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The Study Focuses on the Molecular Identification of MRSA Stains in Clinical *Staphylococcus* Aureus Isolated, Which Produce Enterotoxin Using ISSR Markers

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Annotation

Staphylococcus aureus were isolated from clinical cases of human skin infections and subjected to a molecular study. Genomic DNA extracted Commercial kit method and the yield was observed; it ranged from 240-490 ng/ml. This method gave an A260/A280 ratio approximately 1.7 of DNA quality. This research approaches to investigate the genetic diversity of the methicillin resistant Staphylococcus aureus strains that are clinically collected by performing ISSR PCR technique The purpose of this work is to use ISSR markers to identify and isolate S. aureus from various sources of wounds and gastrointestinal infections, and to examine the genetic diversity of these isolates. DNA was extracted from 20 samples that were collected from 5 isolates of S. aureus. The isolates were described based on their physical and biochemical characteristics. When 9primers from 01primers were used to create ISSR9, five unique bands were produced. The dendrogram of ISSR was reversed, and isolates 2 and 3 had the highest genetic diversity (0.0.987), while isolates 5 and 4 had the lowest genetic similarity (0.065), in contrast to other isolates with high genetic diversity.

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Introduction

It is known that staphylococcal enterotoxins exist. Staphylococcal food poisoning syndrome is one sort of poisoning agent, but they may also be involved in other diseases that cause shock in both people and animals. Staphylococcal enterotoxin A (SEA), SEB, SEC, SED, and SEE are the five toxin categories that have been identified serologically. SEC1, SEC2, and SEC3 are the new divisions resulting from minor variances within the SEC group. Immunological determinants are shared by streptococcal pyrogenic exotoxin A (SPEA), SEB, SEC1, and SEE [1].

Exoproteins known as staphylococcal enterotoxins (SEs) may result in toxin shocks and cause the gastro-enteric syndrome in humans when consumed. As diarrhoea and vomiting are common symptoms of Staphylococcal food poisoning outbreaks, which happen often around the globe, intestinal toxin detection is epidemiological [2]. Due to their similar shapes and biological activity, SEs are pyrogenic toxin superantigens that cause emesis[3].

Heating these proteins (MW: 35 kDa) to 98 ˚C for 15–30 minutes does not inactivate them (Kayser et al., 2005, p232). [4,5]. Recent years have seen the application of various molecular techniques to Investigate S. aureus' genomic diversity and the potential for parallels and differences. One such approach is (ISSR) analysis, which may be used to study genetic diversity in a large number of different strains of microorganism. It utilises less DNA and costs less. [6].

..Additionally, ISSR analysis is widely used in teaching to determine the variety of genes by analysing changes discovered in the area between microsatellitesIt has been used to identify clonal variations, fingerprint genes, and tag genes.

[7]. It has been proposed that this technique, which involves amplifying a DNA segment between two identical microsatellite repeat regions by inserting an orientalist in the opposite direction with a suitable distance using the ISSR method, produces more complicated marker patterns.Furthermore, ISSR primers are designed to anneal at higher temperatures to microsatellite sequences, making ISSR procedures more reproducible than other methods. Furthermore, it has been shown that the multilocus fingerprinting profile acquired by ISSR is a useful approach.

inexpensive, easy to use, very stable, and plentiful [8].The study's objective polymorphisms and the uniquer band seen in S. aureus isolates.

Materials and Methods.

S.aureus isolates.

Table 1 shows five Staphylococcus aureus clinical isolates that were evaluated for microscopic features and biochemical composition. They were then cultivated on nutrient agar and utilised to harvest DNA.

Genomic DNA extraction

Using commercial kits and small-scale procedures, the DNA was extracted (Bionner-Korea). Using a spectrophotometer, the optical density was used to evaluate DNA purity. DNA quality was assessed using UV light and ethidium bromide electrophoresis on an agar gel [9].

