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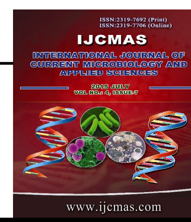

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Original Research Article

Establishment of Horizontal Transformation of *VanA* Gene from another Bacterial species to *Staphylococcus aureus* by Curing

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Keywords

VanA Gene, *Staphylococcus aureus,* vancomycin

A B S T R A C T

Two hundred and ninety nine isolates of *Staphylococcus aureus* were identification from 413 samples collected from Wound and burn patient in Baghdad from period between February - may 2014. Mannitol salt agar was used for preliminary identification of *S. aureus* while chromogenic *S. aureus* agar and chromogenic MRSA *S. aureus* medium as used as confirmatory media as well as *Fem A* gene primer for identification *S. aureus*. After identification these bacteria, we used vancomycin antibiotic sensitive test and after that minimum inhibitory test to these bacteria, and we gated 13 isolates were resistance to vancomycin antibiotic. after that we were extracted the plasmid from this bacteria by plasmid profile method, to identified this resistance gene placed on plasmid or on bacterial DNA and by this plasmid extraction we used PCR to research *VanA* gene, and the result that the *VanA* gene found on plasmid of *S. aureus* also we used curing method to we conformed do this gene removed or not. By this experiment we concluded this Van A gene come from another genus of bacteria by horizontal gene transfer.

Introduction

Staphylococcus aureus is one of the most common causes of nosocomial infections, especially pneumonia, surgical site infections and blood stream infections and continues to be a major cause of community-acquired infections. Methicillin-resistant *S. aureus* (MRSA) was first detected approximately 40 years ago and is still among the top three clinically important pathogens (Van Belkum and Verbrugh, 2001; Deresinski, 2005). The emergence of high levels of penicillin resistance numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA) strains exhibiting two different resistance mechanisms (Tony, 2012).

Initially vancomycin-intermediate *S. aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997, was believed to be due to the thickened cell wall (Hiramatsu *et al.,* 1997), where many vancomycin molecules were trapped within the cell wall.

The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules 6. The second, noted in United States in 2002 (Bozdogan, 2003) among *S. aureus*, was identical to the mechanism seen in vancomycin-resistant *Enterococcus*.

Vancomycin resistant *Enterococcus faecium* harbours the *VanA* operon, which contains five genes, *Van* S, -R, -H, -A and –X8. But Tiwari and Sen have reported a VRSA which is *van* gene-negative (Tiwari and Sen, 2006).

Subsequent isolation of VISA and VRSA isolates from other countries including Brazil (*Oliveira et al.,* 2001), France (Poly *et al.,* 1998), United Kingdom (Howe *et al.,* 1998), Germany (Bierbaum *et al.,* 1999), India (Tiwari and Sen, 2006; Assadullah *et al.*, 2003), and Belgium (Pierard *et al.,* 2004) has confirmed that the emergence of these strains is a global issue.

Approximately 5 years earlier, the Japanese had reported the first strain of *S. aureus* with reduced (or intermediate) susceptibility to vancomycin followed by 2 additional cases from the USA.

These earlier isolates were termed Vancomycin Intermediate *Staphylococcus aureus* (VISA).

Although additional cases have been reported from other countries, to date, no cases of either VISA or VRSA have been identified in Canada.

The definitions of VRSA and VISA are based on the results of laboratory testing which determine the minimum concentration of vancomycin that is required to inhibit the growth of *S. aureus* in a “test tube”.

It should be noted that these definitions are not universal and some countries lump all strains of *S. aureus* that require increased concentrations of vancomycin to inhibit their growth into a single VRSA category (Venubabu *et al.,* 2011).

The source of the *VanA* gene isolated in VRSA appears to have come from co-infection with vancomycin resistant *Enterococcus* (VRE) (Tony, 2012).

Materials and Methods

Collection of samples

A total 413 samples were collected from patient suffering of wound and burn infections in the same period.

Preparation of Culture Media

Mueller-Hinton Agar Medium:

Mueller Hinton agar medium was prepared according to the manufacture's instruction.

Mueller-Hinton broth

Mueller Hinton broth was prepared according to the manufacture's instruction.

Mannitol salt agar

The medium was prepared according to the manufacturer's instruction chromogenic culture media.

1. Chromo agar TM MRSA

This medium were prepared according to the manufacture's instruction

2. Chrom agar *S. aureus*

This medium were prepared according to the manufacture's instruction

McFarland standards solution

This solution was prepared according to Baron *et al.* (1994) as follows**:**

Solution A: 1.175 gm of Barium chloride was dissolved in 100 ml of DW.
Solution B: Prepared by add 1ml of sulfuric acid to 100 ml of DW, add 0.05ml of solution A to 9.95 ml of solution B and mix well and stored dark place until used, this solution gives 1.5X10 cell\ml approximately.

