Detected gene hymolysin in staphylococcus aerues

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Abstract

KEYWORDS

Molecular diagnosis, PCR, S. aureus, hly gene

ABSTRACT

Staphylococcus aureus is a significant human pathogen characterized by diverse virulence factors that contribute to its pathogenicity. this organism remains a leading cause of both hospital –acquired and community –associated infections worldwide the hemolysin gene plays an important role in bacterial virulence through its cytolytic activity against host cells. Understanding the molecular characteristics of this virulence factor is essenatial for improving diagnostic and therapeutic strategies. This study aimed to detect the presence of the .hemolysin gene in S. aureus clinical specimens

Material and methods: Thirty clinical samples were collected and directly inoculated onto blood agar and mannitol salt agar, followed by incubation at37 ºC for 18-24 hours. Bacterial identification was confirmed through standard microbiologyical techniques including gram staining, catalase test, and coagulase test. DNA extraction PCR amplification of the hemolysin gene was performed to detect the hemolysin gene was conducted using specific primer. The amplified products were sequenced and analyzed for genetic characterization then phylogenetic tree for .genes sequenced was constructed by using (MEGA10)

Results: PCR analysis succefully amplified the hemolysin gene for the clinical isolates. eight PCR products sequenced and submitted to GeneBank receving accession numbers LC78668871 LC78668873, LC78668874 LC78668875, LC78668876, LC78668877 LC78668878, LC78668879, LC78668880. were recorded in the database. The prevalence of the hemolysin gene among the tested isolated was 100% indicating its significance in the virulence profile of local S. aureus strains of the through PCR amplification provides a potential rapid ndiagnostic tool for identifying S. aureus infections directly from clinical specimens. The presence of this gene indicates the prevalence of highly virulent and pathogenic strains of S. aureus. Specimens

Conclusion: The PCR for amplification of HLY gene has potential for rapid diagnosis of S. aureus infections by direct testing of clinical specimens. The Sequence gene is one of the modern advanced development technique in molecular biology. In this way genetic relationship can be detected between bacterial isolates rapidly.

Introduction

The staphylococci is a Gram-positive, catalase-positive and oxidase-negative bacterium belonging to the family Micrococcaceae. These spherical cells characterisitically arrange in, grape-like irregular clusters this genus are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. its able to cause a wide spectrum of clinical diseases(Jawetz, 2014). S. aureus being particularly significant due to its ability to produce various virulence factor, including enterotoxins(SE)that are responsible for sever infections.

S. aureus is ubiquitously distributed in the environment,found in soil,air , and water in humans,it commonly colonizeshe nasal passages and skin, where it can exist as part of the normal microbiota. However, under opportunistic conditions,it can cause various dieases ranging from mild skin infections to severe systematic diseases(Fluit, 2012). He pathogenicity of S. aureus is largel attributed to its ability to produce multiple extracellular enzymes and toxins, particular hemolysins.

Hemolysins are an important virulence factors that play a significant role in S. aureus pathogenesis. These toxins are classified into four different types including alpha (α), beta (β), gamma (γ) and delta (δ)) (Aarestrup et al., 1999; Larsen et al., 2002). These pore-forming toxins specifically targt and lyse red blood cells through receptor mediated mechanisms (Da Silva et al., 2005). The mechanism of action involves the formation of β- barrel structures in the cell membranes leading to pore formation. When this toxins bind to their specific receptors they create membrane pore that disrupt cellular ion blance,particlarly Ca2 + and an influx of K +ions +; this imbalance in turn leads to the death of necrotic cells (Powers et al., 2015).

The pathogenic significance of hemolysins extends beyond their direct cytolytic activity .these toxins enable *S. aureus* to evade host immune responses. Facilitate nutrient acquisition through cell lysis and contribute to disease progression (Divyakolu *etal*., 2019). The ability to lyse host cell not only provides nutrients for bacterial survival but also aids in the spread of infection throughout host tissues.

Recent advances in molecular biology and biotechnology have revolutionized the detection and characterization of virulence genes in S. aureus. These modern techniques offer rapid and precise methods for identifying specific virulence factors and antibiotic resistance genes, providing advantages over traditional phenotypic methods. Such molecular approaches are particularly valuable for understanding the distribution and expression of hemolysin genes among different S. aureus strains and their correlation with disease severity.

Material and methods

Sample collection and bacterial isolation

A total number of 30 sample were collected from various specimens clinical. Samples were directly inoculated from transport media, on the blood agar (Himedia) and mannitol salt agar at 37 ºC for 18-24 hours.

Phenotypic identification

After incubation, the isolated colonies were identified by morphological characteristics and biochemical tests according to (Atlas et al.,1995) colonies showing hemolysis in blood agar were selected for futher characterization. The Identification

of S. aureus was confirmed using the morphology and biochemical tests like catalase, coagulase test it shows in table (1).

