Detection of *mec A* ,*van A* and *van B* genes of *Staphylococcus aureus* Isolated from Patients in Al Muthanna Province Hospitals

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Abstract

Background: Infections triggered by methicillin-resistant *Staphylococcus aureus* (MRSA) and onset Resistance to significant anxiety in healthcare environments worldwide from vancomycin-resistant *Staphylococcus aureus* (VRSA)..

Aim: To discover *Staphylococcus aureus* resistant to methacillin and vancomycin by detecting (Mec A ,Van A and Van B) genes by PCR technique.

Methods: A total of 250 samples from patients with different clinical cases whom admitted to Hospitals in Al Muthanna province during a period from January 2019 to July 2019. *Staphylococcus aureus* were isolated and identified by using cultural and biochemical testes.The extracted DNA of isolates were amplified by PCR to detect (*mecA*, *vanA,* and *vanB*) genes.

Results: The results showed that 72/ 250 of *Staphylococcus aureus* isolates contained *mecA* gene, indicating that all isolates are Methicillin resistant *S. aureus* (MRSA), only five isolates contained *van A* gene and only nine isolates contained *van B* gene.

Conclusion: Appropriate monitoring and control measures appear to be crucial to avoid the development and transmission of MRSA and VRSA strains in our nation.

Key words: *Methicillin resistant Staphylococcus aureus(MRSA); vancomycin resistant Staphylococcus aureus(VRSA);mec A, vanA ,vanB genes.*

Introduction

Staphylococcus aureus is a human pathogen as well as a commensal bacterium. *Staphylococcus aureus* colonizes about 30 percent of the human population. At the same time, it is a major cause of bacteremia and endocarditis (IE) and osteoarticular, skin and soft tissue, pleuropulmonary, and devicerelated infections [1]. Horizontal transfer of antibiotic resistance genes and production of multiple virulence factors are thought to play a major role in the emergence and persistence of resistant *S. aureus* strains in the hospital environment [2] . *Staphylococcus aureus* possesses several virulence factors that contribute to colonization and invasion of host tissue, evasion of the hosts' immune systems, and nutrient acquisition [3] . Methicillin resistant *S. aureus* (MRSA) outbreaks have become a serious problem in healthcare settings worldwide. The therapeutic options in MRSA infections are limited to glycopeptides, linezolid, tigecycline, and ceftaroline [4] . Methicillin resistance is mediated by mecA and acquired by horizontal transfer of a mobile genetic element designated staphylococcal cassette chromosome mec (SCCmec) [5] .The gene mecA encodes penicillin-binding protein 2a (PBP2a), an enzyme responsible for crosslinking the peptidoglycans

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in the bacterial cell wall. PBP2a has a low affinity for β- lactams, resulting in resistance to this entire class of antibiotics [6]. In addition to the *mecA* gene, SC*mec* carries the *mecA* regulatory genes *mecI* and *mecR1*, which are divergently transcribed regulatory genes located immediately upstream from the *mecA* promoter. They are similar in molecular organization, structure, function, and mechanism of regulation to Staphylococcal β-lactamase regulatory elements, *blaI* and *blaR1* [7].

Five different types of SCC*mec* elements, types I to V, have been identified. The type I SCC*mec* contains the *mecA* gene as the only resistance element, while the type II and III elements contain, besides *mecA*, multiple determinants for resistance against non-β-lactam antibiotics. Accordingly, type II and III SCC*mec* elements are responsible for multidrug resistance in nosocomial MRSA isolates. Type IV SCC*mec* elements, like type I elements, contain no resistance genes other than *mecA*, and they are significantly smaller than the type II and III elements. This might serve as an evolutionary advantage, making it easier for these mobile genetic elements to spread across bacterial populations [8].

During the last decade, *S. aureus* isolates with decreased susceptibility to vancomycin (vancomycin intermediate *S. aureus*(VISA) and vancomycin resistant *S. aureus* (VRSA) have been reported in various parts of the world. These isolates are associated with increased mortality because of the limited therapeutic options remaining [9].

Vancomycin is a glycopeptide and is very popular and competent antimicrobial drug for treating MRSA infections but unfortunately resistance to vancomycin have also been reported since 1997. This vancomycin resistance is actually due to the presence of Van resistance genes which are acquired from enterococci and are encoded on R-plasmid or chromosome. There are total six genes responsible for vancomycin pyruvate into D-Lactate, ligase that synthesizes D-Alanyl–D-Lactate and a dipeptidase that hydrolyzes D-Alanyl–D-Alanine. The collective action of these three enzymes incorporate DAlanyl–D-Lactate instead of D-Alanyl–D-Alanine into peptidoglycan which prevents the attachment of vancomycin [10]. The vanA gene contributes higher resistance to vancomycin and teicoplanin both glycopeptides while vanB gene which is also an effective resistant gene encodes resistance to vancomycin only. The vanB gene resistance which includes vanA, vanB, vanC, vanD, vanE and vanG whereas vanA and vanB genes are the most important and prevalent genes [11].