Antibiotic stock solutions

Stock solution of Vancomycin was prepared at final concentration of 10 mg/ml according to Clinical Laboratory Standard Institute (CLSI) (CLSI, 2011).

Plasmid Profile test solutions

Plasmid Profile test solution prepared according to Stephenson *et al.* (2003).

Plasmid DNA-Extraction solution

This solution was prepared to isolate plasmid DNA from Enterococcus according to Klaenhammer (1984).

Enzymes

Lysozyme

This solution was prepared at final concentration of 1 mg\ml by dissolving 0.001 gm of lysozyme enzyme powder in 1 ml of TE buffer.

Preparing of the primers

Oligonucleotide primers were prepared depending of manufacturer's instruction by dissolving the lyophilized product in sterile deionized water after spinning down briefly. Working primer solution was prepared by diluting with deionized DW.

The final picomoles depended on the procedure of each primer. The primers used in this study were shown in table 1.

Results and Discussion

Preliminary tests of identification of *Staph aureus*

The isolates of *S. aureus* used in this study 299 were collected from different source, 70 isolates obtained from Microbiology department, the other were collected from burn and wound infections.

Saving of effort and time the policy of this study was (going direct to aim), so we used mannitol salt agar medium to isolate and identify *Staph aureus* since *Staph aureus* cause fermentation of mannitol and change the color of colonies to yellow, it can be said that classical biochemical reaction is no longer of value in this process.

Confirmative tests for *S. aureus*

The confirmative tests for *S. aureus* were solely depended on the appearance of *S. aureus* colonies on chromogenic *S. aureus* agar and chromogenic MRSA.

All *S. aureus* isolates were cultured on chromogenic *Staphylococcus* agar and chromogenic MRSA. The result obtained from this experiment showed that all bacteria l colonies appeared on mannitol salt agar as *S. aureus* were grown on both confirmative media. As well as the 70 isolates obtained from the collection from Microbiology department were also grow on this confirmative medium.

The colonies of *S. aureus* on both confirmative media as moderate raised pink colonies due to the hydrolysis of chromogenic substrates including in media. one of the advantages of MRSA chromogenic media is to differentiate methicillin resistance *S. aureus* from other, on this medium 135 isolates of *S. aureus* were methicillin resistant.

The result obtain from this experiment were shown in table 2. In this table *S. aureus* consisted 72% of all isolates from both source of isolation (burns and wounds).

almost equal isolation percentage of *S. aureus* from both sources(burns and wounds) were recorded (72.39%). MRSA isolation percentage (60.7% from 135 isolates of MRSA) recorded from burn infections higher than wound infection (39.3% from 135isolates of MRSA) (Table 2).

these result were in agreement with Abd-alameer (2009) who showed the rate isolation of MRSA was 33% from burns, but disagreement with VRSA isolation that was all of isolates sensitive to vancomycin and also our results were agreed with Al-Oubaidy (2012) who recorded an isolation rate of *S. aureus* of 56%. While their result were far way from matching with Emran *et al.* (2012) who reported the VRSA percentage was 12% (24/200 isolates) of *S. aureus.*

Other result for confirmation of diagnosis of *S. aureus*, vitek 2 system and PCR technique used (*fem A* gene" house keeping gene") the result of all confirmative test have indicated clearly that all isolates of *S. aureus* previously identified preliminary test were confirmed to be *S. aurus*, the result of PCR amplification study indicted clearly that the band of *femA* gene appeared on gel located at size of 450 bp. this location same to be that typical location for house keeping gene *of S. aureus* according to Kariyama *et al.* (2000). Further, it is a clear from results obtained in this study that aim of this section of research had been full filed, this on one hand, on the other chromogenic media which were used throughout this section of research had proved to be a real able to diagnosis and identification *staphylococcus* in general in particular.

It is obvious from the results obtained in this study that all confirmative tests used to confirmed the diagnosis of the species of *S. aureus* were at equal level and that gives as the opportunity to select any one of these tests to be the gold test for diagnosis and identify *S. aureus.*

A screening test for ability of *S. aureus* isolates to resistant the action of vancomycin antibiotic was detected grossly by used the disc diffusion method.

In this method diameters of inhibition zones were compared to CLSI (2011), as far as *S. aureus* is consider the result of this experiment showed that (9.6 %) 13 isolates were resistance to vancomycin 90% were sensitive to vancomycin.

