Iraqi Journal of Biotechnology, 2022, Vol. 20, No. 1, 19-25

Detection of *vanA* and *vanB* genes Among Vancomycin Resistant *Staphylococcus aureus* Isolated from Clinical Samples in Baghdad Hospitals

Ansam basil AbdulRazzaq[1], AbdulMuhsin Moslim Shami[1], Kais Kassim Ghaima[1]

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad / Baghdad
/Iraq.

Received: May19, 2022 / Accepted: June 19, 2022

Abstract: *Staphylococcus aureus* is an opportunistic pathogen. It is most common in skin and soft tissue. This microbe can cause more diseases as a burn inflammation and tonsillitis through the production of virulence factors. In this study, one hundred and fifty (150) samples were collected from patients with inflammation from different clinical sources and different age groups during the period from December 2020 to the end of April 2021.from main hospitals in Baghdad. All isolates were diagnosed based on microscopic examinations and morphological characteristics using Mannitol salt agar, Blood agar in addition to biochemical tests.The diagnosis was confirmed through Vitek system and API staph kit. Antibiotic susceptibility testing was performed on all identified isolates, and the findings revealed that 12 isolates VRSA.DNA was extracted from bacterial isolates and sepreated by electrophoresis to measure DNA purity and integrity. Molecular diagnosis of bacteria was done using a polymerase chain reaction based on the 16S*rRNA* gene and the results were (100%) as *S. aureus*. In addition the individual interactions polymerized were used to determine the vancomycin resistance genes. It was found that all isolates were vancomycin resistance through the presence of the *van A* gene, while the results of the presence of genes *van B* were (66.6%).

Keyword: Polymerase chain reaction; *Van A*; *Van B*; Vancomycin resistance

Introduction

Staphylococcus aureus is one of the most frequent worldwide causes of morbidity and mortality due to an infectious agent. It is one of the most common microbes associated with nosocomial community-acquired infections, being one of the five most common pathogens to reside in the skin and nasal flora, occurring in roughly 25–30% of all healthy individuals (1). It can cause a range of repercussions from mild to severely life-threatening infections such as wound infections, abscess formation endovascular diseases, toxic shock syndrome (TSS) and Staphylococcal scalded skin syndrome (SSSS) (2). Treatment of these infections has become more difficult because of the emergence of methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VRSA) strains. In addition, VRSA tends to be multidrug resistant (MDR) against a diversity of currently available antimicrobial agents (3).

Existence of MRSA infections and irrational use of Glycopeptides, especially vancomycin, led to the emergence of VRSA phenotype among MRSA (4). Vancomycin resistant *S. aureus* (VRSA) strains may harbor different vancomycin resistance genes such as *vanA* and *vanB* gene (5), which can be acquired via plasmid from Enterococci (6). Also, vancomycin resistance phenotype could be due to the thickness of cell wall or overproduction of D-ala-D-ala caused by a sequential mutation (7). Initially, the emergence of vancomycin intermediate *S. aureus* was reported from Japan in 1997 (8). Then, followed by the first existence of vancomycin-resistant *S. aureus* in the United States in 2002. Thereafter, several reports of vancomycin resistance emerging were raised from throughout the world (9). In Iraq, there are many studies on *S.aureus*, including Abdul-Hameed, and Ayoub (10) indicate All VRSA isolates were MRSA and VRSA showed resistance to a wide range of antimicrobial agents. Alagely, (11) report a high proportion of CA-MRSA predominance of type V *SCCmec* and carry *pvl* gene has been observed in the bacterial isolates. Lafta, (12) showed Chalcone reduced biofilm formation. Sofi, (2020)(13) showed the *icaA* gene considers a directly related to biofilm detection and good target for biofilm detection of *S. aureus*.

Materials and Methods

Bacterial isolation and identification

The samples were collected from the period of December 2020 to the end of April 2021, from burns, wound infections, urine, Abscess, sputum, ear swabs, blood and Cerebrospinal fluid samples from reference deferent patients of both gender, the choice of samples differs according to the clinical manifestations; (i.e. inflammation, fever, abscesses, pain, rapid breathing, irritation at site of infection). Collected and transported in sterilized transport medium containers. The samples were streaked on blood agar and Mannitol salt agar and incubated at 37°c for 24 hrs. Identification of *S. aureus* manual biochemical tests that were used catalase test and oxidase test. For final confirmation biochemical tests embedded in VITEK2 compact system.