Molecular Analysis ISSR assay

The six ISSR primers used were lyophilised and purchased from Pioneer in Korea. They were then dissolved in sterile distilled water, yielding a final concentration of 10 pmol/ml [10]. Providers suggested utilising the primer employed in this study, which is stated in (table-1).

The final concentrations were carried out in a 25µl volume. The following programs were utilised in PCR runs for the ISSR assay: There are 40 cycles total between the first denaturations and the final extensions. The ISSR programs are shown in the following table: To guarantee repeatability, each PCR amplification reaction was performed twice. The result was then examined by electrophoresis in 1.5% agar gels using stain ethidium bromide 0.5µl at 5 vt/cm for two hours.

Data analysis

Estimating the molecular weight

Molecular weight was calculated using the Photo-Capture M.W. application on computers based on a comparison of the RAPD-PCR and ISSR-PCR results rely on the number of bands and molecular weight of bands in a 2000 bp DNA ladder Bioneer (which has 13 bands ranging from 100 to 2000 bp).

Estimate polymorphism, efficiency, and discriminatory power.

Generate data on molecular weight The commercial soft word was scored for each band on the molecular size of the RAPD and ISSR marker results bands (1 for presence, 0 for absence) [11]. Only bands with a steady increase in volume received a score.

The following formula was used to determine each primer's polymorphism:

Polymorphism % = (Np / Nt) × 100

- Nt is the total number of bands for a single primer, while Np is the number of polymorphic bands for random primers. The efficiency and discriminating power of each primer were determined using the following method:

To measure efficiency, divide the total number of bands to the same primers by the number of polymorphic bands to each primer. To calculate discriminating powers, divide the number of polymorphic bands for each primer by the total number of polymorphic bands for all primers (X100%).

Primer efficacy ranged from zero to one. The discrimination power of each primer.

Results and discussion

ISSR-PCR analysis

In order to look for gene diversity among S. aureus isolates and evaluate their differences, ISSR-PCR technique was employed in this study. The amplified products, which included the seven Inter Simple Sequence Repeats (ISSR), generated 178 useable whole bands, 120 polymorphic bands (an average of six polymorphic bands), and 32 polymorphic bands (average range size: 100-2000 bp) (figure 1).

Pr2 primers have the capacity to generate five distinct bands, or high unique bands (table 2). The five S. aureus isolates' genetic similarity ratio ranges from 0.65791 to 0.06899 based on genetic distance (table 3). When comparing isolate numbers 1 and 2, the greatest genetic similarity of 99.8% was found between them, while isolate numbers 3 and 2 showed the lowest genetic similarity of 21.5%, or 0.1025, between them (3 and 4).

Dendrograms were created with Nei and Lei (1979) as a basis. The genetic relationship between seven S. aureus isolates was shown using UPGMA cluster analysisThe results indicated two significant clusters. The first cluster had two primary distances introduced from patient sources, whereas isolate number 2 created a distinct line derived from another patient instance. The second cluster comprised the isolate with the least genetic resemblance.

,which was 0.1025. The isolates in the first group (figure 2), which were introduced from patient wound sources, had a greater genetic similarity of 0.6782.

This research does not provide regional profiling for any of the genetic distances based on amongst isolated. According to reports, the dendrogram produced by ISSR correlates better with genealogy and the ISSR's known pedigree. However, a number of analysis studies that employed ISSR discovered that the technology produces more information with fewer primers than RAPD primers, and one study found that the number of polymorphic bands was still higher with fewer primers [12, 13]. fewer ISSR primers equates to fewer time, DNA, supplies, and samples. In contrast to ISSR markers, RAPD markers lack a specified target. Since ISSR markers are known to be more sensitive than RAPD markers, it was clear from this study that the ISSR-based dendrogram did not match the RAPD-based dendrogram; as a result, both dendrograms agree with the clinical origin groups. Variations in the genotype clustering pattern using ISSR markers may also be related to sampling error in the markers and the degree of polymorphism detected[14].

In comparison with other markers, ISSR markers yielded a higher rate of polymorphism based on polymorphic rate; nevertheless, their effectiveness in producing dendrogram findings was lower.

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