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vicK Gene as Potential Identification Marker for *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is an important human pathogen frequently detected in hospital community and has emerged as an important health concern in human medicine. Identification of *S. aureus* from clinical specimens by phenotypic methods may produce variable characteristics leading to ambiguity. Hence, a rapid and reliable method for identification of *S. aureus* is required which could expedite appropriate antibiotic therapy. This study aimed to evaluate the specificity of polymerase chain reaction (PCR) targeting a signal transduction gene, *vicK*, among *S. aureus* isolates of Hospital Sultanah Nur Zahirah, Kuala Terengganu, Malaysia. A total of 118 bacterial isolates were screened, which consisted of one hundred *S. aureus* isolates, ten *Staphylococcus* spp. and eight non-Staphylococci. Results indicated that PCR targeting *vicK* was able to identify 98% of *S. aureus* isolates with high sensitivity and specificity, while the remaining isolates of *Staphylococcus* spp. and non-Staphylococci did not yield any amplification of the gene. *vicK* thus, is highly specific within interspecies and intraspecies, which is potential to be used as a molecular identification marker for *S. aureus.*

Keywords

vicK Gene, *Staphylococcus aureus*, Identification Marker, Rapid Diagnostic, Two-Component Signal Transduction

1. Introduction

*Staphylococcus aureus*is the most important pathogen associated with skin infection and foodborne disease in humans. The organism is a common microflora of human epithelia exhibiting permanent nasal colonization between 30% - 40% of the human population [1]. Symptomatic *S. aureus* infections may occur fol-

lowing breaks in skin or mucosal barriers, potentially causing severe invasive infections. In extreme condition, *S. aureus* may lead to life-threatening manifestations such as bacteremia, endocarditis, and osteomyelitis [2] [3]. *Staphylococcus aureus* is grouped into the coagulase-positive Staphylococci (CoPS) based on its ability to produce coagulase enzyme, contrasting to the less pathogenic coagulase-negative Staphylococci (CoNS). Although recent reports highlighted the increasing significance of CoNS as opportunistic pathogens, *S. aureus* remains a major health threat in clinical settings [4]. In particular, *S. aureus* has been recognized the most common cause of nosocomial bloodstream infections, where most infections involved the healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) [5] [6]. HA-MRSA infections are usually difficult to treat due to their resistance to multiple antibiotics, contributing to a significant mortality and morbidity [7]. The worldwide concern of *S. aureus* infections increases with recent emergence of community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) which posed a significant impact and economic burden in public health and livestock industry [8] [9] [10].

Due to the importance of *S. aureus* as an opportunistic pathogen, identification of this bacterium in clinical specimens is essential particularly in discriminating *S. aureus* from the CoNS and non-staphylococci. Rapid identification of *S. aureus* allows initiation of the appropriate antibiotic therapy in patients at the early stage of disease onset and prevents development of serious illness. In most clinical laboratories, phenotypic identification of *S. aureus* is routinely carried out based on morphological characterization on agar and biochemical tests [11]. Presumptive isolation of mannitol-positive *S. aureus* is usually determined by growth on mannitol salt agar, producing yellow-coloured colonies as a result of mannitol fermentation [12]. Nonetheless, isolation of mannitol-positive CoNS species in human nasal and clinical specimens has been previously demonstrated in Nigeria and Japan [13] [14]. Coagulase production remains a standard confirmatory test for *S. aureus* [11] [12], however, accurate identification by this test depends on the nature and quality of the plasma used [15]. Furthermore, prolong incubation is sometimes required to achieve reliable results of coagulase test and this represents a great disadvantage for hospital diagnostics [16].

