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Detection of Vancomycin Resistant Gene in *Staphylococcus aureus* Isolated From Different Clinical Samples in Erbil City

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Abstract

Background*: Staphylococcus aureus* is a nosocomial bacterium that causes different kind of diseases, including urinary tract infections., skin infection, meningitis, wound infection, pneumonia and septicemia. The development of vancomycin-resistant Staphylococcus aureus, encoded by the *VanA* gene, is caused by the spread of Staphylococcus aureus among patients.. The aim of present study is to get the rates of *Staphylococcus aureus* infections and to detect vancomycin resistant gene in *Staphylococcus aureus* isolated from different clinical samples.

Material and Methods: A total of 175 isolates of *S. aureus* were collected from different clinical samples including patients attending public hospitals during a period from April 2020 to December 2020. Identification was done depending on biochemical test, Vitek compact system. Vancomycin susceptibility test was done for each sample.

Results: The rate of *Staphylococcus aureus* infections in different specimens were as following: nasal swabs (28%), wound swab (22.8%), urine (26.9%), biopsy (6.3%), sputum (9.1%) and HVS (6.85%). The rate of vancomycin resistance was (10.3%) by vitek 2 system. All *Staphylococcus aureus* isolates were subjected for vancomycin resistance gene by means of increasing of *VanA* gene by polymerase chain reaction technique (PCR), the vancomycin resistant gene size is (732 bp), in which (13.7%) of isolates represented vancomycin resistant gene positive.

Conclusion: Detection of Vancomycin resistant gene by PCR shows higher sensitivity and specificity than other methods.

Key words:

Staphylococcus aureus, Vancomycin, Vitek, *VanA* gen, PCR.

Citation:

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1. INTRODUCTION

Staphylococcus aureus is a member of the Micrococcaceae family. The gold colour appearing of colonies and positive finding outs of biochemical assays separate it from other Staphylococcal species [1]. The ecological niche of *Staphylococcus aureus* in humans is the anterior nares. The pathogen is widely distributed that cause a different type of infections in both hospital and community settings. [2].

Staphylococcus aureus has acquired determinants through flat gene moved of motile genetic elements, which has resulted in resistance to a variety of drug. At the end of 1940s, penicillin-resistant Staphylococci distributed. In hospital patients, the glycopeptide antibiotic is used to treat violent disease caused by MRSA strains. The antibiotic is connecting to the lipid II dipeptide D-Ala4-D-Ala5 & inhibits PBP2 with PBP2a from catalyzing trans glycosylation and trans peptidation, as well as peptidoglycan remodeling [3 and 4].

In 1997, the medical community of Japan was alarmed by reports of decreased or intermediate Vancomycine sensitivity in clinical insulates of *S. aureus* [5-7]. Increase in Vancomycine use has led to the emergence of two different strains of Vancomycin resistant *Staphylococcus aureus* (VRSA) and Vancomycin intermediate *Staphylococcus aureus* VISA) [7]. The *vanA* gene, which is transmitted from *Enterococcus faecalis*, has become synonymous with VRSA. This gene encodes for high-level glycopeptide resistance. Methicillin resistant *Staphylococcus aureus* which is known as MRSA, was detected in British hospital during the next 10 years, multidrug resistant Staphylococci became common in Europe, Australia and the Unitrd States. VRSA has also been isolated in Iran and India [8-10]

A circular chromosome, plasmids and transposons makes up the Staphylococcal genome. Genes performing virulence and resistance to antibiotics are located on the chromosome, as well as the extra-chromosomal genetic material such as plasmids. The peptidoglycan chains are cross-linked by tetrapeptide chains bound to N-acetylmuramic acid and by a pentaglycine bridge specific for *S. aureus*. Peptidoglycan may have endotoxin-like activity, stimulating the release of cytokines by macrophages, activation of complement, and aggregation of platelets. Lipoteichoic acid is a glycerol phosphate polymer linked to a glycolipid terminus anchored in the cytoplasmic membrane[1]

Staphylococcus aureus causes a wide range of infections due to virulence characteristics that allow it to attached to surfaces, penetrate or evade the immune system, and produce severe toxic effects on the host, some of these substances are enzymes; others are considered to be toxins many of the toxins are under the genetic control of plasmids; others are under chromosomal control. [11, 12]

The *VanA* locus usually confers high levels of Vancomycin resistance changes in peptidoglycan production are hypothesized to cause resistance in VARSA strains. [13]. The objective of this study is to find out the rate of *Staphylococcus aureus* infections in different clinical specimens in relation with gender and to find out the incidence of Vancomycin resistance among isolates of *Staphylococcus aureus* in Erbil city by conventional method with detection of the vancomycin resistant gene in *S. aureus* by and PCR and gel electrophoresis.

