



PREVALENCE OF METHICILLIN AND VANCOMYCIN RESISTANCE GENES IN STAPHYLOCOCCUS AUREUS ISOLATED FROM CLINICAL SPECIMENS IN AL-SAMAWAH CITY

¹Mojtaba Mohseni; ²Marwa Abdal Redha Alaskary; ³Saif Mazeel Abed

¹Department of Microbiology, University of Mazandaran, Babolsar, Iran

²Ministry of Education, Al-Muthanna Education Directorate, Iraq, Email: rwamo793@gmail.com

³Ministry of Health, Al-Muthanna Health Directorate, Iraq, Email: Drsaifmazeel@gmail.com,

ORCID: <https://orcid.org/0000-0003-3437-3684>

Corresponding Author: Mojtaba Mohseni

Abstract

Methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), is one of the most common nosocomial infections in humans and animals. The genes *mecA* and *vanA* in the *S. aureus* are the marker of MRSA and VRSA, respectively. The main objective of this study was to detect the prevalence of *mecA* and *vanA* genes which cause resistance against methicillin and vancomycin in the Al- Samawah, respectively. One hundred samples (8 burns, 68wounds, 11 skin sores and 13 urines) were collected from patients in Al-Hussein teaching hospital and medical clinics, Al-Muthanna. Then biochemical tests were performed for conventional identification. Modified Kirby-Bauer disc diffusion method was applied. The DNA of all isolates was extracted, then PCR was then performed for amplification of *mecA*, *VanA* genes. The results confirmed the existence of *S.aureus* in 41 out of 100 isolates. The *S. aureus* isolates showed high incidences of resistance against methicillin and vancomycin about 70% and 80%, respectively. Isolates were also resistant to other antibiotics, such as CX (60%), however the isolates showed more sensitive to following antibiotics including G (40%), CIP (50%), AZM (30%) and T (30%). PCR results revealed that 41 (41.00%) isolates of *S. aureus*. In addition, 25 (60.97%) and 19 (46.3%) isolates of *S. aureus* were harbored *mecA* and *VanA*, respectively. In conclusions, *S. aureus* is a commensal gram- positive bacterium that has captured the attention of the medical community for more than a century. The emergence of MRSA and VRSA strains in the community has increased the risk of infections caused by these strains, as they usually express multidrug resistance. There is a need for continuous surveillance and monitoring of the presence of MRSA and VRSA strains in the community and a clear understanding of the dynamics of the spread of MRSA and VRSA strains will assist in controlling its dissemination

Keywords: *Staphylococcus aureus*; methicillin; vancomycin

Introduction

Staphylococcus aureus (*S. aureus*) infection is one of the most common nosocomial and community-acquired infections in humans and animals (Kuroda et al., 2001). Almost 30% of the human population is asymptotically colonized with commensal *S. aureus* (Tong et al., 2015). *S.aureus* colonization is a global phenomenon affected by various factors, not limited to age, health, economic status and country. *S.aureus* may be colonized in multiple body parts, but the anterior nares are the most stable colonization site. *S.aureus* colonization has been identified as an important risk factor for the development of *S.aureus* infection in community and hospital settings (Chen et al., 2011).

S.aureus can cause a wide range of infections in humans. The most common sites affected are skin and soft tissue; manifestations of infections in these sites include folliculitis, furuncles and carbuncles, impetigo, mastitis, wound infections, and staphylococcal scalded skin syndrome. More serious infections include bacteremia, pneumonia, endocarditis, bone and joint infections, and toxic shock



syndrome. *S. aureus* can also be responsible for outbreaks of food poisoning (Peacock & Paterson, 2015). The organism can grow in temperatures ranging from 7 to 48.5 °C (optimum 30 to 37 °C), pH ranges from 4.2 to 9.3 (optimum 7 to 7.5), and a high concentration of sodium chloride up to 15%. They can grow at low levels of water activity (aw) as 0.83, however, their optimum growth is at aw > 0.99, besides its ability to tolerate desiccation, dry, and stressful environments as hands and food contact surfaces (Centers for Disease Control and Prevention, 2000).

Methicillin resistant *S. aureus* (MRSA) is *S. aureus* that is resistant to early generations of penicillin and penicillinase resistant penicillin (methicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin) antimicrobial agents. Methicillin resistance in Staphylococci is due to conjugated staphylococcal cassette chromosome mec (SCCmec) gene. The Staphylococcal cassette mec (SCCmec) composition consisting of two necessary complexes: mec gene complex and ccr gene complex. The mec gene complex comprises the mecA gene and two regulators (mecRI and mecI). The ccr gene complex encodes integration and excision of the entire SCC element. IR, inverted repeats; DR, direct repeats (Hiramatsu et al., 2014). All SCCmec types contained the mecA gene, which codes for the low-affinity penicillin binding protein 2a (PBP2a). The enzymes that are responsible for crosslinking the peptidoglycans of bacterial cell wall (Kim et al., 2012).

