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Detection of Novel Mutation in LukS Panton-Valentine Leukocidin Gene in Twelve Isolates of *Staphylococcus aureus* from Sudanese Patients

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ABSTRACT

Staphylococcus aureus carrying PVL gene remain major health problem associated with highly virulent infections. Characterization of such gene is important to know the impact and the functional significance of nucleotide variations. PCR and standard sequencing were performed for twelve Sudanese strains from different sources. Protein structures prediction, modeling and physiochemical analysis were done to analyze genetic variation in PVL. Single nucleotide polymorphism at position 610 (A/G) of *lukS-PV* was detected in all tested isolates resulted in nonsynonymous mutation in the amino acid sequence at position 204 (N / D). Using molecular and bioinformatics tools the detected novel mutation in *lukS-PV* may have a diagnostic functional significance.

1. Introduction

The *Staphylococcus aureus* causes widespread human diseases ranging from mild skin infections to fatal necrotizing pneumonia and sepsis (Holmes et al., 2005). Since the late 1960s, *S. aureus* strains have been classified according to their sensitivity to methicillin, into methicillin resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) (Jevons, 1961). The two types of the strains have been reported as the cause of spectrum of infections in hospitals and throughout the communities (Cunha, 2005). Methicillin-resistance is attributed to the *mecA* gene, encoding penicillin-binding protein PBP which presents low affinity to β-lactam antimicrobials (Perez, Dias, & d'Azevedo, 2008). Pathogenicity of *S. aureus* is attributed to a number of virulence factors. Panton-Valentine Leukocidin Gene (PVL) is a pore-forming cytotoxin that targets human and rabbit mononuclear and polymorphonuclear cells (PMNs) (Genestier et al., 2005; Prevost et al., 1995; Prevost, Mourey, Colin, & Menestrina, 2001; Supersac, Prevost, & Piemont, 1993). Studies have shown that PVL is encoded by two genes, this bicomponent, pore forming toxin, encoded by a highly conserved

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_1.9-kb *lukS/F-PV* locus consisting of two adjacent, co-transcribed *lukF* and *lukS* genes (Prevost et al., 1995), has 12 major single nucleotide polymorphisms (SNPs), the majority of which are synonymous. A nonsynonymous mutation at position 527, however, serves as the basis of the H and R isoforms (Dumitrescu et al., 2008; O'Hara et al., 2008; Takano et al., 2008).

The PVL nucleotide sequence is highly conserved and one genetic polymorphism in *lukS-PV* results in a non synonymous amino acid change in *LukS-PV*. Variants containing an arginine (R variant) or a histidine (H variant) are segregated into specific geographic regions (O'Hara et al., 2008). Studies comparing the interaction of PMNs with Rand H variants of PVL determined that this amino acid substitution has no impact on PVL activities. However the question is still arises if there is allelic variations depending on geographical region.

In this study, the presence of PVL was investigated in *S. aureus* strains isolated from Sudanese patients. The objectives of this study were to investigate the allelic variations in their *lukS-PV* and *lukF-PV* gene sequences of Sudanese strains, and to detect the functional analysis of detected new mutation.

2. Materials and Methods

2.1. Study area

This study was carried out mainly in Khartoum state during the years 2014-2015. All the conventional procedures were carried out in Tropical Medicine Research Institute, Department of Microbiology. The isolation and identification of bacterial isolates was done according to standard biochemical tests (Cowan, Steel, Barrow, & Feltham, 1993) and (Collee, Duguid, Fraser, & Marmion, 1996). *S. aureus* isolates were recovered from different sites of infections. *S. aureus* ATCC 25923 was used as control strain in all procedures.

2.2. Testing isolates for methicillin resistance

Standard disk diffusion was done using oxacillin 1µg to differentiate MRSA from MSSA strains, all isolates that gives less than 10 mm in zone diameter were considered as MRSA (CLSI, 2006). *S. aureus* ATCC 25923 was used as control strain in all procedures.

2.3. Molecular detection of mecA and PVL genes

2.3.1. DNA extraction

DNA extraction was done using Qiagen DNeasy kit (69504). Pretreatment of bacterial cells was done according to the manufacturer instructions.

2.3.2. PCR amplification

The specific primers used in this study were synthesized and purchased from Metabion International-Germany. The oxacillin disk diffusion result was confirmed by detection of mecA gene by PCR, the primers used was mecA-1 (5-AAA ATC GAT GGT AAA GGT TGG C-3), mecA-2 (5-AGT TCT GCA GTA CCG GAT TTG C-3). The amplification was performed as described previously (Strommenger, Kettlitz, Werner, & Witte, 2003).