2. Material and Methods:

Samples were collected from different hospitals in Erbil City including Rizgary hospital, Hawler teaching hospital, Central lab and Western emergency hospital during nine months from April to December 2020.

A total of 175 *Staphylococcus aureus* isolate were collected from both gender and different sites including urine, sputum, nasal swab, and wound swab, high vaginal swab and Biopsy.

The identification was carried out using the Vitek 2 method, which is highly automated and employs reagent strips with microliter amounts of antibiotics and test medium in a 64-well configuration. During a shortened incubation time, the vitek 2 system conducts fast identification based on florescence and colorimetry of the bacterial growth pattern.. The device may be set up to run 30-240 tests at the same time. Antibiotics shown in the findings of vitek 2 susceptibility cards that shows growth of bacteria within 4-10 hours [14].

DNA Extraction

Staphylococcal DNA was isolated from the bacterial cultures using (100 prep Fermentas DNA extraction kit), and the *VanA* gene was amplified by PCR (Bioron / Genekam Biotechnology ready to use PCR master mix) [15]

Polymerase chain reaction (PCR)

The primer used for *VanA* gene detection "Vancomycin resistance gene" was (forward primer EA1, 5′-GGGAAAACGACGACAATTGC-3′ and reverse primer EA2, 5′-GTACAATGCGGCCGTTA-3′).

The extracted DNAs were subjected to thirty cycles, (the protocol followed for PCR was initiation denaturation for 3 minute using 94℃ followed by 30 cycles of denaturation for 1minute at 94℃, then annealing for 1minute at 54˚C, followed by an extension for 1minute at 72℃ and a final extension at 72℃ for seven minutes after 30 cycles) [16].

Detection of PCR product

Detection of *VanA* gene was done by using 1% agarose gel (Sigma-Aldrich) stained with ethidium bromide (Sigma-Aldrich), DNA fragments were analyzed by electrophoresis in 0.5X TBE (Tris-borate-EDTA), the samples were scored as positive for *VanA* gene corresponding to a 732bp [16].

3. Results and Discussion:

Staphylococcus aureus is one of the nosocomial pathogens that cause a variety of diseases. *S. aureus* is one of the most frequent nosocomial and community-acquired infections.[17]. Vancomycin resistant because of *VanA* gene encoding, rending *Staphylococcus aureus* resistant to glycopeptide antibiotics, which is the most important group of antibiotic in the treatment of Staphylococcal infection after Beta-lactam antibiotics. Accurate and rapid detection of Vancomycin resistant in *Staphylococcus aureus* is to dominate of the endemicity of Vancomycin resistant *Staphylococcus aureus*.

Distribution of *Staphylococcus aureus* by gender, there was a high prevalence of *S. aureus* infections among male compared to females. The rate of *S. aureus* infections in males was (55.43%) and in females (44.57.03%) as shown in table 1.1. The results of this study showed similar results to that obtained by [18], where the rate of *S. aureus* infection was higher in males (63%) than female (50%).

Table 1.1. The rate *of S. aureus* infection in both males and females among different clinical isolates

Clinical specimen	Gender				Total	
	Female Frequency	Percentage (%)	Male Frequency	Percentage (%)	Frequency	Percentage (%)
Urine	28	16	19	10.9	47	26.9
Sputum	5	2.8	11	6.3	16	9.1
Nasal swab	12	6.9	37	21.1	49	28
Wound swab	17	9.71	23	13.14	40	22.85
Biopsy	4	2.3	7	4	11	6.3
HVS	12	6.85	0	0	12	6.85
Total	78	44.57	97	55.43	175	100

Note *P value* = 0.827, there is no statistical differences between males and females infection with *S. aureus*

From 175 isolates of *S. aureus*, 18 (10.3%) of isolates were resistant to Vancomycin and 157 (89.7%) of isolates were sensitive to Vancomycin. Identification of Vancomycin resistant *S. aureus* were based on the Vitek 2 system, as shown in table (1.2)

Table 1.2. The frequency and percentage of VRSA among *S. aureus* isolates which was detected by Vitek 2 system.

