., Liu Y., Zhao L., He L., Liu Q., and Li M., (2022). The pro-inflammatory effect of Staphylokinase contributes to community-associated Staph. aureus pneumonia. Communications Biology. 5, 1, 618.
12. Michael J Leboffe, and Burton E Pierce., (2021). A Photographic Atlas for the Microbiology Laboratory, Fifth Edition. Morton Publishing Company.
13. Gurung R. R., Maharjan P., and Chhetri G. G., (2020). Antibiotic resistance pattern of Staphylococcus aureus with reference to MRSA isolates from pediatric patients. Future science OA. 6, 4, FSO464.
14. Tripathi N., Zubair M., and Sapra A., Gram Staining. [Updated 2025 Mar 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562156.
15. Al-Akwa A. A. Y., Zabara A. Q. M. Q., Al-Shamahy H. A., Al-labani M. A., Al-Ghaffari K. M., Al-Mortada A. M., and Al-Sharani A. A., (2020). Prevalence of Staphylococcus aureus in dental infections and the occurrence of MRSA

in isolates. Universal Journal of pharmaceutical research. 5, 2, 23-27.

16. M'Aiber S., Maamari K., Williams A., Albakry Z., Taher A. Q. M., Hossain F., Fliti S., Repetto E., and Moussally K., (2022). The challenge of antibiotic resistance in post-war Mosul, Iraq: an analysis of 20 months of microbiological samples from a tertiary orthopaedic care centre. Journal of global antimicrobial resistance. 30, 311-318.
17. Mitiku A., Aklilu A., Biresaw G., and Gize A., (2021). Prevalence and Associated Factors of Methicillin Resistance Staphylococcus aureus (MRSA) Among Urinary Tract Infection Suspected Patients Attending at Arba Minch General Hospital, Southern Ethiopia. Infection and drug resistance. 14, 2133-2142.
18. Arora S., Devi P., Arora U., and Devi B., (2010). Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care hospital in Northern India. Journal of laboratory physicians. 2, 02, 078-081.
19. Valsesia G., Rossi M., Bertschy S., and Pfyffer G. E., (2010). Emergence of SCCmec type IV and SCCmec type V methicillin-resistant Staphylococcus aureus containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central Switzerland: external invaders or persisting circulators?. Journal of clinical microbiology. 48, 3, 720-727.
20. Yamaguchi T., Ono D., and Sato A., (2020). Staphylococcal Cassette Chromosome mec (SCCmec) Analysis of MRSA. Methods in molecular biology (Clifton, N.J.). 2069, 59-78.
21. Hussein N., (2016). Prevalent Genotypes of Staphylococcus aureus Strains Isolated From Healthcare Workers in Duhok City, Kurdistan Region, Iraq. International Journal of Infection. 3, 2, e35375.
22. Al Hamdan A. S., Alghamdi A. A., Alyousif G. F., Hamza F. A., Shafey M. M., AlAmri A. M., and Sunki A. A., (2022). Evaluating the Prevalence and the Risk Factors of Gram-Negative Multi-Drug Resistant Bacteria in Eastern Saudi Arabia. Infection and drug resistance. 15, 475-490.
23. Sadeghi Moghaddam T., Namaei M. H., Afshar D., and Yousefi M., (2022). High frequency of SCCmec type IV and multidrug-resistant SCCmec type I among hospital acquired methicillin-resistant Staphylococcus aureus isolates in Birjand Imam Reza Hospital, Iran. Iranian journal of microbiology. 14, 1, 67-75.