MRSA is the predominant *S. aureus* isolated from infected wounds (Percival et al., 2015). Beginning the late 1980s, vancomycin became the antibiotic of choice for treatment for patients infected with MRSA in hospital settings (Pardos et al., 2016). However, after a few years of marketing, vancomycin-resistant *S. aureus* strains emerged from Japan. *S. aureus* acquire vancomycin resistance plasmid gene from vancomycin resistant Enterococcus (VRE) through transposon Tn1546. VRSA is due to the acquisition of the *vanA* gene (Zhu et al., 2010). vancomycin-resistant *S. aureus* (VRSA) characterized by changing the target side of PBP and its substrate by changing D-ala-D-ala to D-ala-D-lac by binding to the C-terminal of the pentapeptide (Bitrus et al., 2018). With increased global dissemination of vancomycin resistance, detection of new antimicrobial agents or vaccine development are urgently required (Shariati et al., 2020). Despite the global recognition of MRSA and VRSA as major nosocomial pathogens, there is limited data regarding the prevalence and distribution of *mecA* and *vanA* genes in *S. aureus* isolates in Al-Muthanna governorate. The dynamics of antibiotic resistance in this region, particularly in both hospital and community-acquired infections, remain poorly understood. This lack of local epidemiological data hampers effective infection control and antimicrobial stewardship strategies, it is hypothesized that *S. aureus* isolates from clinical specimens in Al-Muthanna exhibit a high prevalence of methicillin and vancomycin resistance, mediated by the presence of *mecA* and *vanA* genes, and that these isolates may also display multidrug resistance against commonly used antibiotics. The current study was planned to determine the antibiotic resistance pattern of clinical isolates of *S. aureus*, and detection of resistance genes (i.e., *mecA* and *vanA*) which cause resistance against methicillin and vancomycin, respectively and its prevalence in the AL-Samawah.

Material and methods

Isolation of *S. aureus* from clinical specimens

To isolate Staphylococcus aureus, a total of 100 clinical specimens, including wounds, burns, skin sores, and urine, were collected from Al-Hussein Teaching Hospital in Al-Muthanna Governorate (Tables 1). All samples were initially inoculated into nutrient broth and incubated at 37 °C for 24 h. Following incubation, serial dilutions were prepared for each sample, and 100 µL from the appropriate dilution was spread onto blood agar and mannitol salt agar plates. The plates were incubated at 37 °C for 24 h. Identification of *S. aureus* was performed based on cultural characteristics and confirmed by *16S rRNA* gene analysis (Lemaire, 2008).



Table (1): Types and distribution of clinical specimens

Clinical specimen	Gender		Total	
	Female frequency	Male frequency	frequency	Percentage (%)
Burns	5	3	8	8%
Wound	25	43	68	68%
Skin sore	6	5	11	11%
urine	7	6	13	13%

Antibiotic susceptibility test

Antibiotic susceptibility analysis will be performed by modified Kirby Bauer’s Disk diffusion technique with commercially available antibiotic disc. Characterization of stains as susceptible, intermediately resistant or resistant was done as based on the size of the inhibition zone according to the manufacturer’s instruction which matched the interpretive criteria recommended by (CLSI 2020) as Vancomycin (VA), Methicillin (MET), Cefoxitin (CX), Ciprofloxacin (CIP), Gentamicin (G), Azithromycin (AZM), Tetracycline (T)

Genomic DNA extraction

DNA was extracted from Bacterial isolates, using the classical method by Presto Mini gDNA Bacteria Kit (Geneaid, USA) protocol for bacterial genome was used.

Primers

Primers of this study were manufactured by Macrogen company, (Korea). All of these primers were summarized in table (2).

Table (2): Primer list

Gene	Oligo Name	5' - Oligo Seq - 3'	PCR product (bp)
16SrRNA	16S_Staph_F	GGTCTTGCTGTCACTTATAGATGG	164
	16S_Staph_R	CGGAAGATTCCTACTGCTG	
<i>mecA</i>	MecA-F	AAAATCGATGGTAAAGGTTGGC	533
	MecA-R	AGTTCTGGAGTACCGGATTTGC	
<i>vanA</i>	VanA-F	GGCAAGTCAGGTGAAGATG	713
	VanA-R	ATCAAGCGGTCAATCAGTTC	

PCR Amplification of 16S rRNA gene.

Molecular identification of *S. aureus* was done according to Alves, et al. (2018), where 16S rRNA specific primers (primer list table 3-1) were used to produce 164bp product. PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). PCR mixtures and conditions of this assay was summarized in table (3).



Table (3): Uniplex PCR mixtures and conditions for identification of *16S rRNA* gene.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	35
Reverse Primer	2.5 µl	Annealing	56 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

Detection of *mecA* and *vanA* genes by PCR

PCR Amplification of *mecA* gene

Identification of *mecA* gene was done according to Murakami, *et al.* (1991). PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). Primers were listed in table (2-6). PCR mixtures and conditions of this assay was summarized in table (4).

Table (4): Uniplex PCR mixtures and conditions for identification of *mecA* gene.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	30
Reverse Primer	2.5 µl	Annealing	55 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

PCR Amplification of *vanA* gene.

Identification of *vanA* gene was done according to Azimian, *et al.* (2012). PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 µl (GoTaq® G2 Green Master Mix, Promega, USA). Primers were listed in table (2-6). PCR mixtures and conditions of this assay was summarized in table (5).