***Staphylococcus aureus* isolates**	**Frequency**	**Percentage (%)**
Vancomycin resistant *Staphylococcus aureus*	18	10.3
Vancomycin sensitive *Staphylococcus aureus*	157	89.7
Total	175	100

Among 18 isolates of VRSA the rate of Vancomycin resistance in males was (55.6%) and in females (44.4%), as shown in table (1.3)

Table 1.3. The frequency and percentage of VRSA in relation to gender depending on vitek 2

Rate of VRSA infections in both genders				**Total VRSA**	**Percentage**
Female Frequency	Percentage	Male Frequency	Percentage (%)		
8	44.4	10	55.6	18	100

Note *P value* = 1.000, There is no statistical differences between male and females infections with VRSA, the reason is due to sample size of males were larger than females.

In the present study the susceptibility test for *S. aureus* to antibiotics indicated that the most effective antibiotics was Ampiclox followed by Nitrofuration, and the less effective antibiotic which shows high rate of bacterial resistance was clindamycin followed by benzoic acid and tetracyclin, as shown in table (1.4), these results agree with that obtained by [19] in which the isolates showed high rate of resistance to clindamycin and tetracyclin.

Table 1.4. The results of susceptibility test to antibiotics.

Antibiotics	Number of resistant isolates	Percentage(%)
Vancomycin	18	10.3
Clindamycin	50	28.6
Benzoic acid	40	22.9
Cefxitin	38	21.7
Tetracyclin	38	21.7
Eruthromycin	36	20.6
Amoxicillin	36	20.6
Oxacillin	32	18.3
Cefixime	20	11.4
Rifampicin	16	9.1
Gentamycin	12	6.9
Ciprofloxacin	10	5.7
Meropenim	10	5.7
Trimithoprim	4	2.3
Rifampin	4	2.3
Nitrofurantoin	2	1.1
Ampiclox	1	0.6

According to antibiotic test by Vitek 2 system the rate of vancomycin resistance was 10% , result of this study disagree with the results obtained by [20] in which the rate of vancomycin resistance was 0% among *S. aureus* isolates there results shows high resistance to Amoxicillin and Penicillin.

The development of such resistant strains is a major factor in human infection treatment failure. The problem is exacerbated by the unregulated use of antibiotics, which, along with inadequate diagnostic procedures and incorrect administration by inexperienced physicians, creates a significant challenge for the prevention and management of this disease [21-23].

Polymerase chain reaction (PCR)

The PCR result for detection of *VanA* gene with a molecular size 732 bp, showed in figure [1] demonstrating some strains of positive isolates for *VanA* gene.

In the present study (10.3%) VRSA strains were identified from of *S. aureus* isolates according to conventional methods, while higher rate (13.7%) were showed positive result for *VanA* gene (*VanA* for vancomycin resistance) by PCR. The maximum rate of VRSA in our result might be illustrate through the use of numerous preventive and after surgery antibiotics in conjunction with expanded hospitalization. [22- 25]. Our result disagrees with the result obtained by [16] in which half of the isolates which were detected by conventional method showed positive *VanA* gene.

One of the expected pathways of Vancomycin resistance in *S. aureus* is conjugative transfer of plasmids harboring Tn1546 and therefore the VanA gene cluster from Vancomycin resistant *Enterococcus* spp. [21 and 26].