Determination the Minimum Inhibitory Concentration for Vancomycin antibiotic of Enterococcus and *S. aureus*

the *S. aureus* 13 isolates show a value of MIC range from 64-512 µg /ml and the picture of resistance was observed in which summarized the result MIC determination of *S. auerus* which range from 64 to 512 µg \ml.

The result present in this study have a good agreement with Anvari *et al.* (2012) who showed MIC result were 128 µg/ml. MIC

value of one isolate was 512 μg/ml and this result agreement with Azimian *et al.* (2012) which show MIC for all your isolates 512 μg\ml.

The results of the present study have been revealed that all isolates of *S. aureus* obtained in this study contained plasmids regardless the number of plasmids 3 isolates contain one plasmid of size 7 Kb, one isolate contain plasmid of 5Kb, two isolates contain 2 plasmid one of size 7 Kb and other of size more than 10 Kb. While 3 isolates were contain two plasmids one of 5 Kb and other of 7 Kb and lastly, one isolate contain two plasmids one 7Kb and other of more than 10 Kb. These results were agreed with Fred (2008) and Marck (2015).

We used *Van A* gene primer to research this gene in plasmid extracted from this bacteria and the gel electrophoresis showed all 13 isolates of *S. aureus* contain *Van A* gene carried on plasmids and the bands localized on local 733 bp (Figure 3) and this local agreement with Al-Talib *et al.* (2009).

The result of the present study indicated clearly that resistance of antibiotic Vancomycin is a plasmid born character. This assumption conformed by the curing method in which both members of genus staph. aurus and genus Enterococcus lost liability for vancomycin resistance after being curing as indicated by figure 5 and these results were in agreed with Ambrina *et al.* (2010).

In an effort, to track the movement of the plasmid inter or intra generic of genus *Enterococcus* or genus staphylococcus in our view, the *E. faecalis or E. faecium* may be the source of resistance of vancomycin concluded that the movement of plasmid of resistance is coming from the large pool to the smallest one and in this case is from *Enterococcus* to *Staphylococcus*.

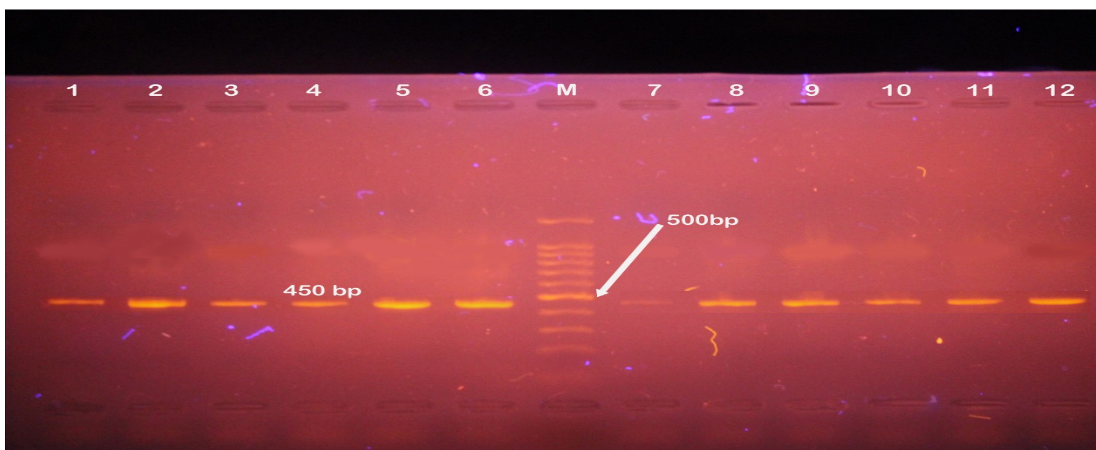
Table.1 Oligonucleotide primers sequences used for PCR amplification of *VanA* and *FemA* genes

Genes	Sequence (5' to 3')	Size (bp)	References
VanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	733	17
*fem*A	F- CGATCCATATTTACCATATCA R –ATCACGCTCTTCGTTTAGTT	450	18

Table.2 *S. aureus* isolates; numbers and isolate rate

Type of isolates	Number of samples	**Number of *S. aureus***	**Rate of isolation**		**Number and rate MRSA**			**Number and rate VRSA**		
			typically	totally	N	typically	totally	N	typically	totally
Burn	232	162	54.18	39.22	82	60.7	19.8	8	4.9	1.9
wound	181	137	45.82	33.17	53	39.3	12.8	5	3.6	1.2
account	413	299	100	72.39	135	100	32.6	13	8.5	3.1