Table (5): Uniplex PCR mixtures and conditions for identification of *vanA* gene.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	30
Reverse Primer	2.5 µl	Annealing	55 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1



Gel electrophoresis

Gel electrophoresis is applied to ensure the validity of DNA extraction PCR results. 2% agarose concentration for the quality of the extracted DNA stained with 500µl of a 10mg/ml stock solution per 100 ml green star dye with 100 volts for 36 minutes. The results were confirmed by using Gel Documentation system (DUALED Blue / White Transilluminator, Bioneer, Korea).

Statistical analysis

Statistical analysis was performed using the chi-square (χ^2) test to evaluate the association between categorical variables. The test was applied to determine whether there were statistically significant differences between the observed and expected frequencies. A p-value of less than 0.05 was considered statistically significant

Results and Discussion

Isolation of *S. aureus* from clinical specimens

A total of 100 clinical specimens, including 68 wounds, 8 burns, 11 skin sores, and 13 urine samples, were collected from patients at Al-Hussein Teaching Hospital and affiliated medical clinics in Al-Muthanna Governorate. The specimens were cultured in nutrient broth and incubated at 37 °C for 24 h. Depending on colony morphology, the cells of *Staphylococcus aureus* appeared as Gram-positive cocci arranged in clusters, pairs, or singly (Sizar et al, 2020). On mannitol salt agar, the colonies were surrounded by yellow zones, indicating mannitol fermentation. From these samples, 41 *S. aureus* isolates were obtained, comprising 29 from wounds, 5 from burns, 5 from skin sores, and 2 from urine.

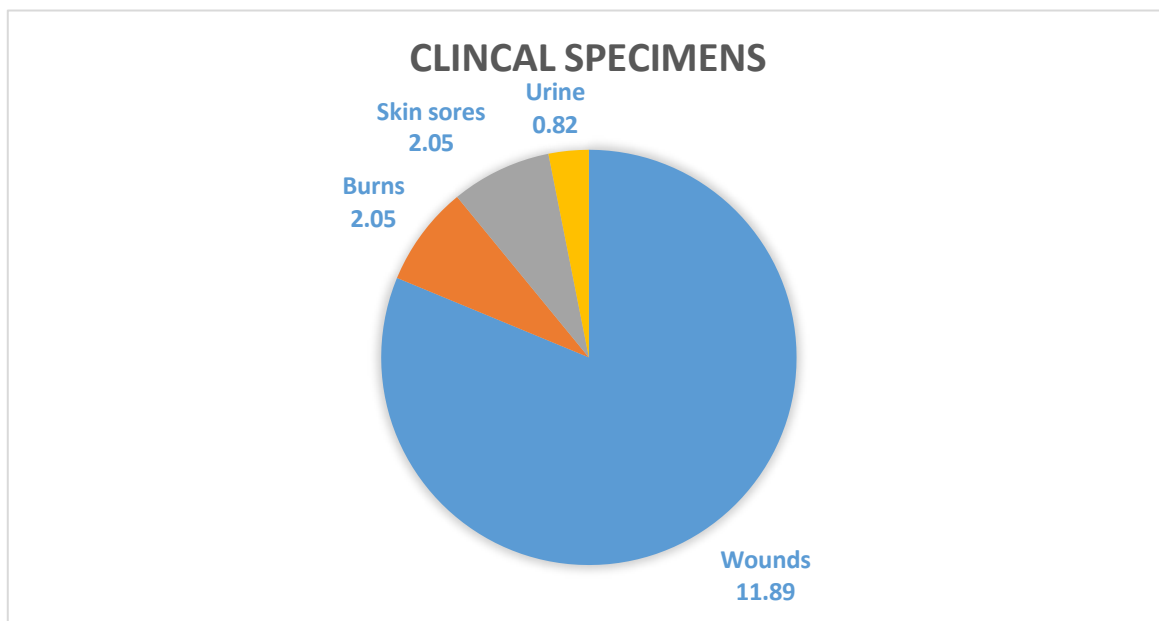


Figure (1): The isolation of *S.aureus* percentage %

The identification of *S. aureus* among the 100 clinical samples using *16S rRNA*-specific primers revealed that 41 isolates (41%) tested positive, while 59 samples (59%) were negative. Although the P value was <0.072, which does not reach the conventional level of statistical significance ($p < 0.05$), the results demonstrate that *16S rRNA* PCR is effective for confirming the presence of *S. aureus* in clinical specimens. as shown in the figure (2) and table (6).