Antibiotic susceptibility Test

Antimicrobial susceptibility testing was performed by Kirby- Bauer test by using Mueller Hinton medium according to (CLSI, 2018).Which included antimicrobial agents as follows: Azithromycin (ATH), Cefoxitin (FOX), Chloramphenicol (C)**,** Erthromycin (E), Gentamycin (GM), Levofloxacin(LEV), Methicillin (ME), Penicillin G (PG), Rifampin(RA), Tetracycline (TE), Vancomycin (VA).

Identification of *S. aureus* and Vancomycin Resistance genes by molecular method

DNA Extraction

DNA was extracted from *S.aureus* clinical isolates using a commercial purification system Genomic DNA Mini Kit (Geneaid, Taiwan); this kit was designed for the isolation of DNA from Gram positive and Gram-negative bacteria. DNA was extracted by this kit using bacterial protocol (for gram positive bacteria).

Conventional polymerase chain reaction for 16s *rRNA Van A,* and *Van B genes*

Genes encoding the vancomycin resistance determinants, *vanA* and *vanB*, were investigated by PCR using specific primers Table (1) PCR amplification was carried out in a 25µl reaction mixture Table (2) with each primer as the following steps: an initial denaturation step at 94°C for 5 min; followed by 30 cycles of 94°C for 45 sec, 58°C for 45 sec and 72°C for 45 sec for *vanA* gene, and an initial denaturation step at 94°C for 5 min; followed by 30 cycles of 94°C for 45 sec, 58°C for 45 sec and 72°C for 45 sec for *vanB* gene, then finally elongation step at 72°C for 6 min. The PCR products were separate in 1.5% agarose gel.

Table (1) Primer sequences used in this study.

Primer	Primer sequence (5-3)	Product size
16SrRNA	F AAGCAACGCGAAGAACCTTA	207
	R TGTCACCGGCAGTCAACTTA	
Van A	F CATGGCAAGTCAGGTGAAGA	223
	R CCGGCTTAACAAAAACAGGA	
Van B	F GTGACAAACCGGAGGCGAGGA	433
	R CCGCCATCCTCCTGCAAAAAA	

*F: Forward R: Reverse

Table (2): Component of PCR Master Mix Reaction.

PCR Master mix reaction components		Volume
PCR Premix		12.5µL
DNA template		5µL
Primers	Forward	1µL
	Reverse	1µL
nuclease-free H_2O		5.5µL
Total volume		25µL

Results and Discussion

The antibiotic susceptibility test was carried out for *Staphylococcus aureus*, by Kirby Bauer disk diffusion method on a Mueller-Hinton agar according to (14). All identified *S. aureus* were exposed to 11 antimicrobial agent resistance patterns in table (3).

Table (3): Antimicrobial Susceptibility test of *Staphylococcus aureus*

Antimicrobial Agent	*Staphylococcus aureus*						P-value
	S		I		R		
	No.	%	No.	%	No.	%	
Azithromycin (ATH)	24	34.2	3	4.2	43	61.4	0.0001 **
cefoxitin(FOX)	17	24.2	0	0	53	75.7	0.0001 **
Chloramphenicol(C)	41	58.5	18	25.7	11	15.7	0.0007 **
Erthromycin(E)	25	35.7	5	7.1	40	57.1	0.0001 **
Gentamycin (GM)	48	68.5	5	7.1	17	24.2	0.0001 **
Levofloxacin(LEV)	49	70	0	0	21	30	0.0001 **
Methicillin (ME)	14	20	15	21.4	41	58.5	0.0042 **
Penicillin G (PG)	0	0	0	0	70	100	0.0001 **
Rifampin(RA)	51	72.8	0	0	19	27.1	0.0001 **
Tetracycline (TE)	30	42.8	3	4.2	37	52.8	0.0001 **
Vancomycin(VA)	58	82.8	2	2.8	10	14.2	0.0001 **

**** (P<0.01). S: Sensitive I: Intermediate R: Resistant**

Molecular identification of *S. aureus* by*16S rRNA gene, VanA* gene, *Van B* gene

All Phenotypically positive isolates of *S. aureus* strains were subjected to molecular identification using the specific initiator of the *16S rRNA* gene to confirm its diagnosis by PCR. The results showed that all bacterial isolates belong to *S. aureus* (Figure 1).