The examples of resistant strains are vancomycin intermediate *S. aureus* (VISA), vancomycin resistant *S. aureus* (VRSA) and heterogeneous VISA (hVISA) strains. The mechanism of resistance for both vanA and vanB genes encompass three sets of enzymes harbored by glycopeptide-producing bacteria. The enzymes are dehydrogenase that reduces clusters (vanB1, vanB2, and vanB3) are generally carried by large elements (90-250 kb) which are transferable by conjugation from one chromosome to another and often contain the transposon Tn1547 [12]. Studies have also been conducted on detection of vanB gene in clinical isolates of *Staphylococcus aureus* strains but a few cases have been reported so far [13].

Materials and Method

A total of 250 samples from different clinical cases (wound, urine, high vaginal , throat, skin, ear, nose and pus swabs) were taken from patients of different ages and gender whom were admitted to Al Hussein Educational Hospital, Children's Hospital and the women's Educational, Rumaitha General Hospital, El Khidhir General Hospital and Public Health Laboratory in Al Muthanna province. The samples were collected during a period from January 2019 to July 2019. only 72 samples were identified as *Staphylococcus aureus* by using cultural and biochemical testes.

Genomic DNA was extracted from bacterial isolates by using Genomic DNA Mini Bacteria Kit. The extracted DNA was checked by using Nanodrop (THERMO. USA) that measured DNA concentration (ng/μL) and checked the DNA purity by reading the absorbance at (260 /280 nm) .

Polymerase chain reaction master mix for each gene was prepared by using (Maxime PCR PreMix kit) and this master mix done according to company instructions. Polymerase chain reaction assay was performed for detection antibiotic resistance genes (*mecA, vanA,* and *vanB* gene) in *Staphylococcus aureus*. Table (1).

Table (1): The PCR primers for *Staphylococcus aurous* antibiotic resistance genes

Primer	Sequence 5'-3'		Amplicon
mecA gene	F	GTGAAGATATACCAAGTGATT	147bp
	R	ATGCGCTATAGATTGAAAGGAT	
vanA gene	F	GGCAAGTCAGGTGAAGATG	713bp
	R	ATCAAGCGGTCAATCAGTTC	
vanB gene	F	GTGACAAACCGGAGGCGAGGA	430bp
	R	CCGCCATCCTCCTGCAAAAAA	

Then the Polymerase chain reaction products were visualized in an ethidium bromide-stained 1% agarose gel using a UV Transilluminator.

Statistical Analysis

All the results of the present study were analyzed statistically by Social Science Statistics and the Statistical Package For Social Sciences version 23 for Windows Software(Inc., Chicago, IL, USA). Chi-square test (X^2) was used for the assessment of differences between the variables studied. The P values less than 0.05 were considered statistically significant and high significant respectively. [14,15].

Results and Discussion

Bacterial Isolation & Identification

The present study included 72 isolates from *Staphylococcus aureus* which isolated from Urine , High Vaginal Swab, Pus, Wound, Nose, Ear, Throat and Skin Swab. All isolates had ability to grow on the mannitol salt agar which considered selective and differential media for genus *Staphylococcus* [16]. The colonies appeared round, smooth, raised, mucoid and shiny . Consequently, the isolates belong to the genus Staphylococcus .

All isolates had the ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones and turned the colour of the medium from pink to yellow as showed in figure (1).

(A) (B)

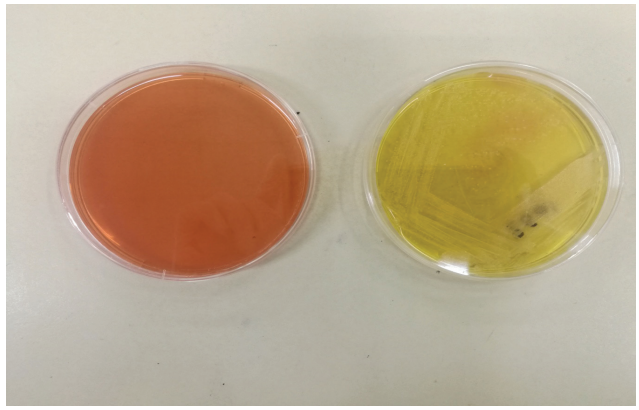


Figure 1: Manitol salt agar medium(A) ; colonies of Staphylococcus aureus ferment mannitol and form large golden colonies surrounded by wide yellow zones (B).