Table (1) biochemical testes of S. aureus

Oxidase	Catalase	Haemolysis	Gram – staining	Coagulase	Motility	Growth on Mantol salt agar
_	+	+	+	+	_	+

Molecular diagnoses methods of Hemolysin Gene

DNA extraction

DNA isolation and PCR conditions. DNA extraction was carried out with a mercantile DNA isolation kit (Promega, USA) according to the manufacturer's instructions.

Primer Design

Specific primer targeting the (hly) gene was designed using primer 3 software and he NCBI Gen Bank website. Primers were synthesized by Bioneer Corporation (Korean (company) Sequence of primer as shown in Table (2)

Table (2) Sequence of the nitrogenous bases of a specific primer

Gene Sequencing	**Size (bp)**	**Gene**
F:GGTTTAGCCTGGCCTT R: CATCACGAACTCGTTC	534	*Hly*

Gel electrophoresis

Electrophoresis was carried out using prepared agarose gel

By 5.1% under a voltage difference of 100 volts and a current of 80 amperes and for one hour, to read the result of the PCR polymerase chain reaction according to (Sambrook and Russell,2001).

The entire volume of the PCR tubes was 50µl, and it was made up of the following: 10µl of Master Mix, 1µl of both the forward and reverse primers that were particular to each gene, 5µl of bacterial DNA, and the remaining volume was filled with nuclease-free water. The protocol for the PCR amplification of gene was described in table (3). Phylogenetic analysis was conducted using MEGA10 software (Kumar et al., 2018) to construct phylogenetic trees and analyze evolutionary relationships.

Table (3) Amplification conditions of gene

Cycle	**Time**	**Temperature**	**Step**
1	2 min	94C°	Initial denaturation-1
2	30 sec	94C°	Denaturation-2
35	30 sec	62C°	annealing-3
2	12 sec	72C°	extension-4
2	2 min	72C°	Final extension-5
1	α	4C°	Hold-6

Results and Discussion

Phyenotypic charactrization

microscopic examination revealed Gram-positive cocci arranged in the clusters of grape-like shape and do not form For spores and non-motile by motility assay test . Mannitol salt agar or MSA is selective differential medium for Staphylococcus

aureus. It contain: Nacl 7.5%, Mannitol, and phenol Red. The cause of selectivity due to presence of high salt concentration. The cause of differential because contains mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH).

The results of the biochemical tests for the isolates were as shown in the table(1) Colonies of S. aureus were observed after an incubation period of (18-24) hours on blood agar plates, colonies surrounded clear zone of haemolysis, which confirmed the diagnosis as S. aureus according to (Leboffe and Pierce, 2012). Coagulase positive staphylococci.

Molecular detection of Hemolysin Gene

PCR analysis revealed the presene of hly gen in 29 out of 30 isolates (96.7%)consistent with previous finding (Abdul-Kareem & Husain, 2015; od'Su, 2005). Eight isolates showing pronounced hemolytic activity were selected for detailed molecular characterization of their hemolysin gene. the ampilification products confirmed the presence of the hly gene ,aligning with observation reported by (Al-Nashi and Al- Mansouri ,2017).figure (1)

Sequence Analysis and Gen Bank submission

Eight PCR products were succefully and deposited in Gen Bank under accession numbers LC78668871 LC78668873, LC78668874 LC78668875, LC78668876, LC78668877 LC78668878, LC78668879, LC78668880**.** Sequence analysis demonstrated that hly gene is widely distributed among clinical S. aureus isolated, supporting its potential roles as a molecular marker for pathogenic strains (figure 2).

Role of hemolysin in virulence

Hemolysin represent an important virlence factor in S. aureus pathogenesis(Paul etal.,2004). These cytolytic exotoxins damgage host cell plasma membrane and have been associated with biofilm formation(Caizza, and Otole,2003).the ability to produce hemolysin enables S. aureus to lyse various host cell populations including immune cells ,it helps the bacteria to spread within the host and contribute to disease progression(He etal., 2018).

The prevlence of the hly gene in Staphylococcus aureus is important for these isolates Various diseases that affect humans and animals This result was similar to (Ariyanti et al.,2011). The Hemolysis is excoprotein which has enzymatic and toxin activity so the bacteria that form hemolysis are pathogenic this agree with (Williams et al., 2000). The Sequence gene is one of the modern advanced development technique in molecular biology. In this way mutation and genetic relationship can be detected between bacterial isolates rapidly (Ahmed & Hamim,2020).while the extact indcidence of hemolysin mediated infections requires further investigation current suggests its role may have been undersitmated .