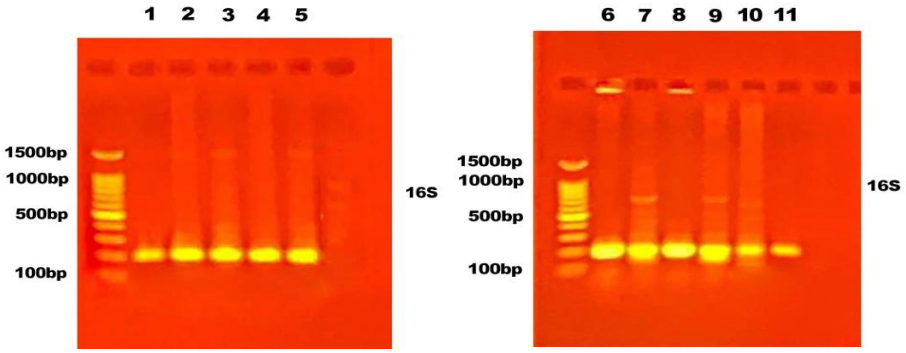

Figure (1): Agarose Gel Electrophoresis of PCR Amplified Products for*16S rRNA* (207 bp) *S. aureus*, l00bp DNA ladder, (2% Agarose, 75 V for 1 hr)

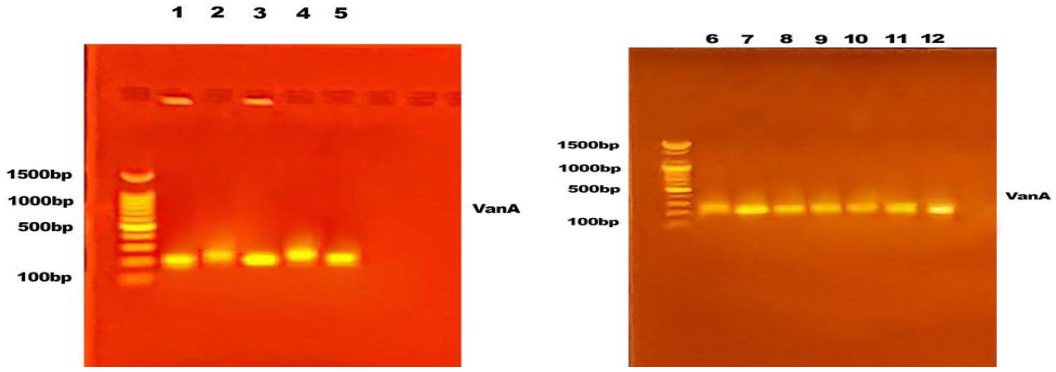

Figure (2): The gel electrophoresis of PCR product of *Van A* gene (223 bp) of *S.aureus*, 100bp DNA ladder; (2% Agarose, 75V for 1 hr)

In this study, the extracted DNA sample from VRSA has been selected to detect the *Van A* gene as an indicator of vancomycin resistance and considers positive strains as VRSA. The results of the detection of *Van A* 12/12 gene was (100%) (Figure 2)

The PCR technique has been used for molecular detection using specific primers for *Van B* gene. The results of the detection of *van B* were (66.6%) (Figure 3).

