Partial Sequencing of IS*1216V* Transposase Gene of *Staphylococcus Aureus* Isolated from Food Samples

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ABSTRACT

Background: Insertion sequence is a short DNA sequence encode for proteins implicated in the transposition activity. Transposase catalyzes the enzymatic reaction allowing the insertion sequence to +9*lo2 move. ;qqa;.

Objective: To study the sequencing of transposase gene, *tnp*, IS*1216V* of *S. aureus* isolated from food and then compared with that documented in National Center for Biotechnology Information (NCBI).

Methods: Food samples of animal and plant origin were collected, and screened for presence of *S. aureus*, IS*1216V* was identified in the Tn*1546*-like elements in the genomes of all *Staphylococcus aureus* isolates.

Results: About 75% of total food samples were positive to *S. aureus* especially in the food of animal origin. *tnp* amplification showed that, 85% of isolates gave positive result. Sequencing of amplified part of IS*1216V tnp* of *S. aureus* isolates showed that, *tnp* gene had high identity (78-79%) with the reference strains of NCBI.

Conclusion: High percentage of local food samples were contaminated with *S. aureus* especially of animal origin. Most of the *S. aureus* isolates showed the presence of transposase gene (*tnp*) of IS*1216V*. Sequencing showed some dissimilarity between the sequence of transposase gene (*tnp*) of IS*1216V S. aureus* isolated from local foods and strains recorded in database of NCBI.

Key Words: IS*1216V, Staphylococcus aureus*, transposase gene

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INTRODUCTION

Insertion sequence (IS) is a short DNA sequence that acts as a simple transposable element. They are small as compare with other transposable elements, around 700 to 2500 bp in length and only code for proteins implicated in the transposition activity. These proteins are usually the transposase which catalyzes the enzymatic reaction allowing the IS to move, and a regulatory protein which either stimulates or inhibits the transposition activity. The coding region in an IS usually flanked by inverted repeats[(1,2,3)].

IS*1216V* is known to be ubiquitous in *vanA* (Vancomycin resistance) gene. The types of *vanA* gene were identified previously according to the distributions of IS and found that, IS*1216V* and IS*1251* were identified in the Tn*1546*-like elements in the genomes of all *S. aureus* isolates from Sulaimani hospital [(4)] . IS known as IS*1216* were the most frequently detected insertion sequence within Tn*1546*. IS*1216V* has been extensively found among transposons containing *vanA* in enterococci [(5)] such transposons responsible for antibiotic resistance transmission. The goal of the present study was to amplify and sequence a part of transposase gene, *tnp*, of IS1216V from *S. aureus* isolated from food.

METHODS

Twenty eight food swabs were collected from different types of food, 8 were from chicken meat samples, 9 from beef, one from cheese, one from cream, 2 from milk, 5 from homemade soup and 2 from banan (Table 1). Swabs were streaked on Mannitol salt agar and the colonies that appeared on it were identified as *Staphylococcus aureus* according to Forbes *et al.*, (2002) [6] by biochemical tests.

The obtained isolates were subjected to amplification by polymerase chain reaction (PCR) technique to amplify part of transposase gene (*tnp*) of IS*1216V* using. Direct colony was used to obtain whole genomic DNA that serve as template for PCR. Single primer, GCGGATCCGGTTCTGTTGCAAAGTTT

(forward and reverse) and the amplification protocol consisted of one cycle 2 min 94°C followed by 34 cycle of 1 min 94°C, 1min 45°C and 1 min 72°C, final extension was 2 min 72°C. These primer and protocol were adapted according to Walczak *et al.*, (2005) [7] . The amplified product was electrophoresed according to Sambrook and Russell (2001) [8]. Three of PCR products were sequenced by sending the products to NICEM Company, USA. The results were analyzed using genius software according to national center for biotechnology information (NCBI).

RESULTS

Twenty one isolates of *Staphylococcus aureus* were identified (75% from total food samples were positive) and they were as follows: 7 isolates, 87.5% from chicken meat samples; 8 isolates, 88.9% from beef; one isolate, 100% from cheese sample; one isolate, 100% from cream sample; 2 isolates, 100% from milk samples; one isolate, 20% from homemade soup samples and one isolate, 50% from banana samples (Table 1).

Table 1: Number and Percentage of *Staphylococcus aureus* Isolated from Food Samples.