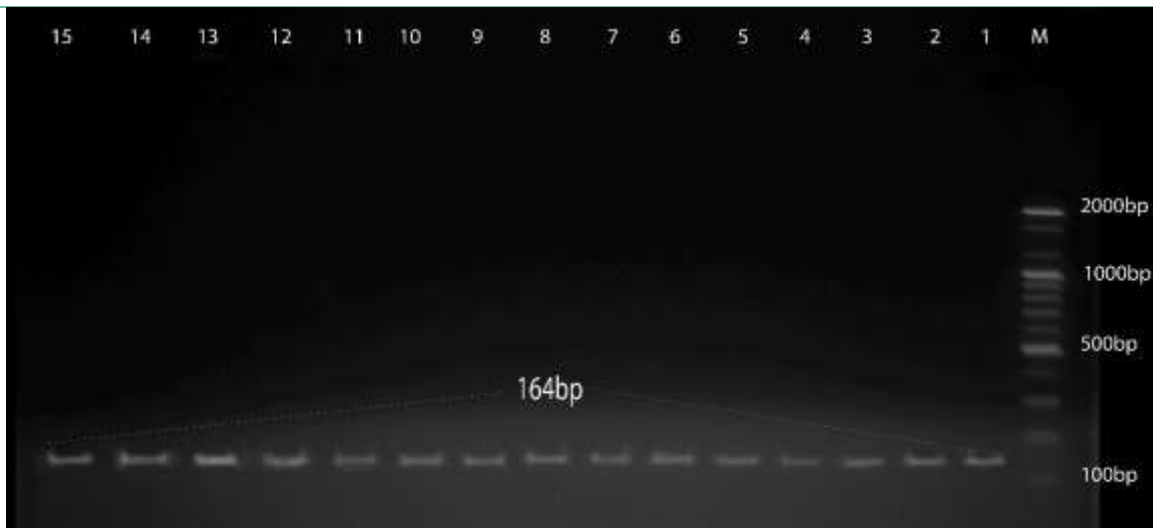


Figure (2): Identification of *S. aureus* isolates by 16S-rRNA gene. Lanes 1-15 represent the identified 164bp gene products, Lane M represent 100bp DNA ladder

Table (6): Identification of *S.aureus* among all studied samples using *16 SrRNA*-specific primer.

Results	N	Percentage	P value
Positive	41	41	<0.072
Negative	59	59	
Total	100	100%	

* represent a significant difference at $p < 0.05$

S. aureus is part of the skin microbiota of up to a third of the general population; the nasal vestibules (35%) and the perianal region (30%) are the main reservoirs, followed by the axillary and interdigital regions (5%-10%), where dissemination can occur, causing infections. Therefore, infections in healthcare settings caused by multi resistant *S. aureus* have become quite relevant in the latest decades, being responsible for high indices of morbidity and mortality(Breves et al., 2015).

MRSA, a leading clinical pathogen, has successfully colonized and transmitted between communities and health-care settings for six decades, and the epidemiology of antibiotic resistance surveillance for MRSA has always been a concerning worldwide issue(Wang et al., 2022). Due to the widespread occurrence of MRSA, the empiric therapy for MRSA was changed to vancomycin. This has lead to the emergence of vancomycin intermediate and vancomycin resistant *S.aureus* (Dhanalakshmi et al., 2012).The increasing rate of drug resistance associated with methicillin resistant Staphylococcus aureus and the emergence of vancomycin resistant trait is a great problem in human disease treatment and management. The present study was carried out to determine the prevalence, antibiotic susceptibility profile of MRSA, and vancomycin resistant *S. aureus* among patients and healthy subjects in Al-Samawah southern of Iraq

Several studies revealed that a total of 100 samples were screened out of which 70 isolates were catalase and coagulase positive. Several works have reported the detection, screening and identification of *S. aureus* following catalase test, coagulase test and growth on Mannitol salt agar (Hasan et al . 2016) .The finding that the prevalence of Staphylococcus aureus was highest in wounds (36.4%), burns (3.5%), skins sore (3.5%) and (1.4%) from urine. These results are consistent with previous studies(Kejela & Dekosa, 2022). A study involving a thousand healthy individual also demonstrated 22.5% and 16.6% of individuals colonized by Staphylococcus aureus and MRSA, respectively, in nostrils, forearm and hands(Gould et al., 2011) Concern over VRSA colonization and transmission is not caused exclusively by nasal carriers, but also by medical devices as vehicles of this transmission.



Thus, this colonization is considered a public health problem, and it is of interest to investigate whether health professionals are also nasal carriers of MRSA (Breves et al., 2015).

Susceptibility test

Antibiotic susceptibility analysis will be performed by modified Kirby Bauer's Disk diffusion technique with commercially available antibiotic disc. Characterization of stains as susceptible, intermediately resistant or resistant was done as based on the size of the inhibition zone according to the manufacturer's instruction which matched the interpretive criteria recommended by WHO. The antibiotics used were penicillin (P, 10 µg), azithromycin (AZM, 15 µg), ciprofloxacin (CIP, 5 µg), tetracycline (T, 30 µg), gentamicin (G, 10 µg), doxycycline (DO, 30 µg), vancomycin (VA, 30 µg), ofloxacin (OF, 5 µg), chloramphenicol (C, 30 µg), ampicillin (A, 10 µg), ceftiofur (CX, 30 µg) and oxacillin (OX, 1 µg). Methicillin (MET 5 µg). *S. aureus* isolates showed various response to antibiotics depending on antimicrobial resistance percentages the highest resistance noticed in VA (80%) and Met (70%) followed by, CX (60%), however the isolates showed more sensitive to following antibiotics; G (40%), CIP (50%) AZM (30%), T (30%) as shown in figure (3).