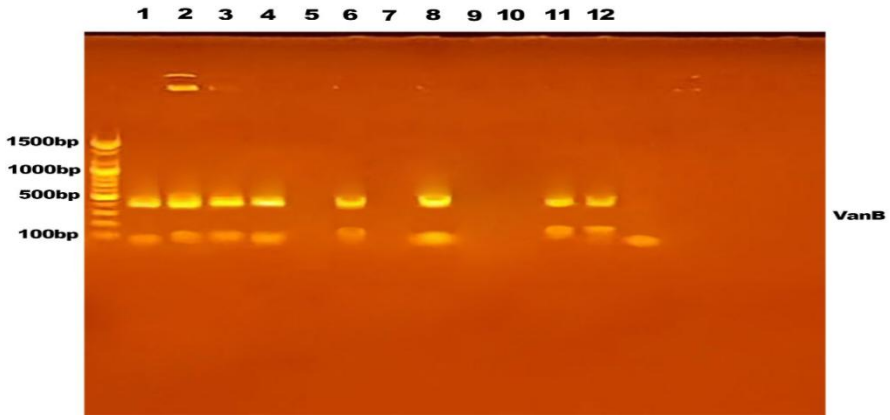

Figure (3): The gel electrophoresis of PCR product of *Van B* gene (433 bp) of *S. aureus* 100bp DNA ladder, (2% Agarose, 75V for 1 hr)

The results of the current study were in agree with those of Mashaly *et al.*, (2019)(15) who indicated that *VanA* gene among 92 MRSA isolates showed that 76 (82.6%) isolates were *VanA* negative and 16 (17.4%) isolates were *VanA* positive.

Also, Thati *et al.* (16), and Mahmood and Flayyih (2014)(17) revealed that *VanA* gen was present in 86%, and 4.5% respectively. Jubair and Khlebos (18) indicated that 8 isolates (19%) had *mecA* gene methicillin resistance and 3 isolates (7.1%) had *van A gene* vancomycin resistance in all isolates. Abdul-Hameed and Ayoub, (10) found PCR amplification for *van A* gene among 22 VRSA isolates; showed that 12(55%) have *van A* gene, the remaining 10(45%) did not have *van A* gene. Another study done by Zaki *et al.* (19)In *S. aureus* VISA was found in thirteen (12.6%) isolates VRSA was reported in five isolates. *Van A* gene was detected among four isolates with intermediate susceptibility and in two resistant strains.

Van B was more commonly associated with intermediate resistance pattern in six isolates and in one resistant strains and none of the isolates had *Van C.* Results of the present study were defferent with Bamigboye *et al.* (20) that found the strain displayed a vancomycin MIC value of 16 µg/ml and carried a *mecA* gene but did not contain *vanA* or *vanB* gene. In other study by Saadat *et al.*(21) found *vanA* and *vanB* resistant genes were detected in 34% and 37% of clinical isolates, respectively.

In other study conducted by Maharjan *et al.* (22) declared that of five VRSA-positive isolates, two isolates were found to be positive for the *vanA* gene whereas the *vanB* gene was not detected in his study.

From the results of this study, we conclude that the presence of *van A* alone was sufficient to ensure that bacteria are resistant to vancomycin and do not necessarily need *van B*, but that its presence *van B* alone may not make them resistant at all, And the extent of resistance apparently depends on *van A* because all resistant isolates with different resistance to vancomycin were necessarily carrying *van A* and not *van B*, and therefore we conclude that *van A* is the origin of resistance to vancomycin, meaning that it is possible that it or its product (protein) is a target to overcome resistamce .

References

1- Cheung, G. Y.; Bae, J. S.; Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. Virulence, 12(1): 547-569

2- Saeed, A.; Ahsan, F.; Nawaz, M.; Iqbal, K.; Rehman, K. U.; and Ijaz, T. (2019). Incidence of Vancomycin Resistant Phenotype of the Methicillin Resistant Staphylococcus aureus Isolated from a Tertiary Care Hospital in Lahore. Antibiotics (Basel, Switzerland), 9(1)3:1-10.

3- Wu, Q.; Sabokroo, N.; Wang, Y.; Hashemian, M.; Karamollahi, S.; and Kouhsari, E. (2021). Systematic review and meta-analysis of the epidemiology of vancomycin-resistance *Staphylococcus aureus* isolates. Antimicrobial Resistance & Infection Control, 10(1): 1-13.