Microscopic examination was applied to the all 72 isolates after staining by Gram stain and the cells appeared as Gram-positive cocci arranged in grape-like irregular cluster. For further identification, the catalase test performed and all 72 isolates gave positive results. A coagulation test was performed to identify bacterial isolates at the species level, and all isolates showed the ability to produce coagulation. Isolates examined for colony characterization after sub culturing on 5% human blood agar and incubated for 24 hrs. at 37˚C . The colonies appeared large, round, golden colony surrounded with a halo clear zone of hemolysis . It is often *β*–hemolysis type on blood agar.

Screening for resistant genes by Polymerase chain reaction

Polymerase chain reaction (PCR) was performed for 72 isolates *Staphylococcus aureus* to detect the presence of *mecA* genes(figure 2). The results showed that all 72 isolates contained *mecA* gene, indicating that all isolates are resistant to antibiotic Methicillin (MRSA)

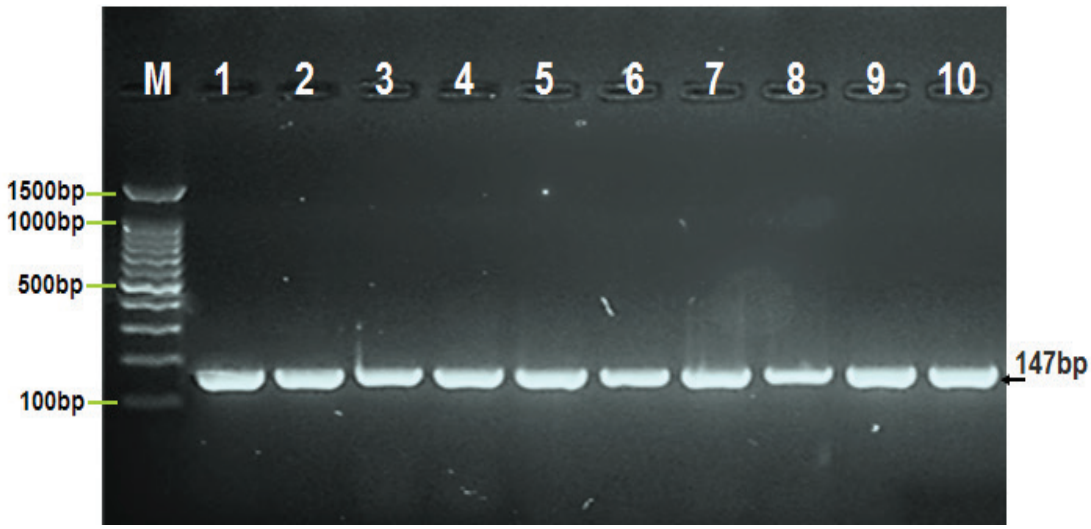


Figure (2): Agarose gel electrophoresis image that showed PCR product analysis for methicillin antibiotic resistance *mecA* gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-10) some positive *mecA* gene Staphylococcus aureus isolates at 147bp product size.

Polymerase chain reaction (PCR) was performed for all 72 isolates of *Staphylococcus aureus* to detect the presence of *vanA* gene (figure 3). The results showed that only five isolates contained *vanA* gene, indicating that only five isolates are resistant to antibiotic vancomycin.