Food sample	No of samples tested (%)	*S. aureus* Positive (%)	*S. aureus* Negative (%)
chicken meat	8 (28.6)	7 (87.5)	1 (12.5)
Local beef	9 (32.1)	8 (88.9)	1 (11.1)
Local cheese	1 (3.6)	1 (100)	0 (0)
Local cream	1 (3.6)	1 (100)	0 (0)
Local Milk	2 (7.1)	2 (100)	0 (0)
Homemade Soup	5 (17.9)	1 (20)	4 (80)
Banana	2 (7.1)	1 (50)	1 (50)
Total	28 (100)	21(75)	7 (25)

Out of 20 *S. aureus* isolates that subjected to PCR, 17(85%) isolates gave positive result to *tnp* amplification, the amplicon size was about 200bp (figure 1).

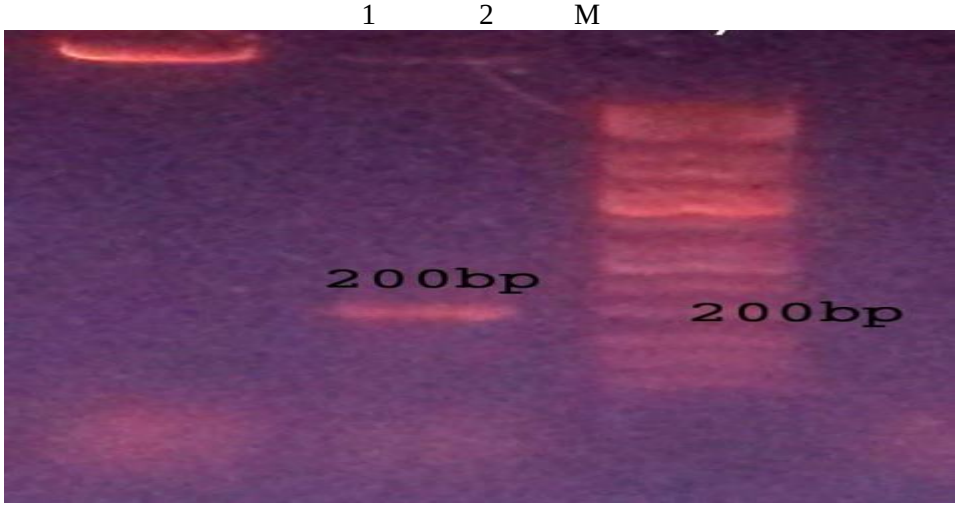

Figure 1: Electrophereticogram of amplified part of *tnp* gene of IS*1216V* of *S. aureus*. M:100bp ladder, 1,2 lanes are negative and positive results respectively. The electrophoresis was done by 1.5% agarose gel at 7V/cm for 90 minutes.

Sequencing of amplified part of *tnp*, of IS*1216V* for three randomly selected *S. aureus* isolates showed that, two amplified products of *tnp* gene were identical to 78% of that of strain: PM1(accession no. AB699882.1)

the third was identical to 79% of that of strain: PM1. The third was also showed 79% identity to *tnp* gene of *S. aureus* subsp. *aureus* M013 (accession no. CP003166.1) as showed in figures 2, and 3.

Figure 2: Sequence and identity a part of *S. aureus tnp* gene of this study and that of PM1 strain as reported in NCBI (accession no. AB699882.1).

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Query 33 AGCTGGAGTTCCCGATGATACCTTGTTTTCAGGAAA-ACCTTACAGCCGGAGAGGGTGTC91
          ||||||  ||||| || |||||||| |||||| | | |||  | | ||| |||| ||| |
Sbjct 58 AGCTGGGATTCCCAATAATACCTTGATTTCAGTACAGACCGAAAACCCGAAGAGAGTGCC 117

Query 92TGCTTTTCGGGTTTTCTTATATACTCCACGAACGGCTCCCTTGCGATTAATCGAGGTCGA 151
         | ||||||||||||||||||||| ||| |||| |||| || |||  ||||||| ||| ||
Sbjct 118 TTCTTTTCGGGTTTTCTTATATAATCCTCGAATGGCTTCCATGCCTTTAATCGTGGTAGA 177

Query  152  GGCTGAGCGGAGACTTCGATGTTAACTTATTCGCGCCTCT  191
             ||| | ||| | ||||||||   || ||||| ||| ||||
Sbjct  178  GGCAGTGCGTAAACTTCGAT-AGAATTTATT-GCGTCTCT  215