Figure.1 Agaros gel electrophoresis of *femA* gene (bp amplification) for *S. aureus* (lane M100 bp DNA ladder)



Lane 1(S64) Lane 2(S70) lane 3 (S41)lane 4(S31) lane 5(S66) lane 6 (S58)lane 7M100 lane 8(S43)lane 9(S8) lane 10(S2) lane 11(S72) lane 12(S56) lane 13(S69)

Figure.2 Plasmid content for some isolates of *S. aureus*

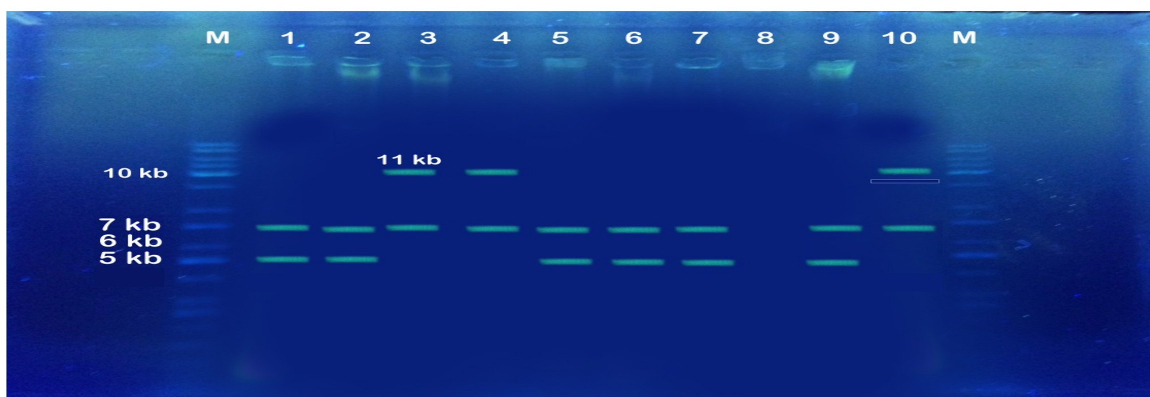
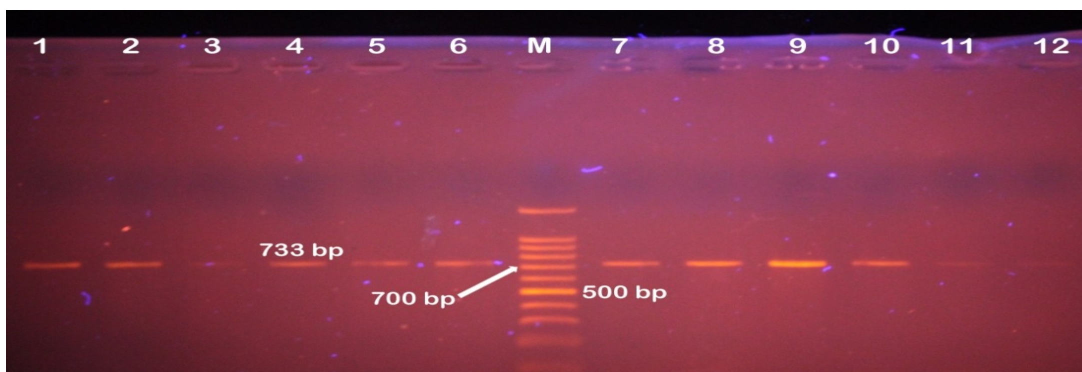


Figure.3 Agaros gel electrophoresis *Van A* gene (733 bp amplification) for *S. aureus* (lane M100 bp DNA ladder)



Lane 1(S64) Lane 2(S70) lane 3 (S41)lane 4(S31) lane 5(S66) lane 6 (S58)lane 7M100 lane 8(S43)lane 9(S8) lane 10(S2) lane 11(S72) lane 12(S56) lane 13(S69)

Figure.4 Agarose gel electrophoresis *Van A gene* (733 bp amplification) for *S. aureus* (lane M100 bp DNA ladder)

Lane 1(S64) Lane 2(S70) lane 3 (S41)lane 4(S31) lane 5(S66) lane 6 (S58)lane 7M100 lane 8(S43)lane 9(S8) lane 10(S2) lane 11(S72) lane 12(S56) lane 13(S69)

Figure.5 Lost plasmid content from VRSA after curing experiment

Lane 1(S64) Lane 2(S70) lane 3 (S41)lane 4(S31) lane 5(S66) lane 6 (S58)lane 7M100 lane 8(S43)lane 9(S8) lane 10(S2) lane 11(S72) lane 12(S56) lane 13(S69) 1.5% agarose,70 vol. for 60 min

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