Also a single PCR assay targeting *Staphylococcus aureus* species specific *lukS*/*F-PV* gene (a determinant of leukotoxin) was performed, the primers used was *luk-PV*-1, (5-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3) and *luk-PV*-2, (5-GCA TCA ACT GTA TTG GAT AGC AAA AGC-3) (Lina et al., 1999). The amplifications were performed as described by Lina *et al*., (1999).

The PCR products were analyzed on a 1.5% agarose gel. The gel electrophoresis was performed at 80V for 45 min. and the analysis was done by using an automated gel photo documentation system.

2.3.3. DNA sequencing

DNA purification and standard sequencing was performed for both strands of PVL genes by Macrogen Company (Seoul, Korea).

2.3.4. In Silico analysis

Sequence similarity was performed using the public Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.-gov/blast/Blast.cgi). Using BioEdit (Hall, 1999) software the obtained sequences of PVL genes were compared by multiple alignments with control sequence (ATCC 49775, GeneBank accession NO AB006796) and those of other strains (Egypt (FJ821791), USA (EF571829), China (AB678712), and UK (EF571788) acquired from GeneBank (http://www.ncbi.nlm.-nih.gov/). In GeneMarks version 4.25 (http:/exon.gatech.edu/genemark/genemarks.cgi) the gene sequence was translated into amino acid sequence (Besemer, Lomsadze, & Borodovsky, 2001). The stability of protein was tested by using I-mutant version 3. Phyre2 soft ware had been used to predict the protein secondary structure (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015). Mutation analysis and protein tertiary structure was done online by project hope software (http://www.cmbi.ru.nl/hope/report/2064?10) (Venselaar, Te Beek, Kuipers, Hekkelman, & Vriend, 2010).

3. Results

Isolates from different sites of infection were PVL positive. Five of them were MRSA and seven isolates were MSSA (Table1).

3.1. PCR results

PCR results revealed typical bands for *mecA* gene (532bp) for MRSA isolates, and typical (433bp) for PVL gene (Figure 1).

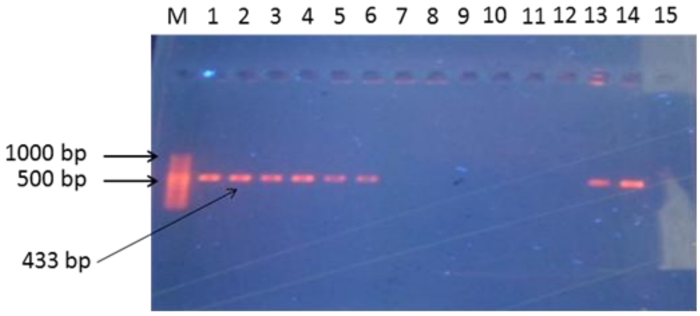

Figure 1. PCR for detection of lukS/F-PV, M: 100 bp molecular ladder, lane 1: positive control, lane 15: negative control, lane 2, 3, 4, 5, 6, 13 and 14 typical PVL positive isolates, lane: 7, 9, 10, 11 and 12 were PVL negative isolates.

Table 1. Characteristics of mutated *LukS-PV* positive isolates

Isolate No.	Source	Site of infection	*mecA*	PVL
12	Community	Paronychia	-	+
49	Hospital	Skin abscess	-	+
90	Community	Wound	-	+
116	Hospital	Osteomyelitis	+	+
120	Community	Wound	+	+
129	Hospital	Osteomyelitis	-	+
146	Community	Skin abscess	+	+
154	Community	Skin abscess	-	+
160	Community	Throat infection	-	+
171	Community	Ear infection	+	+
172	Hospital	Wound	+	+
198	Community	Pneumonia	-	+

3.2. In Silico Analysis

The nucleotide sequences of *LukS-PV* of the twelve isolates showed 99% identity when compared with other sequences obtained from Gen-Bank using BLAST nucleotide algorithm.

Multiple nucleotide sequence alignment revealed single nucleotide polymorphism at position 610 (AAT / GAT) when compared to reference sequence. The mutation had occurred in *lukS-PV* region in the twelve isolates. This mutation resulted in amino acid change from

Asparagine to Aspartic acid (N→D) at position 204 of *LukS-PV* gene, when compared with the control and other selected sequences from data base (Figure 2).

3.3. Protein secondary structure

Prediction of the secondary structure showed that the control containing 9% alpha helix, 58% beta strand and 34% disordered, while the mutated protein containing 8% alpha helix, 59% beta strand and 34% disordered (Figure 3A and B).