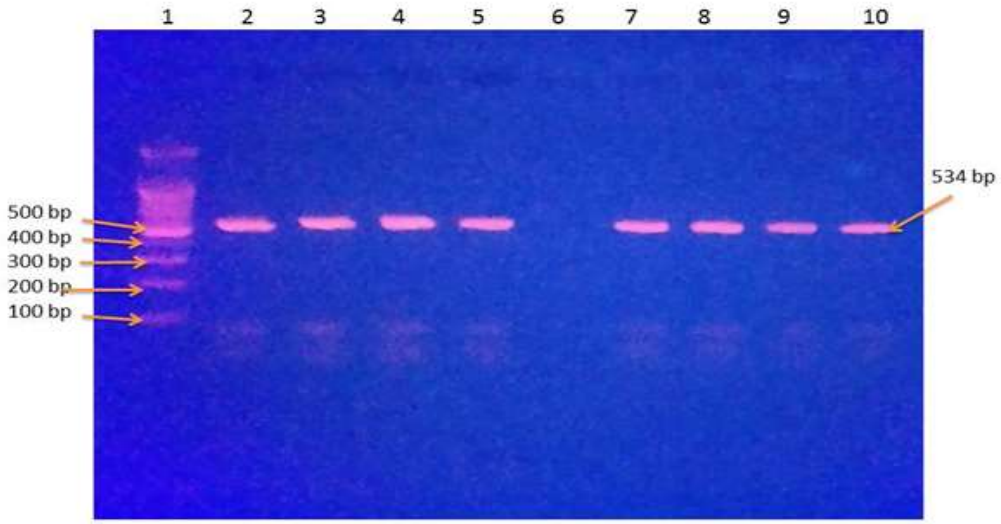

Figure 1. Agarose gel electrophoresis Detection of PCR product of hly gene(bp534)

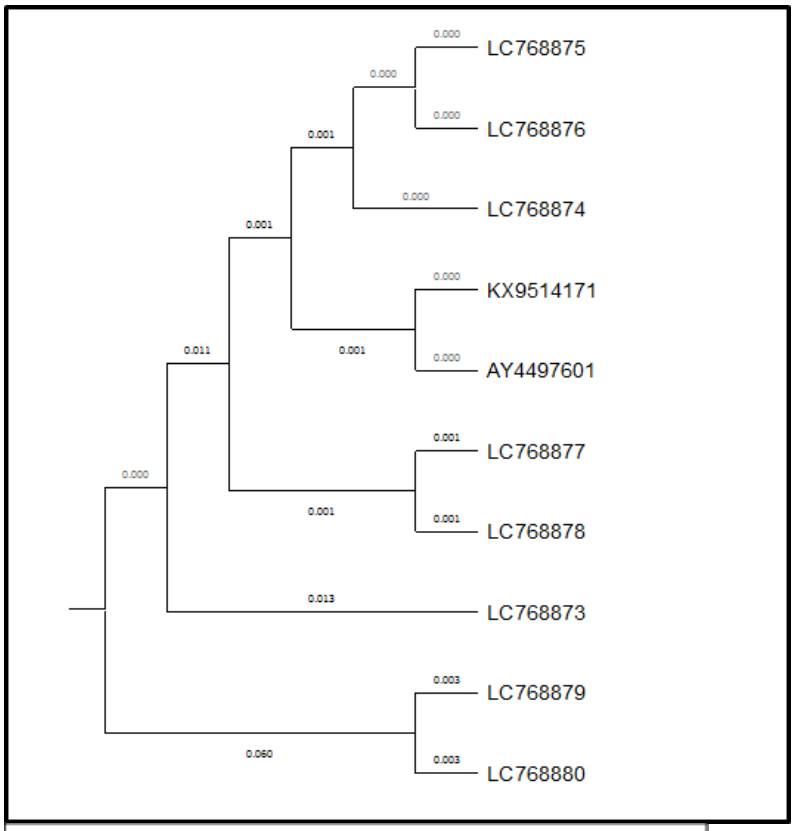

Figure 2. Phylogenetic tree

Conclusions

This study demonstrated that: the Hly gene is highly prevalent among S. aureus isolated from clinical isolates. Indeed, although their true incidence in staphylococcal

infection cases that caused has still need to be clarified, it is now thought likely that their role has been underestimated.

The current study used the genome sequence of S. aureus to search for a S. aureus-specific diagnostic marker for this human pathogen The pathogenicity of this bacteria is due to its production Hemolysin enzyme.

Eight PCR product of was recorded globally in Gene bank under the official accession numbers of LC78668871 LC78668873, LC78668874 LC78668875, LC78668876, LC78668877 LC78668878, LC78668879, LC78668880

This findings contribute to our understanding of S. aureus pathogenicity and may aid in developing diagnostic strategies.

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