Partial Sequencing of IS1216V Transposase Gene of Staphylococcus Aureus Isolated from Food Samples

Ibtisam G. Aud 2Yusra M. Mohsin 3Enas I. Jasim, 4Duaa S. Shawkat, 5Zainab H. Sharhan, 6Jameelah G. Oudah

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*l02 move :qqaa.. Objective: To study the sequencing of transposase gene, tnp, IS1216V 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, IS1216V was identified in the Tn1546-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 IS1216V tnp of S. aureus isolates showed that, tnp gene had high identity (78-79%) with the reference strains of NCBI.

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).

IS1216V 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, IS1216V and IS251 were identified in the Tn1546-like elements in the genomes of all S. aureus isolates from Sulaimani hospital (6). IS known as IS1216 were the most frequently detected insertion sequence within Tn1546. IS1216V 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) by biochemical tests.

The obtained isolates were subjected to amplification by polymerase chain reaction (PCR) technique to amplify part of transposase gene (tnp) of IS1216V using. Direct colony was used to obtain whole genomic DNA that serve as template for PCR. Single primer, GCGGATCCGGTTCTGTTGCAAAGTTT

www.jkmc.uobaghdad.edu.iq 24 Al-kindyy College Medical Journal 2018:14 No.2
(forward and reverse) and the amplification protocol consisted of one cycle 2 min 94°C followed by 34 cycle of 1 min 94°C, 1 min 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).

| 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 IS1216V 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 IS1216V 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).

![Sequence alignment](image)

**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).

![Sequence alignment](image)

**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, and from this point of view, these sources of food contamination can be considered important sources of food poisoning.
Transposase gene (tnp) of IS1216V 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 IS1216V in S. aureus isolates as well as other insertion sequences like ISS7. The results obtained in this study were resembled to that of Clark et al (2005) who found that an IS1216V-like element inserted before nucleotide 3099 of Tn1546 and an IS1216V encoding multidrug resistance and originating in enterococci, had emerged in S. aureus (strain PM1) in Taiwan. Mohammed and Khder (2011) from Sulaimani, in Iraq showed that IS1216V and IS1251 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 IS1216V 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 IS1216V 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 IS1216V. Sequencing showed some dissimilarity between the sequence of transposase gene (tnp) of IS1216V of isolated S. aureus from local foods and strains recorded in database of NCBI.

REFERENCES

1. Campbell, Neil A. and Reece, Jane B. Biology (6th ed.). San Francisco: Benjamin Cummings. (2002). pp. 345–346

2. Siguer, P. Gourbeyre, E. Chandler, M. Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol. Review. 2014; 38(5): 865–891.

3. Opinnen, T. and Camilli, A. Transposon insertion sequencing: a new tool for systems-level analysis of microorganisms. Nature. Review. Microbiol. 2013; 11:435–442.

4. Mohammed, A.D. and Khder, S.D. Distribution of insertion sequences in Tn1546 element in vancomycin-resistant Staphylococcus aureus in Sulaimani, Kurdistan of Iraq. J. Comput. Biol. Bioinf. Res. 2011; 3(4): 42–46.

5. Novais, C. Ana R. Freitas, A.F. Sousa, J. C. Fernando Baquero, F. Coque, T. M. and Peixe, L. V. Diversity of Tn1546 and Its Role in the Dissemination of Vancomycin-Resistant Enterococci in Portugal. Antimicrob. Agent. Chemother. 2008; 52(3): 1001–1008.

6. Forbes, B.A.; Sahm, D.F.; and Weissfeld, A.S. Diagnostic Microbiology, 10th ed. Mosby, USA 2002.

7. Walczak, P.; Konopacka, M. and Otlewska, A. Genetic diversity among lactococcus sp and Leuconostoc sp strains using PCR-RFLP of insertion sequence ISS1-type, IS904 and IS982. Polish J. Microbiol. 2005. 54(3):183-189.

8. Sambrook, J.; and Russell, D. Molecular Cloning: Laboratory Manual. 3rd ed. Cold Spring Harbor, New York USA. 2001.

9. Argudin, M. A. Mendoza, M. C. and Rodicio, M. R. Food Poisoning and Staphylococcus aureus Enterotoxins. Toxins. 2010; 2(7): 1751–1773.

10. Sawanobori, E., Hung, W., Takano, T., Hachuda, K., Horiuchi, T., Higuchi, W., Hung, W., Iwao, Y., Nishiyama, A., Reva, L., Reva, G. and Lee, J. Emergence of Panton-Valentine leukocidin-positive ST59 methicillin-susceptible Staphylococcus aureus with high cytolytic peptide expression in association with community-acquired pediatric osteomyelitis complicated by pulmonary embolism. J Microbiol. Immun. Infect. 2015; 48(5): 565–573.

11. Clark, N. C., Linda M. W., Jean B. P. and Fred C. T. Comparison of Tn1546-Like Elements in Vancomycin-Resistant Staphylococcus aureus Isolates from Michigan and Pennsylvania. Antimicrob Agents Chemother. 2005; 49(1): 470–472.