4- Baetz, B.; Boudrioua, A.; Hartke, A.; and Giraud, C. (2021). Alternatives to Fight Vancomycin-Resistant Staphylococci and Enterococci. Antibiotics, 10(9):1-20.

5- Lamichhane-Khadka, R.; Dulal, S.; Cuaron, J. A.; Pfeltz, R.; Gupta, S. K.; Wilkinson, B. J.; and Gustafson, J. E. (2021). Apt (Adenine Phosphoribosyltransferase) Mutation in Laboratory-Selected Vancomycin-Intermediate *Staphylococcus aureus*. Antibiotics, 10(5) 583:1-17.

6- Lakhundi, S., & Zhang, K.. (2018). Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clinical Microbiology Reviews, 31(4):1-8.

7- Shariati, A.; Dadashi, M.; Moghadam, M. T.; van Belkum, A.; Yaslianifard, S.; and 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): 1-16.

8- Abdul-Hameed, R. A. and Ayoub, N. S. (2015). Vancomycin resistance among methicillin resistant *Saphylococcus aureus* isolates from general hospitals. Al-Kindy College Medical Journal, 11(1): 6-9.

9- Alagely, H. S. (2016) Molecular differentiation between community acquired (CA-MRSA) and hospital acquired (HA- MRSA) methicillin – resistant *Staphylococcus aureus*. PhD. Thesis. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

10- Lafta, E.T. (2020) The Role of Chalcone on the Formation of Biofilms and Expression of Virulence Genes in Methicillin-resistant *Staphylococcus aureus*. PhD. Thesis. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

11- Sofi, D. K. (2021) The Impact of Adenovirus and *Staphylococcus aureus* in Ocular Infections Incidence and Estimation of Gene Expression of Adhesion Involved icaA Gene. Master thesis. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

12- Clinical and laboratory standards institute, CLSI. (2018). performance standards for antimicrobial susceptibility testing. Second informational supplement. CLSI document M100-S22. Clinical and laboratory Standard Institute, Wayne. Pya.

13- Mashaly, M.; El-Mashad, N.; and El-deeb, H. (2019). Detection of VanA type vancomycin resistance among MRSA isolates from an emergency hospital in Egypt. Comparative Clinical Pathology, 28(4): 971-976.

14- Thati, V; Shivannavar, CT; Gaddad, SM. (2011) Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. The Indian Journal of Medical Research, 134(5): 704-708.

15- Mahmood, H.A.; Flayyih, M.T. (2014) Detection of van A gene of vancomycin resistant *Staphylococcus aureus* by PCR technique. International Journal of Advanced Research,2(7): 209–216.

16- Jubair, H. H.; and Khlebos, A. H. (2016). Molecular Detection of Methicillin and Vancomycin Resistance in *Staphylococcus aureus* Isolated From Burn and Wound Infection Patients. Al-Kufa University Journal for Biology, 201: 46-50.

17- Zaki, M.E.S.; Abouelnour, A.; El-Kannishy, S.M.H.; Hassan, R. (2019) Molecular Study of Vancomycin Resistance in Hospital Acquired *Staphylococcus* Infection. International Journal of Drug Development and Research, 11: 11-14.

18- Bamigboye, B. T.; Olowe, O. A. and Taiwo, S. S. (2018). Phenotypic and molecular identification of vancomycin resistance in clinical *Staphylococcus aureus* isolates in Osogbo, Nigeria. European Journal of Microbiology and Immunology, 8(1): 25-30.

19- Saadat, S.; Solhjoo, K.; Norooz-Nejad, M. J.; and Kazemi, A. (2014). VanA and vanB positive vancomycin-resistant *Staphylococcus aureus* among clinical isolates in Shiraz, South of Iran. Oman medical journal, 29(5): 335-339.

20- Maharjan, M.; Sah, A. K.; Pyakurel, S.; Thapa, S.; Maharjan, S.; Adhikari, N.; ... and Thapa Shrestha, U. (2021). Molecular Confirmation of Vancomycin-Resistant *Staphylococcus aureus* with vanA Gene from a Hospital in Kathmandu. International Journal of Microbiology, 2021,1-8.