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Full Length Research Paper

Distribution of insertion sequences in Tn*1546* element in vancomycin-resistant *Staphylococcus aureus* in Sulaimani, Kurdistan of Iraq

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In 2008, nine clinical isolates of vancomycin-resistant *Staphylococcus aureus* containing Tn*1546* were recovered in Sulaimani, Iraq. The genetic diversity in Tn*1546*-like elements has been documented previously. The differences described thus far have included the integration of insertion sequence (IS) elements IS*1216V* and IS*1251.* With polymerase chain reaction (PCR) and deoxyribonucleic acid (DNA) sequence analysis of Tn*1546*, the distribution of ISs among 9 *vanA*-containing *S. aureus* isolates were investigated. Only one VRSA element was identical to the prototype Tn*1546* element. Structural analyses of the *van* gene detected IS*1216* and IS*1251* in the genomes of 8 isolates. In addition, IS*19* was detected in the *vanS-vanH* region of one of the 8 isolates. Two of the 8 vancomycin-resistant *Staphylococcus aureus* (VRSA) elements showed a deletion, which eliminated the *orf1* region, and IS*1216* inserted in place of it and also another copy of IS*1216* inserted into the *vanSH* region. The distribution of ISs associated with Tn*1546*-like elements among the Sulaimani isolates was found to be different from that of American vancomycin-resistant Staphylococci population. From this study, it was concluded that identification and analysis of the IS within the *vanA* gene could be a useful tool in epidemiological investigations.

Key words: Vancomycin-resistant *Staphylococcus aureus* (VRSA), insertion sequence (IS), Tn*1546*, Sulaimani, Kurdistan.

INTRODUCTION

Vancomycin-resistant enterococci (VRE) containing the *vanA* gene have been isolated from humans and animals worldwide (Woodford, 1998). Epidemiologic studies of VRE indicate that there are geographic differences (Dahl et al., 1999). *Staphylococcus aureus* is a major cause of potentially life-threatening infections acquired in health care settings and in the community worldwide. Only four vancomycin-resistant *S. aureus* (VRSA) isolates have been reported so far from the USA (Arthur et al., 1993; Brown et al., 2001; Dahl et al., 1999; Darini et al., 1999; Donabedian et al., 2000).

There has been no report of the *van* gene-mediated VRSA (Vancomycin-resistant *Staphylococcus aureus*), from Asia, except for vancomycin-intermediate *S. aureus* (ISA) in Japan (Dutka-Malen and Courvalin, 1995), Korea (Handwerger and Scoble, 1995), India (Hashimoto et al., 2000), Southern Asia (Jensen et al., 1998). Vancomycin, a glycopeptide antibiotic, acts against Gram-positive bacteria only by inhibiting the incorporation of N-acetyl-muramic acid- N-acetyl-glucose amine polypeptide into the growing peptidoglycan (PG) chain. This is achieved by interacting with D-Alanine-D-Alanine which subsequently blocks the release of terminal D- alanine and intra-chain bond formation. Vancomycin-resistant *Enterococcus faecium* harbours the *vanA* operon, which contain five genes, including vanS, -R, -H, -A and -X, respectively (Perichon et al., 2000).

It had been known that the *vanA* gene cluster is carried as a part of Tn*1546*-like elements and this indicate that the horizontal transfer of Tn*1546*-like elements play an important role in the dissemination of *vanA*-type VRE. Therefore, investigation of the genetic variations within

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Tn*1546*-like elements would be essential to have an understanding of the mechanism of evolution of the VRSA, particularly in cases that involve horizontal gene transfer.

The majority of the variations comprises of integration of insertion sequences (ISs) with or without a deletion at the insertion site, point mutations, and deletions (Brown et al., 2001). Epidemiological studies of *vanA* gene indicate that there are geographic differences associated with this genetic element (Stobberingh et al., 1999). Thus, it is important to understand the underlying molecular mechanisms for the dissemination of VRSA in order to have an effective mechanism for controlling the threat associated with this potentially hazardous microbe in Iraq and elsewhere.

Generally, the primary mechanism for dissemination of the *van* gene in *Staphyllococci* could be the clonal dissemination of VRSA where horizontal transfer of resistance gene cluster also plays a prominent role. Pulsed-field gel electrophoresis (PFGE) has been widely carried out to have an understanding of this clonal dissemination of VRE and structural analyses of the *van* gene have already been introduced to establish an understanding of the horizontal transfer of the resistance gene cluster. In the present study, a set of nine isolates of vancomycin-resistant *Staphylococcus aureus* (VRSA) containing Tn*1546*, recovered from Iraq were analyzed to understand the distribution pattern of insertion sequences in Tn*1546*.