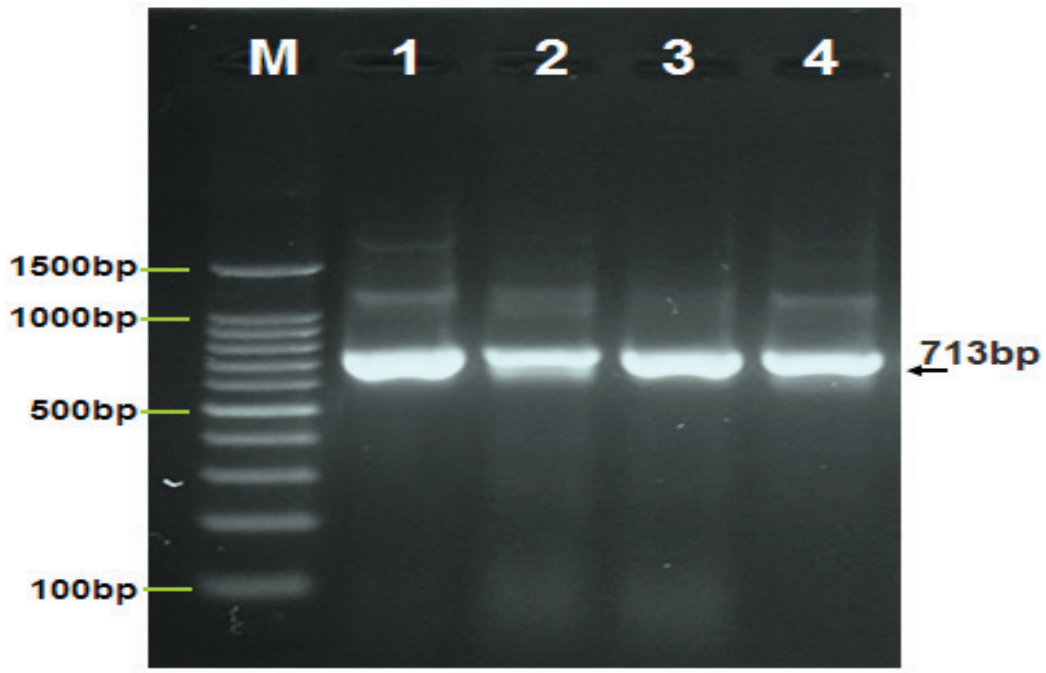
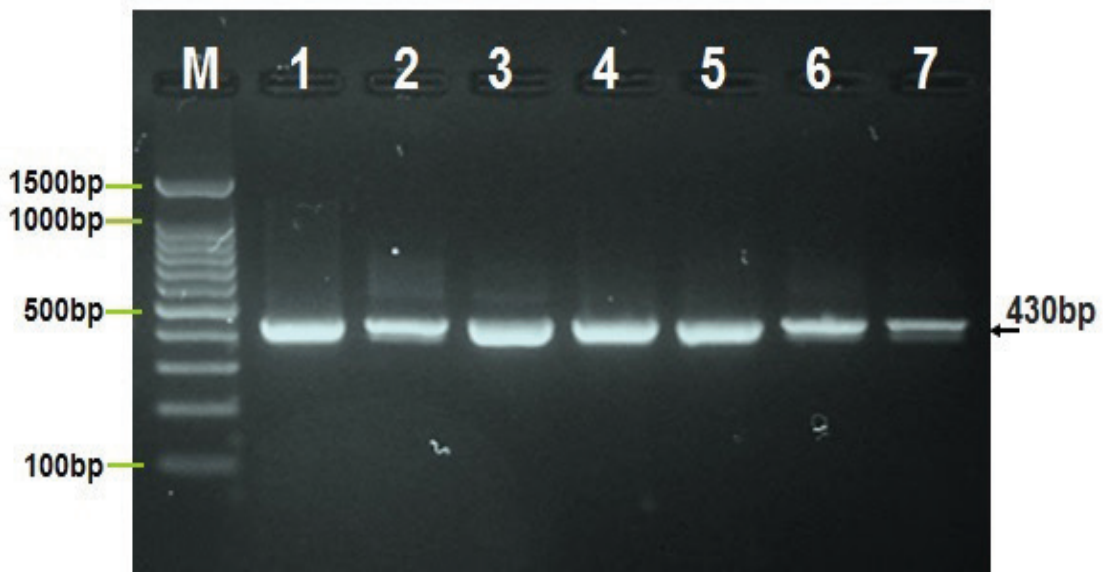


Figure (3): Agarose gel electrophoresis image that showed PCR product analysis for vancomycin antibiotic resistance *vanA* gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-4) only positive *vanA* gene Staphylococcus aureus isolates at 713bp product size.

