

A Review on PCR Method for Detection of *Staphylococcus aureus* Enterotoxins

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Abstract:

Foodborne intoxications (staphylococcal food poisoning) is mediated by Staphylococcal enterotoxins and cause an extensive variety of diseases. To date, more than 25 superantigen (Sags) have been designated in *Staphylococcus aureus* (*S. aureus*) strains. Outbreaks of staphylococcal food poisoning are brought on by SEs generated by some *S. aureus* strains. The most frequent side effects brought on by eating SEs in food include vomiting, diarrhoea, nausea and cramps. High prevalence of a particular type of enterotoxin may differ from region to region. Hence, finding and identifying SEs quickly and properly is extremely difficult and critical. The molecular biological analysis based on PCR is dependable, offers results much more quickly, and is sensitive and accurate. Here, a thorough literature review has been used to present a PCR based overview of SEs detection.

Key words: *Staphylococcus aureus* enterotoxins, staphylococcal enterotoxins food borne diarrhoea, staphylococcal enterotoxins PCR.

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I. Introduction

Staphylococcus aureus (*S. aureus*) exotoxins are responsible for tissue damage and many functions as superantigens promoting the commencement of shock-like syndromes¹. Staphylococcal enterotoxins (SEs) cause staphylococcal food poisoning, whereas TSST-1 and ETs are responsible for toxic shock syndrome (TSS) and staphylococcal scalded-skin syndrome (SSSS), respectively. TSST-1 and SEs belong to the superantigen (SAG) family. To date, more than 25 SAGs have been described in *S. aureus* strains². A minimum of 80% of clinical strains of *S. aureus* harbor at least one superantigen³. Numerous types of staphylococcal enterotoxins have been reported, including: A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, V, W, X, Y and Z and their allied genes are *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *she*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, *ser*, *ses*, *set*, *selu*, *selu2*, *selw*, *selx*, *sely* and *selz* respectively^{4,5,6}. All SEs are similar in structure and function, but they differ in their antigenic properties⁷. *S. aureus* produces a number of cytotoxic molecules, which can be divided into four classes which include the four hemolysins (alpha, beta, delta and gamma) and Panton-Valentine Leukocidin (PVL), also known as PV-leukocidin¹. It is encoded by two genes, *lukS-PV* and *lukF-PV*⁸. These toxins form pores in the membrane of host defence cells by the synergistic action of two secretory proteins, designated *LukS-PV* and *LukF-PV*⁹. It is usually linked to complicated skin and soft tissue infections and was found in a majority of community associated MRSA isolates and infrequently present in hospital isolates^{10,11}.

If SEs are created in a pure form that may be utilised as a purposeful adulterant, they pose a threat to both food safety and food security. A brief summary of the PCR-based techniques used to identify SE in food and medical sources is given in this paper. The discussion of conclusions concludes.

II. Material and Methods

The search for data in this review article was carried out by collecting numerous literatures of primary data in the form of international journals. Using online media through websites such as Academic Journals, ResearchGate, ScienceDirect, National Library of Medicine (NLM), PubMed, and the Journal toxins by using the keywords "*Staphylococcus aureus* enterotoxin", "staphylococcal enterotoxin food borne diarrhoea", "staphylococcal enterotoxin PCR".

III. Prevalence of Food-Borne Poisoning Associated Diarrhoea and SEs

The second most frequently reported types of food-borne infections are staphylococcal diseases, which are contracted from consuming enterotoxin-contaminated food. The inadequate pasteurization/decontamination

of the initially contaminated product source is to blame for the high staphylococcal food poisoning incidence¹². Food poisoning was caused, according to epidemiological research, by sweet meat (coconut balls) contaminated with *S. aureus*¹³. The findings of a study on a food poisoning outbreak in an army unit with 42 cases suggested that *S. aureus* toxin production was the cause of the outbreak. This could be linked to a slowdown in the meat's preparation¹⁴. As the toxicity of the toxin has already been preformed, the sickness has a brief incubation period that lasts only a few minutes to a few hours. Nausea, vomiting, cramps, diarrhoea, and stomach pain are among symptoms. In the USA, SEA is thought to be responsible for about 80% of food poisoning outbreak cases, whereas SEB is responsible for 10% of the cases^{15,16}. Around 64% of *S. aureus* isolates tested positive for enterotoxin genes, according to a study done in Iran. The seb gene was present in 18.5% of the strains in that study, and the sea gene was present in 45.2% of them. Just 3.9% of the strains tested positive for tst¹⁷. Animals with mastitis are by far the most likely to isolate SEC as a toxin^{18,19,20,21}. Based on their biological and serological properties, new enterotoxins (SEG-SEQ and SER-SEU) were the culprit in 5% of cases^{22,23}.

IV. SEs Detection Using Polymerase Chain Reaction

Techniques used in molecular biology include polymerase chain reaction (PCR) and nucleic acid hybridization. By employing gene-specific nucleotide sequences as probes, nucleic acid hybridization techniques have been used as a supplementary method of evaluating strains carrying toxin genes. The discovery that oligonucleotide probes targeting several enterotoxins genes would enable accurate detection and separation of toxigenic *S. aureus* isolates by colony blot hybridization was initially made by Gemski and coworkers²⁴. Moreover, PCR has been extensively utilised to identify SEs by amplifying the relevant genes²⁵. Wilson and coworkers employed two sets of primers to amplify the staphylococcal nuclease gene (nuc) and the SEB and SEC genes (entB and entC1), to identify SEs by PCR for the first time²⁶. New variations of seG and seI in *S. aureus* AB-8802 were discovered by PCR analysis of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products²⁷. Presently, a number of PCR versions, including multiplex PCR, real-time, and reverse-transcriptase PCR, have been developed to identify SEs^{28,29,30,31,32,33}. Real-time PCR has been shown to be a quick and accurate identification method for finding enterotoxin gene clusters in raw milk sources²³. Typically, PVL is employed as a marker for community-acquired MRSA, which is responsible for deep skin infections, including soft tissue infections. In India, a RT-PCR based study was conducted and the result revealed that 9% of the CA-MRSA isolates were positive for *pvl* gene³⁴. In a research using clinical samples directly, the Triplex Real Time PCR assay's sensitivity and specificity were both hundred percent³⁵. Another study used *S. aureus* clinical isolates to quickly detect PVL on bacterial suspensions³⁶. Multiplex PCR has the particular benefit over other PCR-based methods in that it can simultaneously detect several SEs using various primers. The multiplex PCR was expected to identify up to 10 genes with high specificity, dependability, and efficiency³⁷. For the purpose of detecting pyrogenic exotoxin genes in staphylococcal isolates, Lovseth et al. (2004), created a modified multiplex PCR³⁸. Another multiplex PCR assay for all of the identified enterotoxin genes (sea-sej) and *tst* has been developed by Monday and Bohach (1999), although this again requires special primer sets for detecting specific genes³⁹. A novel multiplex PCR was developed for the identification of 19 SAg genes of *S. aureus* from raw meat isolates in Korea⁴⁰. In a single-reaction multiplex PCR, Jeyasekaran et al. (2011), demonstrated that the MPCR technique may be utilised to simultaneously detect *S. aureus* enterotoxin C-producing strains from clinical and environmental samples.

V. Conclusion

SEs are part of a wide family of structurally and functionally similar bacterial exotoxins generated by staphylococci and streptococci. Traditional SEs detection techniques, similar animal tests and serologic tests, require a lot of labour and fall short of real-time detection requirements. A dependable, sensitive, accurate and significantly quicker technique with definitive results is PCR-based molecular biological analysis.

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