MATERIALS AND METHODS

Bacterial strains

During the period from 2008 to 2009, 10 clinical isolates of *vanA*-containing *S. aureus* isolates were collected from patients at the Sulaimani emergency hospital, Iraq. Organisms were identified by conventional biochemical reactions using the API 20 Strep system (BioMérieux).

DNA extraction and PCR

Extraction of bacterial DNA was performed with a Qiagen DNeasy kit (Qiagen, Germany) according to the instructions of the manufacturer. The vancomycin resistance genotypes were determined by PCR with oligo-nucleotide primers specific for the *van* gene sequences as described previously (Dahl et al., 1999; Dutka-Malen and Courvalin, 1995). For structural analysis of Tn*1546*-like elements, PCR amplification of internal regions of Tn*1546* was performed. The primer sequences for specific Tn*1546* regions were listed in Table 1. Primers ISV*650F* and ISV*132R* were designed based on the published sequence of IS*1216V* using the OLIGO program (version 6.0; National Biosciences Inc., Plymouth, Minn.). The melting temperatures of the individual primers were computed by using the same software (Table 1).

Sequence analysis

PCR amplicons of *vanA* gene cluster were purified using Gene clean kit (Qiagen, Germany). The purified PCR products were directly sequenced by using an ABI 377 genetic analyzer. DNA fragments amplified with a combination of a Tn*1546* and IS*1216V*-specific primer pairs were also purified and subsequently sequenced to determine the exact integration site and orientation of the S*1216V* insertion. The DNASIS program for Windows (version 2.6; Hitachi Software Engineering, South San Francisco, Calif.) was used for bioinformatic analysis of nucleotide sequences.

RESULTS AND DISCUSSION

The horizontal transfer of the resistance gene cluster has been regarded as the main mechanism in the dissemination of *van* gene (Lee et al., 2001; Shin et al., 2003). VanB VRE was predominantly isolated in Korean hospitals in the initial years, whereas isolates recovered between years 1998 and 2000 were predominant VanA type (Lee et al., 2001; Shin et al., 2003). Hence, structural analysis of the *vanA* gene cluster is critical to investigation of the epidemiology of *vanA*-containing *Staphylococci* in this research program. The *vanA* gene cluster is carried as a part of Tn*1546*-like elements. The heterogeneity of Tn*1546* has previously been reported and comprises of point mutations, deletions, and integration of the IS elements. Among these variations, the presence of IS elements accounts for a major part of the heterogeneity.

Till date, IS*1216V*, IS*1542*, IS*1251*, and IS*1476* have been reported in VanA VRE. IS*1216V* is known to be ubiquitous in *vanA* elements (Jensen et al., 1998; Willems et al., 1999) whereas the other three IS elements appear to be geographically restricted. For example, IS*1542* is frequently found in clinical as well poultry-originating VRE isolates from the United Kingdom and Ireland (Schouten et al., 2001; Woodford et al., 1998). IS*1251* and IS*1476* have been reported in the *vanA* elements of enterococci from the United States (Donabedian et al., 2000; Handwerger and Scoble, 1995) and Canada (Mackinnon et al., 1997), respectively.

The present study involved characterization of the structures of Tn*1546*-like elements among *S. aureus* isolates from burnt patients admitted to emergency hospital from different parts of Sulaimani governorate using PCR and nucleotide sequencing analysis. Three main types of *vanA* gene clusters were identified according to the distributions of ISs that is, IS*1216V*, IS*1251* and IS*19* in the Tn*1546*-like elements. In contrast, IS*1476* and IS1542 were not detected in the Sulaimani isolates. IS*1216V* and IS*1251* were identified in the genomes of all isolates from Sulaimani emergency hospital. Isolate no. 2 was characterized by a Tn*1546* sequence identical to the prototype Tn*1546* element (GenBank accession no. M97297).

The DNA sequences of the other 7 isolates were not identical to those of the prototype Tn*1546* element and divided into two groups, one contained IS*1216* and IS*1251,* the other contained IS*19*, IS*1216* and IS*1251.* Sequence analysis of isolate no.3, was characterized by

Table 1. Nucleotide sequences of PCR (primers).