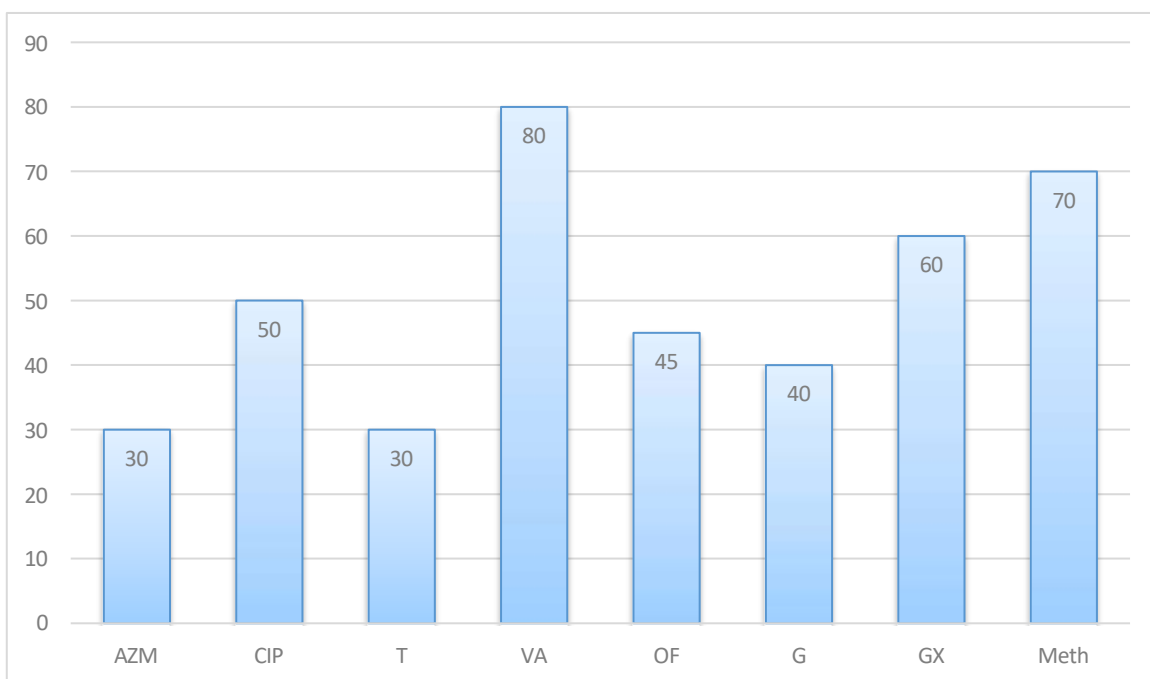


Figure (3): Antimicrobial Resistance Percentage %

The findings were agreed with (Dadashi et al., 2018). The analyses showed that the frequency of MRSA infections was 43.0% (95% confidence interval 36.3–50.0%) among confirmed *S. aureus* isolates. Further stratified analyses indicated that the prevalence of MRSA was higher in studies performed after the year 2000. Also, the results agreed with (Boswihi et al., 2018) who's reported that the 1,327 MRSA isolates investigated by using antibiotic susceptibility testing, SCCmec typing and spa typing. Antibiotic susceptibility testing showed that all MRSA isolates were susceptible to vancomycin, teicoplanin and linezolid. Besides beta-lactam resistance, resistance was observed for fusidic acid (619; 46.6%), kanamycin (563; 42.4%), erythromycin and clindamycin (521; 39.2%), trimethoprim (521; 39.2%), ciprofloxacin (508; 38.3%), tetracycline (427; 32.1%), chloramphenicol (120; 9.0%), and high-level mupirocin (49; 3.7%).

However, the results agreed with (Saeed et al., 2020) that reported that *S. aureus* isolates showed high incidences of resistance against methicillin (76%) and moderate incidences of resistance to vancomycin (14%). Isolates were also resistant to several other drugs, such as ceftiofur (76%), ertapenem (83%), ampicillin (81%), tobramycin (78%), moxifloxacin (76%), and tetracycline (74%). An encouraging finding was that 98% of isolates were susceptible to tigecycline, indicating its possible



role in the treatment of methicillin-resistant *S. aureus* (MRSA) and VRSA, as well as the multi-drug-resistant *S. aureus*. The *mecA* gene was detected in 33.3% of tested isolates (10/30), while the *vanA* gene was also detected in 30% (9/30) of the tested isolates (Saeed et al., 2020).

Basil AbdulRazzaq et al., (2022) who's reported that one hundred and fifty (150) samples were (100%) as *S. aureus*. found that all isolates were vancomycin resistance through the presence of the *van A* gene. The rate of vancomycin resistance is (10.3%) by vitek 2 system, the vancomycin resistant gene size is (732 bp), in which (13.7%) of isolates represented vancomycin resistant gene positive (Mahmood & Anwer, 2021). While Awayid and Mohammad (2022) showed the sensitivity of linezolid, quinupristin-dalfopristin, rifampin, daptomycin, and vancomycin different. Moreover, multidrug resistance of MRSA was shown to be more than 90% for penicillin and 91.1% for erythromycin. It was revealed that SCCmec III was resistant to at least four to five different antibiotics. ST585 (2.9%), ST240 (8.8%), ST45 (14.7%), ST22 (17.6%), and ST239 (higher rate) were the five sequence types found in STs (55.8%) (Sami Awayid & Qassim Mohammad, 2022).