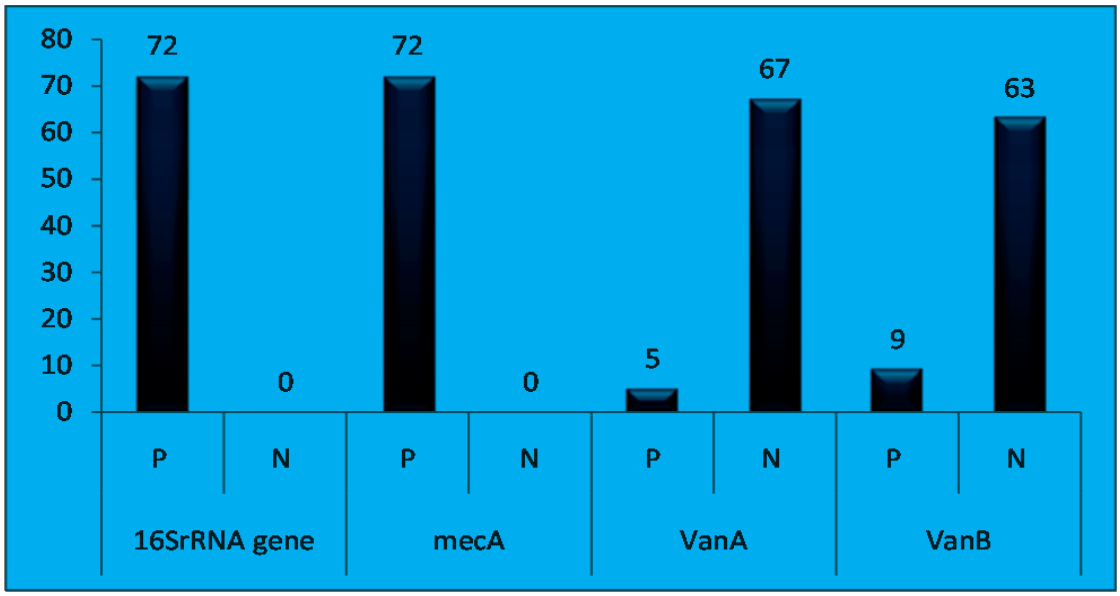
Polymerase chain reaction (PCR) was performed for all (72) isolates *Staphylococcus aureus* to detect the presence of van B gene figure(4). The results showed that only nine isolates contained van B gene, this indicate that only nine isolates are resistant to antibiotic



Vancomycin

Figure (4): Agarose gel electrophoresis image that showed PCR product analysis for vancomycin antibiotic resistance *vanB* gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-7) only positive *vanB* gene Staphylococcus aureus isolates at 430bp product size.

This genetic study included Polymerase chain reaction (PCR) assessment of mecA , vanA and vanB over expression and the results showed that *mecA* gene was over expressed in 72 (100 %) isolates, *vanA* gene was only expressed in 5 (6.9 %) and *vanB* was only expressed in 9 (12.5 %) isolates , as shown in Figure (5).



Figure(5): Distribution of *mecA , vanA and vanB* genes in *Staphylococcus aureus* isolates .

The results showed that all isolates of *Staphylococcus aureus* are contain mecA gene, this means that all isolates were methicillin resistance *Staphylococcus aureus* (MRSA), our study approach with [17] in Iran , reported that all 46 MRSA isolates harbored mecA gene. However we disagree with [18] in Qadisiyah**,** who showed that (19%) of isolates were have *mecA* gene methicillin resistance. [19] in Bangladesh, was disagree with this study, they suggest that prevalence of mecA in the isolates was 72%.

The results showed that five isolates of *Staphylococcus aureus* are contain vanA gene, and this means only (6.9%) isolates were Vancomycin resistance (VRSA) and this approach came with [20] in Egypt , who reported 20.13% of the VRSA emerged contain vanA gene. Also Fasihi *et al*. [21] in Iran, agreed with the present study, they found that two MRSA isolates were identified as VRSA and both isolates were vanA gene positive.In addition we agree with [22] in Iran **,** they report that vanA gene was found in two VRSA isolates. However we disagree with the results of [23] in Egypt, they reported the occurrence of vanA gene-negative VRSA in Egypt. Other study done by [24] in Pakistan,who disagree with our results ,they found no vancomycin resistant strain in their setup.

Our results showed that nine isolates of *Staphylococcus aureus* are contain vanB gene, this mean only (12.5 %) isolates were Vancomycin resistance (VRSA) and approach came with [25] in sudan , reported that only three VRSA contained vanB. Also [26] in Bangladesh , found that VRSA strains were positive for the vanB gene but negative for the vanA gene. Although our result disagree with [22] in Iran, who showed that vanB gene was not detected in any VRSA isolates. El-Banna *et al*. [20] in Egypt, described that VanB gene were not found in any isolates.In conclusion, the *Staphylococcus aureus* isolates have *mec A* gene, which is characteristic of Methicillin-resistant *Staphylococcus aureus* (MRSA), and only five isolates on the *van A* gene is proof that they are resistant to vancomycin *S .aureus* (VRSA). Also, only nine isolates possessed the van B gene and are evidence of vancomycin resistance *S .aureus* (VRSA).

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

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