Primer	Sequences (5′ →3′)
	Tn*1546*-specific primers
42F	ATT TTC CTG ACG AAT CCC TCG
349R	TCG GAA AAC AAG GTG AGC TTA GA
164F	AAC CTA AGG GCG ACA TAT GGT G
921R	AAA AGG AGC CAC AT CTA CCG
170F	AGG GCG ACA TAT GGT GTA ACA
1913R	CGT CCT GCC GAC TAT GAT TAT TT
949F	GCA TGT AGT GAT GAA ACA CCT AGC TGC
2976R	TGA AGA TGA ATG GAT ACT GGG GAC C
1871F	ACC GTT TTT GCA GTA AGT CTA AAT
3726R	AGC CCT AGA TAC ATT AGT AAT T
3514F	ACT GTA ATG GCT GGT GTT AAC
3978R	CAT AGT TAT CAC CCC TTT CAC TAT
3907F	ATG CTT ATA AAT TCG GCC C
4794R	ATC CAA TCC CCA AGT TTC CC
3992F	TTA TTG TGG ATG ATG AAC ATG
4511R	TCG GAG CTA ACC ACA TTC
4676F	AAC GAC TAT TCC AAA CTA GAA C
5769R	GCT GGA AGC TCT ACC CTA AA
5235F	ATA TCA CGT TGG ACA AAG C
7035R	TTA CGT CAT GCT CCT CTG AG
8082F	ACT TGG GAT AAT TTC ACC GG
8505R	TGC GAT TTT GCG CTT CAT TG
8448F	GAT GAA CGC TCT CAT CAT GC
9138R	TTC CTG AGA AAA CAG TGC TTC A
8544F	GCA TAT AGC CTC GAA TGG
9580R	TCG TCA AGC TTG ATC CTA C
10446F	AAT ACT GTT GGA GGC TTT CTT GG
10577R	GGT ACG GTA AAC GAG CAA TAA TAC G
	IS*1216V*-specific primers
650F	ACC TTC ACG ATA GCT AAG GTT
132R	AGG ATT ATA TAA GAA AAC CCG

a 1,499-bp sequence designated “IS*1251*-like” in the *orf2-vanR* intergenic region.

This element was inserted downstream from position 5820 in the opposite orientation relative to the transposon but was in the same position and orientation as an insertion described by Donabedian et al. (2000) in a Tn*1546*-like element from an *E. faecium* isolate, and an 810 bp IS*1216V* insertion in the *vanX-vanY* intergenic region. Isolate no. 3 and 4 was characterized by two copies of IS*1216V* at the left ends of Tn*1251*-like elements, the first copy revealing truncation of the 5′ region of the Tn*1546*-like element resulting in the loss of nucleotides 1 to 3100, which eliminated the *orf1* region. Upstream from the truncated Tn*1546* element was a 419-bp sequence with homology to bases 58293 to 58711 of *E. faecalis* V583 pTEF1 (GenBank accession no. AE016833).

This sequence, represented by the dotted line in Figure 1, was followed by an 810-bp sequence designated an “IS*1216V*-like” element that is in the same 5′→ 3′ orientation as the transposon, and a second copy of IS*1216* in the *vanX-vanY* intergenic region as well as IS*1251* in the *orf2-vanR* intergenic region. Isolate no. 5, 6, 7, and 8 was characterized by two copies of IS*1216V* at the left ends of Tn*1546*-like elements and in the *vanX-vanY* intergenic region as well as IS*1251* in the *orf2-vanR* intergenic region. Isolate no 9, was characterized by the presence of IS*19* in the *vanS-vanH* intergenic region, in addition to IS*1251* in the *orf2-vanR* intergenic region and IS*1216V* in the *vanX-vanY* intergenic region. IS*1216V* was present in the *vanX-vanY* intergenic regions of the genomes of 8 isolates, but at various points of