Query 34 AGCTGGGAAGAACGATAATAACTTGTTTTCAGTGAACACTTAACAGCCGAGGAGAGTGTC 93
           ||||||||    | |||||| |||| |||||||  | ||  || | |||| ||||||| |
Sbjct 58 AGCTGGGATTCCCAATAATACCTTGATTTCAGTACAGACCGAAAACCCGAAGAGAGTGCC 117

Query 94 GTCTCTTCGGGTTTTCTTATATACTCCACGAACGGCTTCCATGCCATTAATCGTGGGATA 153
           ||| |||||||||||||||| ||| |||| |||||||||||| |||||||||| | |
Sbjct 118 TTCTTTTCGGGTTTTCTTATATAATCCTCGAATGGCTTCCATGCCTTTAATCGTGGTAGA 177

Query  154  TAGCTGTGCGGGAACTTCGATATTAACTTATTGCAGTCTTTTTATTGGA  202
            || |||||  |||||||||||  || ||||||| |||| |||| ||||
Sbjct  178  -GGCAGTGCGTAAACTTCGATA-GAATTTATTGC-GTCTCTTTACTGGA  223
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Figure 3: Sequence and identity a part of third *S. aureus tnp* gene of this study and that of PM1 strain as reported in NCBI (accession no. AB699882.1).

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Query 34 AGCTGGGAAGAACGATAATAACTTGTTTTCAGTGAACACTTAACAGCCGAGGAGAGTGTC 93
         ||||||||    | |||||| |||| |||||||  | ||  || | |||| ||||||| |
Sbjct 26 AGCTGGGATTCCCAATAATACCTTGATTTCAGTACAGACCGAAAACCCGAAGAGAGTGCC 26

Query 94 GTCTCTTCGGGTTTTCTTATATACTCCACGAACGGCTTCCATGCCATTAATCGTGGGATA 153
        ||| | |||||||||||||||| ||| |||| |||||||||||| |||||||||| | |
Sbjct 94 TTCTTTCCGGGTTTTCTTATATAATCCTCGAATGGCTTCCATGCCTTTAATCGTGGTAGA 53

Query 154    TAGCTGTGCGGGAACTTCGATATTAACTTATTGCAGTCTTTTTATTGGA  202
                || |||||  |||||||||||  || ||||||| |||| |||| ||||
Sbjct 54  -GGCAGTGCGTAAACTTCGATA-GAATTTATTGC-GTCTCTTTACTGGA  2643899
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DISCUSSION

The results showed a high percentage of local food samples were contaminated with *S. aureus* (75%) especially food of animal origin (chicken, beef, cheese, cream and milk) that showed the highest percentages(Table-1), which suggest the origin of *S. aureus* was from animals in this study. *Staphylococcus aureus* is main cause of food poisoning [9], and from this point of view, these sources of food contamination can be considered important sources of food poisoning.

Transposase gene (*tnp*) of IS*1216V* of food *S. aureus* isolates was partially amplified. Most of the isolates showed the presence of such gene. It was previously showed the presence of such gene as a part of IS*1216V* in *S. aureus* isolates [10] as well as other insertion sequences like IS*S1*[7]. The results obtained in this study were resembled to that of Clark *et al* (2005)[11] who found that an IS*1216V*-like element inserted before nucleotide 3099 of Tn*1546* and an IS*1216V* encoding multidrug resistance and originating in enterococci, had emerged in *S. aureus* (strain PM1) in Taiwan. Mohammed and Khder (2011) [4] from Sulaimani , in Iraq showed that IS*1216V* and IS*1251* were identified in the genomes of all isolates from Sulaimani inserted within the *vanA* gene, the gene responsible for antibiotic resistance.

Sequencing of some amplified products showed that, there is dissimilarity between the sequence of transposase gene (*tnp*) of IS*1216V* of isolated *S. aureus* from foods and that of recorded *S. aureus* in database of NCBI. The similarity was reached to 78-79%, however, the dissimilarity was noticed among the sequences of transposase gene (*tnp*) of IS*1216V* of *S. aureus* strains in database of NCBI itself (data not showed). The dissimilarity of transposase gene (*tnp*) of *S. aureus* isolated from food samples as compare with that of database represent the diversity in gene sequences among transposase genes of *S. aureus* of any source as well as of any geographic distribution.

In conclusion, high percentage of local food samples were contaminated with *S. aureus* especially of animal origin. Most of the *S. aureus* isolates showed the presence of transposase gene (*tnp*) of IS*1216V*. Sequencing showed some dissimilarity between the sequence of transposase gene (*tnp*) of IS*1216V* of isolated *S. aureus* from local foods and strains recorded in database of NCBI.

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