Furthermore, the results agreed with (Selim et al., 2022) whose reported that from 292 urine samples, 103 bacterial strains (35.3%) were identified as *S. aureus*. Antibiotic resistance to erythromycin was found in most bacterial isolates, whereas tobramycin antibiotic sensitivity was found in most of them. Vancomycin resistance was found in 23 of all *S. aureus* isolates in this study. Analysis for β -lactamase found that 71% of *S. aureus* isolates were positive in all isolates. There was a single plasmid with a molecular weight of 39.306 Kbp in five selected VRSA isolates that was subjected to plasmid analysis (Selim et al., 2022)

Detection of *mecA* and *vanA* genes by PCR

Detection of *mecA* gene

The *mecA* gene was identified by PCR assay in all positive isolates of *S. aureus*. The results revealed that 60.97% (25/41) of *S. aureus* carried *mecA* gene as in table (7). The PCR products for identification of *mecA* gene among *S. aureus* isolates shown in figure (4).

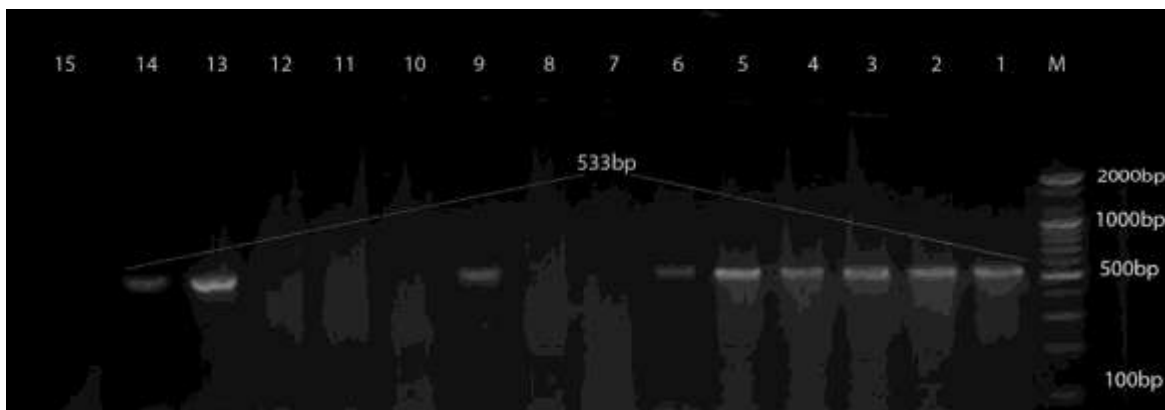


Figure (4): PCR products for identification of *mecA* gene among *S. aureus* isolates. Lanes (1-6,9, 13-14) represent the identified gene with 533bp amplicon, Lane M represent 100bp DNA ladder.

Table (7): Detection of *mecA* gene among *S. aureus* isolates

Results	N	Percentage	P value
Positive	25	(60.97)	0.160
Negative	16	(39.03)	
Total	41	100%	

* represent a significant difference at $p < 0.05$



Detection of *vanA* gene

The *vanA* gene was identified by PCR assay in all positive isolates of *S. aureus*. The results revealed that 46.3% (19/41) of *S. aureus* carried *vanA* gene as in table (8). The PCR products for identification of *vanA* gene among *S. aureus* isolates shown in figure (5).

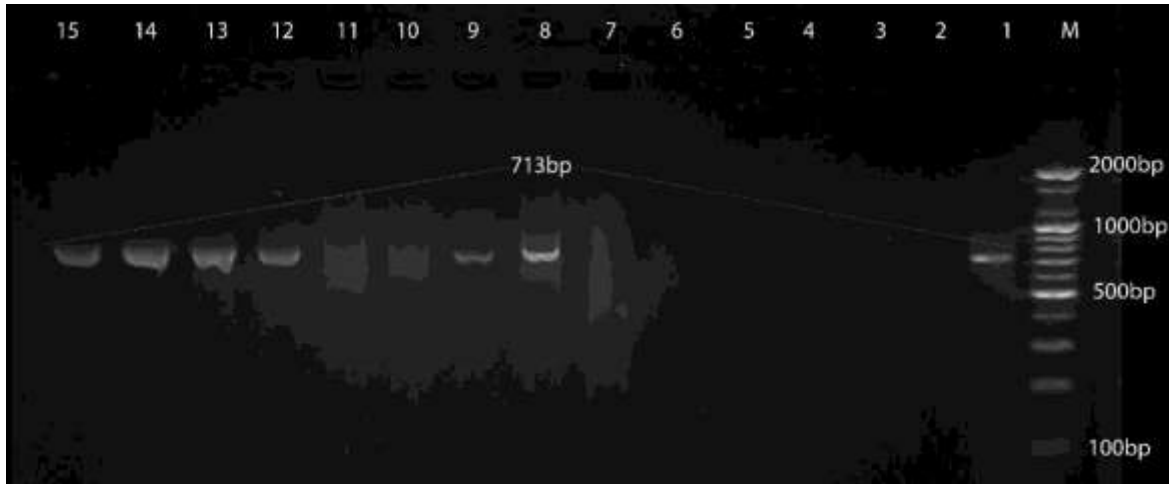


Figure (5): PCR products for identification of *vanA* gene among *S. aureus* isolates. Lanes (1, 8-9, 12-15) represent the identified *vanA* gene with 713bp amplicon, Lane M represent 100bp DNA ladder.