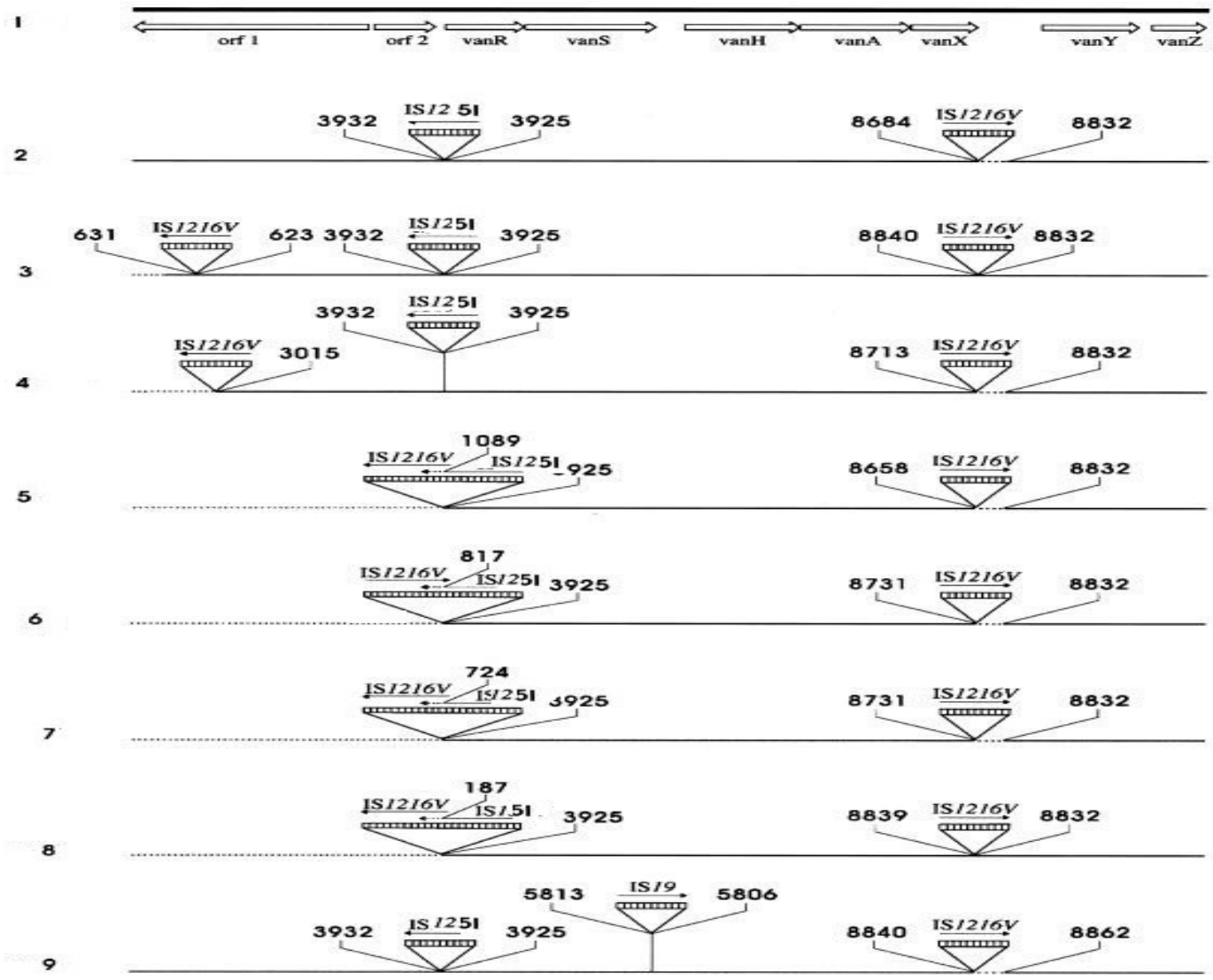

Figure 1. Genetic maps of Tn*1546* types of *S. aureus* isolates from Sulaimani emergency hospital. The positions of genes and open reading frames (orf1 and orf2) and the direction of transcription are marked by open arrows at the top. Boxes with vertical lines represent IS elements

integration. Among these 8 isolates, the insertions in 4 isolates were accompanied by small deletions adjacent to the insertion site. Also, IS*1216V* was present at the left ends of Tn*1546*-like elements of 6 isolates, with or without large deletions encompassing the *orf1* and/or *orf2* region. IS*1251* was detected in the *orf2-vanR* intergenic regions of all 8 isolates (Figure 1)

IS*1216V* was inserted at the left ends of the *vanA* elements, with a deletion that included the *orf1* and/or *orf2* regions. IS*1216* was found in the Tn*1546*-like element at right ends of Van A gene exactly at intragenic of *Van* XY at the same position in all the isolates as describe previously (Darini et al., 1999; Schouten et al., 2001; Woodford, 1998). Interestingly, the 3´ end of IS*1251* belonging to isolates no. 6, 7, and 8 was deleted at various points by the IS*1216V* insertion. This finding suggests that IS*1216V* at the left end of Tn*1546* was acquired later than IS*1251*. Moreover, importantly, to our knowledge, our study is the first to demonstrate the presence of IS*19* in the *vanS-vanH* intergenic region of the *vanA* gene cluster. Perichon et al. (2000) reported that IS*19* was inserted in the d-Ala-d-Ala ligase gene of VanD strain *E. faecium* BM*4416*, resulting in inactivation of the *ddl* ligase. However, IS*19* has never been documented in Tn*1546*-like elements. The movement of ISs frequently causes structural alterations in Tn*1546*-like elements. Furthermore, several investigators have documented the functional changes associated with IS integration with or without the adjacent deletion, as in the loss of VanY activity by an IS*1476* insertion (Mackinnon

et al., 1997) and inactivation of the *ddl* ligase by IS*19* (Perichon et al., 2000).

In our study, it was unlikely that the integration of an IS would affect the function of the *vanA* gene cluster. The genetic differences among Tn*1546*-like elements have been investigated in several studies (Brown et al., 2001; Hashimoto et al., 2000; Jensen et al., 1998; Simonsen et al., 2000; Stobberingh et al., 1999; Willems et al., 1999; Woodford et al., 1998). However, the Tn*1546* subtypes of the enterococci investigated were not comparable, since various molecular techniques were used. Finally the identification of ISs within the *vanA* gene cluster to analyze and compare the structures of Tn*1546*-like elements could be a useful tool in epidemiological studies.

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