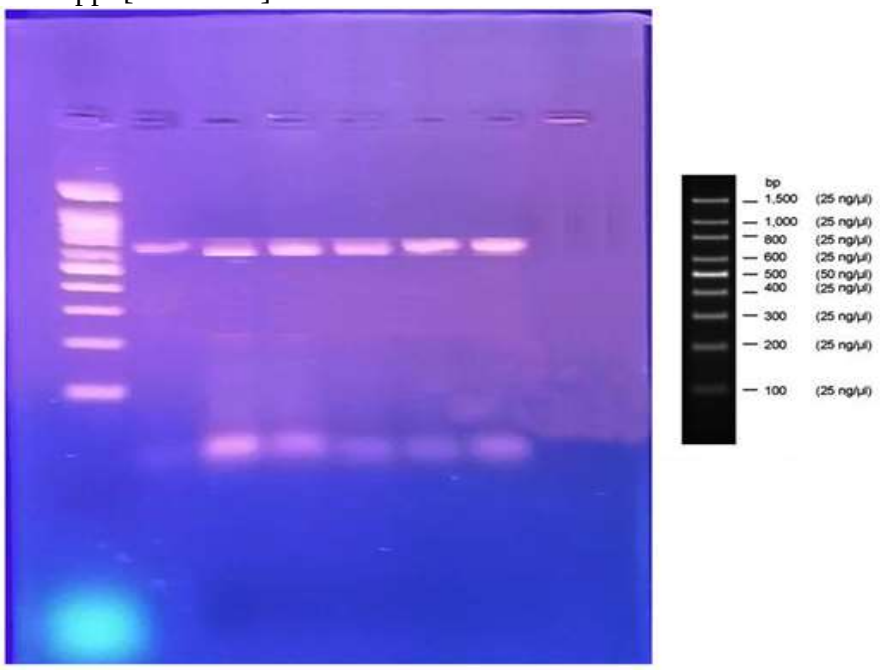

Figure 1. Gel electrophoresis for detection of *VanA* gene with a molecular size 732 bp, first lane on the left molecular size marker, lane 2-7 *S. aureus* isolates show *VanA* gene and line 8 negative control.

Conclusion: We can conclude from this research that *S. aureus* isolated from different clinical samples showed high resistance to Clindamycin and Benzoic acid.
VRSA strain is probably hazard to the health of population, despite the low number of cases. For preventing establishment and spread of VRSA strains, intensive surveillance of vancomycin resistance, appropriate antibiotic usage, and adherence to infection control recommendations in health-care settings are required.

The detection of vancomycin resistant gene by PCR have higher sensitivity and specifity than other methods.

The appearance of these resistant strains prompt researchers to do a series of studies to assess the colonization and infection with VRSA in patients to define risk factors and to evaluate the effect of interventions on rates of colonization and infection.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة:

الخلفية العلمية: المكورات العنقودية الذهبية هي بكتيريا في المستشفيات تسبب أنواعًا مختلفة من الأمراض ، بما في ذلك التهابات المسالك البولية ، والتهاب الجلد ، والتهاب السحايا ، والتهاب الجروح ، والالتهاب الرئوي ، وتسمم الدم. إن تطور المكورات العنقودية الذهبية المقاومة للفانكومايسين ، المشفرة بواسطة جين VanA ، ناتج عن انتشار المكورات العنقودية الذهبية بين المرضى. الهدف من الدراسة الحالية هو الحصول على معدلات عدوى Staphylococcus aureus واكتشاف الجين المقاوم للفانكومايسين في Staphylococcus aureus معزولة من عينات سريرية مختلفة.

المواد والطرق: تم جمع ما مجموعه 175 عزلة من بكتيريا S. aureus من عينات سريرية مختلفة بما في ذلك المرضى الذين يحضرون إلى المستشفيات العامة خلال الفترة من أبريل 2020 إلى ديسمبر 2020. تم التعرف اعتمادًا على الاختبار الكيميائي الحيوي ، نظام Vitek المضغوط. تم إجراء اختبار حساسية الفانكومايسين لكل عينة.

النتائج: كان معدل الإصابة بالمكورات العنقودية الذهبية في العينات المختلفة كالتالي: مسحات الأنف (28٪) ، مسحة الجرح (22.8٪) ، البول (26.9٪)، الخزعة (6.3٪) ، البلغم (9.1٪) و HVS (6.85 ٪). كان معدل مقاومة الفانكومايسين (10.3٪) بنظام vitek 2. تم إخضاع جميع عزلات Staphylococcus aureus لجين مقاومة الفانكومايسين عن طريق زيادة جين VanA بواسطة تقنية تفاعل البوليميراز المتسلسل (PCR) ، وكان حجم الجين المقاوم للفانكومايسين (732 زوجًا أساسي) ، حيث يمثل (13.7٪) من العزلات الجين المقاوم للفانكومايسين إيجابيًا.

يُظهر اكتشاف الجين المقاوم للفانكومايسين بواسطة تفاعل البوليميراز المتسلسل حساسية وخصوصية أعلى من الطرق الأخرى.