Table (8): Detection of *vanA* gene among *S. aureus* isolates

Results	N	Percentage	P value
Positive	19	(46.3)	0.639
Negative	22	(53.7)	
Total	41	100%	

* represent a significant difference at $p < 0.05$

However The analysis of antimicrobial resistance genes among the 41 *Staphylococcus aureus* isolates revealed that 25 isolates (60.97%) harbored the *mecA* gene, which is associated with methicillin resistance. Additionally, 19 isolates (46.3%) carried the *vanA* gene, indicating potential vancomycin resistance. These findings suggest a high prevalence of genes conferring resistance to critical antibiotics among the tested isolates as in table (9).

Table (9): Distribution of antimicrobial resistance genes (*mecA* and *vanA*) among *S. aureus* isolates.

Genes	No.	(%)
<i>mecA</i>	25	60.97
<i>vanA</i>	19	46.3

The results agreed with (Dhungel et al., 2021) who's reported that Among 144 culture positive isolates, *S. aureus* (27.1%; 39/144) was the predominant bacteria. Among 39 *S. aureus* isolates, all isolates were found resistant to penicillin followed by erythromycin (94.9%; 37/39), gentamicin (94.9%; 37/39) and cefoxitin (87.2%; 34/39). Out of 39 *S. aureus*, 87.2% (34/39) were MRSA. Among



34 MRSA, 8.8% (3/34) were vancomycin intermediate *S. aureus* (VISA). None of the MRSA was resistant to vancomycin. All of the 3 VISA isolates were obtained from inpatients. Of 39 *S. aureus*, 82.1% (32/39) harbored *mecA* gene. Similarly, the entire VISA isolates and 94.1% (32/34) of the MRSA isolates were tested positive for *mecA* gene (Dhungel et al., 2021).

Moreover, Dehbandi et al. (2019) who's reported that the presence of *vanA* gene was investigated in VRSA and intermediate resistance (VISA) isolates by PCR method using specific primers. Over 40% of isolates were resistant to commonly-used antibiotics, including gentamicin (46.67%), ceftazidime (45%), and carbenicillin (43.34%). Only few, however, were sensitive to gentamicin (33.33%) and ceftazidime (35%). Vancomycin was the most effective antibiotic against *S. aureus* isolates (56.66% sensitivity). Eleven isolates (18.34%) were resistant (VRSA) and 15 isolates (25%) were intermediate resistance (VISA) to vancomycin. Molecular analysis of *vanA* gene in 11 VRSA and 15 VISA showed that 8 VRSA (72.72%) and 6 VISA (40%) isolates were positive for *vanA* gene. Our current study showed that there is a significant increase in the rate of resistance to methicillin and vancomycin, and this is due to several reasons, including misuse and overuse of antibiotics, taking antibiotics without consulting a doctor, lack of adherence to personal hygiene, lack of infection prevention and control measures, and lack of community awareness.

Conclusion

S. aureus is an important nosocomial pathogen, and the emergence of Methicillin-resistant (MRSA) and Vancomycin-resistant (VRSA) strains poses a serious public health concern. In this study, the presence of *mecA* and *vanA* genes in 60.97% and 46.3% of isolates, respectively, highlights the prevalence of genetic determinants of resistance against methicillin and vancomycin. High resistance rates were also observed against other critical antibiotics, while some isolates remained susceptible to gentamicin, ciprofloxacin, azithromycin, and tetracycline. These findings emphasize the need for continuous surveillance, antimicrobial stewardship, and effective infection control measures to prevent the spread of multidrug-resistant *S. aureus* in both hospital and community settings.

References

1. Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., ... & Hiramatsu, K. (2001). Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *The Lancet*, 357(9264), 1225-1240.
2. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiology reviews*, 28(3), 603-661.
3. Chen, C. J., Hsu, K. H., Lin, T. Y., Hwang, K. P., Chen, P. Y., & Huang, Y. C. (2011). Factors associated with nasal colonization of methicillin-resistant *Staphylococcus aureus* among healthy children in Taiwan. *Journal of Clinical Microbiology*, 49(1), 131-137.
4. Peacock, S. J., & Paterson, G. K. (2015). Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annual review of biochemistry*, 84(1), 577-601.
5. Centers for Disease Control and Prevention, 2. (2000). *Centers for disease control and prevention*. Centers for Disease Control and Prevention.
6. Kim, C., Milheiriço, C., Gardete, S., Holmes, M. A., Holden, M. T., de Lencastre, H., & Tomasz, A. (2012). Properties of a novel PBP2A protein homolog from *Staphylococcus aureus* strain LGA251 and its contribution to the β -lactam-resistant phenotype. *Journal of Biological Chemistry*, 287(44), 36854-36863.
7. Percival, S. L., McCarty, S. M., & Lipsky, B. (2015). Biofilms and wounds: an overview of the evidence. *Advances in wound care*, 4(7), 373-381.
8. Pardos de la Gandara, M., Curry, M., Berger, J., Burstein, D., Della-Latta, P., Kopetz, V., ... & Tomasz, A. (2016). MRSA causing infections in hospitals in greater metropolitan New York: major shift in the dominant clonal type between 1996 and 2014. *PLoS One*, 11(6), e0156924.