3.4. Protein tertiary structure

Project Hope software was used to predict the protein 3D structure as shown in figure 3-C. It revealed several differences in the structure and charge between the mutated and the wild-type protein (Table 2).

3.5. Physiochemical analysis

The result of I-mutant 3 demonstrated that there is a decrease in the stability of the protein due to mutation.

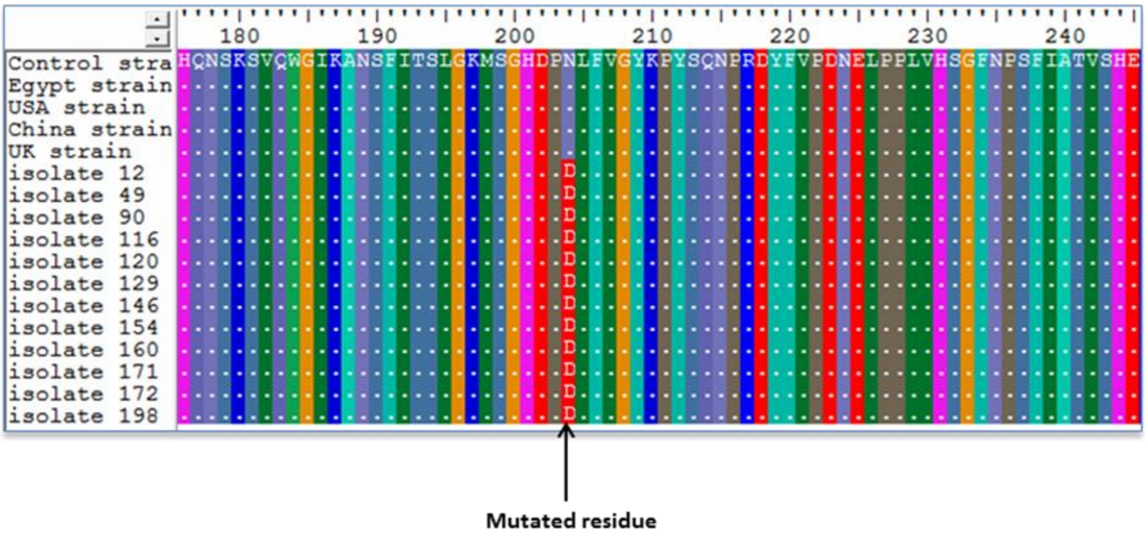

Figure 2. Amino acids sequence alignment of mutated *lukS-PV* (204 N / D) compared with the control strain (wild type) and other sequence obtained from GeneBank.

Table 2. Comparison of wild type and mutated *lukS-PV* residue as analyzed by Project Hope and I-mutant software.

Position of mutation	Feature	Control residue	Mutant residue
Position 204, Asparagine to Aspartic acid (N→D)	Charge Stability Structure Hydropathy class	Neutral stable Located in α-helix Hydrophilic	Negatively charged Decrease stability Located in disordered region Hydrophilic

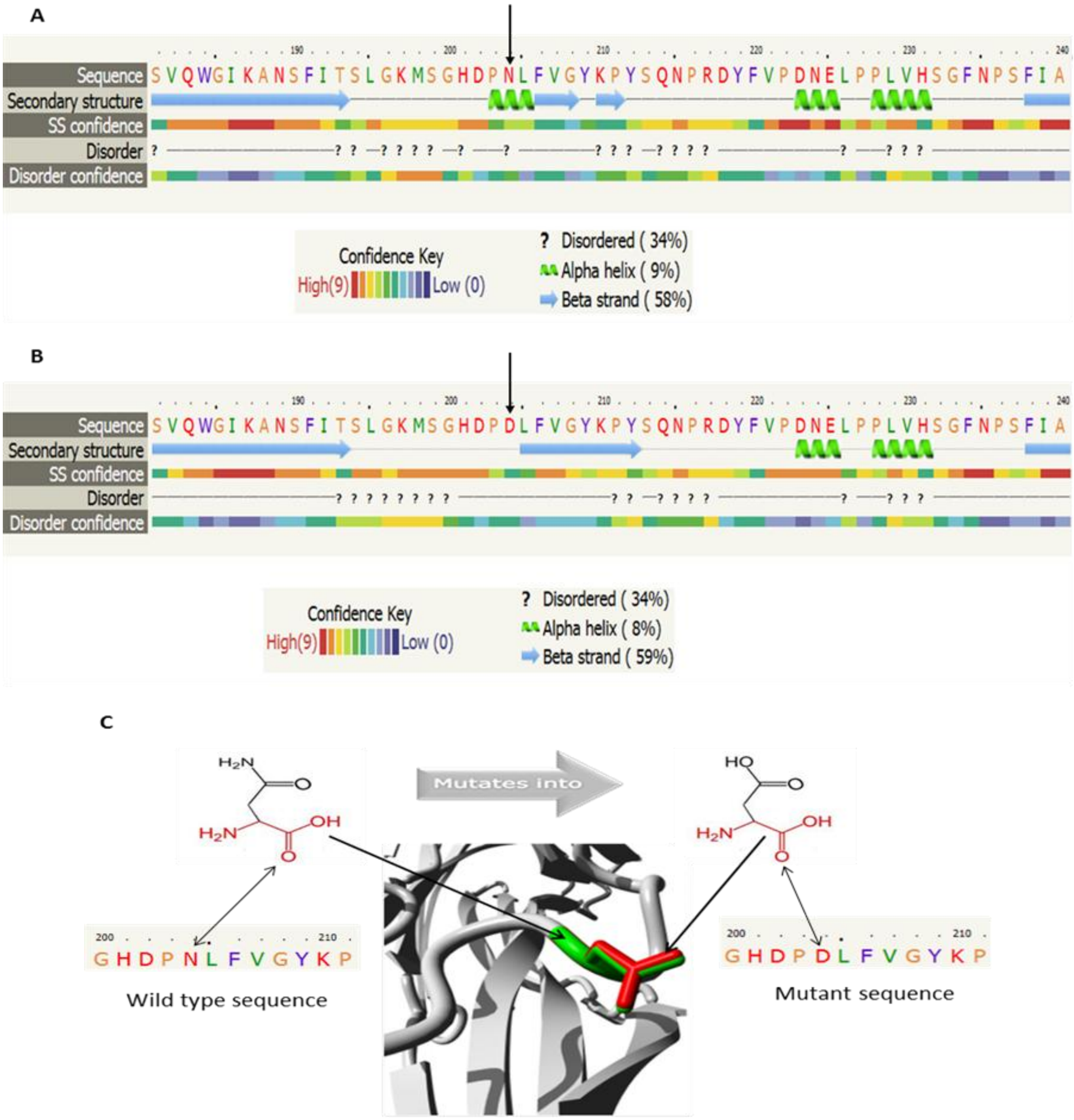

Figure 3. A. *lukS-PV* secondary structure of wild type strain. **B**. *lukS-PV* secondary structure of mutated isolates from Sudan. **C**. Predicted 3D structure showed the position of mutation as done by Project Hope software.

4. Discussion

Different types of point mutation were detected in PVL gens as described by several previous studies (O'Hara et al., 2008). The novel mutation detected in this study, was observed in isolates from different sources (community & hospital) and with different properties (MSSA & MRSA). Homology of Blast algorithm tool and the multiple sequence alignment revealed a single nucleotide substitution (610 A / G). The translation of amino acids sequences showed a substitution of the amino acid Asparagine in the wild type residue to Aspartic acid in our mutant residue. This residue is part of an interpro domain named Lekocidin/portin (IPR016183). The residue is buried in the core of a domain. Slightly differences were observed in the predicted secondary structure of *LukS-PV* of wild type and mutant isolates. The Asparagine is located in the alpha helix region of wild type strains while the Aspartic acid of mutant isolates is located before the beta strand. The predicted 3D structure obtained from Project Hope software revealed difference in the site chain of Asparagine and Aspartic acid. The wild-type residue is neutral while the mutant residue is negatively charged. I-mutant result showed that the mutant residue is less stable than wild-type, this may be due to that the mutation site is buried in a core of the domain. This detected single mutation may be geographically dependant, as it only detected in the local strains rather than the control strain. On the other hand the variation of amino acid detected in the current study has no correlation with the presence of *mecA* gene this is because seven isolates out of twelve were *mecA* negative. O'Hara *et al*, mentioned that variation at amino acid 176 correlates strongly with the geography of the isolate harboring the PVL sequence and is also correlated with the presence of *mecA*. (O'Hara et al., 2008). However the detection of such new variant of PVL gene may be important as it can be used as a diagnostic value for such strains.

Conclusion

The detected novel mutation in *LukS-PV* gene may slightly affect the stability of the protein. Using molecular approach and bioinformatics tools including protein modeling we revealed a- new variant of *LukS-PV* gene which may have a chance in the future research.

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Conflicts of interest

Authors have no conflict of interest.

Refereces

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