9. Zhu, W., Murray, P. R., Huskins, W. C., Jernigan, J. A., McDonald, L. C., Clark, N. C., ... & Patel, J. B. (2010). Dissemination of an Enterococcus Inc18-Like vanA plasmid associated with vancomycin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy*, 54(10), 4314-4320.
10. Bitrus, A. A., Peter, O. M., Abbas, M. A., & Goni, M. D. (2018). Staphylococcus aureus: A review of antimicrobial resistance mechanisms. *Veterinary Sciences: Research and Reviews*, 4(2), 43-54.
11. Shariati, A., Dadashi, M., Moghadam, M. T., van Belkum, A., Yaslianifard, S., & Darban-Sarokhalil, D. (2020). Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate Staphylococcus aureus clinical isolates: a systematic review and meta-analysis. *Scientific reports*, 10(1), 12689.
12. Lemaire, S., Kosowska-Shick, K., Julian, K., Tulkens, P. M., Van Bambeke, F., & Appelbaum, P. C. (2008). Activities of antistaphylococcal antibiotics towards the extracellular and intraphagocytic forms of Staphylococcus aureus isolates from a patient with persistent bacteraemia and endocarditis. *Clinical microbiology and infection*, 14(8), 766-777.
13. Sizar, O., Leslie, S. W., & Unakal, C. G. (2017). Gram-positive bacteria.
14. Breves, A., Miranda, C. A. C., Flores, C., Filippis, I. D., & Clementino, M. M. (2015). Methicillin-and vancomycin-resistant Staphylococcus aureus in health care workers and medical devices. *Jornal brasileiro de patologia e medicina laboratorial*, 51(3), 143-152.
15. Wang, W. Y., Chiu, C. F., Lee, Y. T., Hsueh, P. R., & Tsao, S. M. (2022). Molecular epidemiology and phenotypes of invasive methicillin-resistant vancomycin-intermediate Staphylococcus aureus in Taiwan. *Journal of Microbiology, Immunology and Infection*, 55(6), 1203-1210.
16. Dhanalakshmi, T. A., Umapathy, B. L., & Mohan, D. R. (2012). Prevalence of Methicillin, Vancomycin and Multidrug Resistance among Staphylococcus aureus. *Journal of Clinical & Diagnostic Research*, 6(6).
17. Hasan, R., Acharjee, M., & Noor, R. (2016). Prevalence of vancomycin resistant Staphylococcus aureus (VRSA) in methicillin resistant S. aureus (MRSA) strains isolated from burn wound infections. *Tzu Chi Medical Journal*, 28(2), 49-53.
18. Kejela, T., & Dekosa, F. (2022). High prevalence of MRSA and VRSA among inpatients of Mettu Karl referral hospital, southwest Ethiopia. *Tropical Medicine & International Health*, 27(8), 735-741.
19. Gould, I. M., Cauda, R., Esposito, S., Gudiol, F., Mazzei, T., & Garau, J. (2011). Management of serious methicillin-resistant Staphylococcus aureus infections: what are the limits?. *International journal of antimicrobial agents*, 37(3), 202-209.
20. Dadashi, M., Nasiri, M. J., Fallah, F., Owlia, P., Hajikhani, B., Emaneini, M., & Mirpour, M. (2018). Methicillin-resistant Staphylococcus aureus (MRSA) in Iran: a systematic review and meta-analysis. *Journal of global antimicrobial resistance*, 12, 96-103.
21. Boswihi, S. S., Udo, E. E., Monecke, S., Mathew, B., Noronha, B., Verghese, T., & Tappa, S. B. (2018). Emerging variants of methicillin-resistant Staphylococcus aureus genotypes in Kuwait hospitals. *PloS one*, 13(4), e0195933.
22. Saeed, A., Ahsan, F., Nawaz, M., Iqbal, K., Rehman, K. U., & Ijaz, T. (2019). Incidence of vancomycin resistant phenotype of the methicillin resistant Staphylococcus aureus isolated from a tertiary care hospital in Lahore. *Antibiotics*, 9(1), 3.
23. basil AbdulRazaq, A., Shami, A. M., & Ghaima, K. K. (2022). Detection of vanA and vanB genes Among Vancomycin Resistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. *Iraqi journal of biotechnology*, 21(1).
24. Selim, S., Fariad, O. A., Almuhayawi, M. S., Saleh, F. M., Sharaf, M., El Nahhas, N., & Warrad, M. (2022). Incidence of vancomycin-resistant Staphylococcus aureus strains among patients with urinary tract infections. *Antibiotics*, 11(3), 408.



-
25. Dhungel, S., Rijal, K. R., Yadav, B., Dhungel, B., Adhikari, N., Shrestha, U. T., ... & Ghimire, P. (2021). Methicillin-Resistant Staphylococcus aureus (MRSA): Prevalence, antimicrobial susceptibility pattern, and detection of mec a gene among cardiac patients from a tertiary care heart center in Kathmandu, Nepal. *Infectious Diseases: Research and Treatment*, 14, 11786337211037355.
 26. Dehbandi, N., Izadi Amoli, R., Oskoueian, R., & Gholami, A. (2019). The prevalence of vanA gene in clinical isolates of vancomycin-resistant Staphylococcus aureus in a hospital in Mazandaran, Iran. *Caspian Journal of Environmental Sciences*, 17(4), 319-325.