Advanced molecular approaches provide rapid and reliable means of identifying *S. aureus* from clinical samples. Through these methods, *S. aureus* has been identified efficiently using DNA microarray, real-time PCR, fluorescence *in situ* hybridization, surface enhanced laser desorption/ionization time of flight and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [17] [18] [19] [20] [21]. These methods however, require highly expensive equipments, which limit their use in healthcare diagnostics. In contrast, PCR-based approaches are more cost effective and highly efficient to be used for rapid identification of *S. aureus.* Standard microbial identification utilizes 16S ribosomal DNA sequence analysis, useful for identification at species level [22]. PCR targeting constitutively expressed genes such as *nuc, femA, sodA* and *coa* allows rapid identification of *S. aureus* without further requirement of gene sequencing

[23] [24] [25] [26]. Moreover, several *S. aureus* genes associated with antibiotic resistance and enterotoxins have been shown to facilitate identification of the bacteria from various sources with high sensitivity and specificity [27] [28]. Polymorphisms however occur in some of these genes which limit their use as a reliable identification marker for *S. aureus* [29] [30].

PCR targeting a signal transduction gene, *vicK* has been recently described as highly specific and sensitive of detecting a low copy genetic material of *S. aureus* [31] [32]. *vicK* encodes the bacterial two-component signal transduction system (TCS) that senses and responds towards the environmental stimuli especially when invading the host [32]. The gene regulons of TCS are species-specific, which play diverse roles in modulating of cell division, cell-wall biosynthesis and membrane integrity [33]. This study aimed to evaluate *vicK* as a potential rapid identification marker for *S. aureus* by determining the frequency of *vicK* detection in *S. aureus* clinical isolates.

2. Materials and Methods

2.1. Bacteria Isolates

A total of 110 Staphylococci clinical isolates were obtained from the Pathology Laboratory, Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu, Malaysia, within five months from September 2008 until January 2009 (**Table 1**). The isolates comprised of one hundred *S. aureus* and ten *Staphylococcus* spp.; *Staphylococcus epidermidis* (4), *Staphylococcus haemolyticus* (2), *Staphylococcus hominis* (2), *Staphylococcus saphrophyticus* (1) and *Staphylococcus cohnii* (1). The organisms were isolated from various body sites of hospitalized patients contracted with the bacterial infection (**Table 2**). Identification of *Staphylococcus* spp. to species level was preceded by sequencing of the bacterial 16S rDNA. *Staphylococcus aureus*(ATCC 29213) was used as a positive control for PCR amplification. For non-Staphylococci negative controls, eight different types of bacteria comprising of three Gram-positives (*Streptococcus uberis*, *Micrococcus* sp., *Bacillus* sp.) and five Gram-negatives (*Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella* sp., *Salmonella* sp. and *Aeromonas* sp.) were obtained from

Table 1. Staphylococci clinical isolates collected from Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu, Malaysia, from September 2008-January 2009.

Bacteria species	Number of isolates	Source
Staphylococcus aureus	100	HSNZ
Staphylococcus spp.		
Staphylococcus epidermidis	4	HSNZ
Staphylococcus haemolyticus	2	HSNZ
Staphylococcus hominis	2	HSNZ
Staphylococcus saphrophyticus	1	HSNZ
Staphylococcus cohnii	1	HSNZ
Total	110	

the Microbiology Laboratory, School of Fundamental Science, Universiti Malaysia Terengganu (UMT) (**Table 3**). These isolates were sub-cultured on nutrient agar and incubated at 37˚C overnight.

2.2. Identification of Bacteria

Primary bacterial identification was performed by basic microbiological methods using colony morphology and biochemical tests. *Staphylococcus aureus* were sub-cultured on blood agar for examination of morphological characteristics and haemolytic activity. The bacteria were further examined by Gram staining, catalase test, coagulase test and growth on mannitol salt agar.

2.3. DNA Extraction

DNA was prepared by *boiling* method as previously described with minor

Table 2. The source of *Staphylococci* isolated from patients of Hospital Sultanah Nur Zahirah.

Organism	Clinical specimens	Number of isolates
Staphylococcus aureus	Pus	62
	Blood	14
	Eye	10
	Cerebrospinal fluid	7
	Urine	3
	Unknown*	3
	Secretion	1
Staphylococcus spp.	Blood	6
	Cerebrospinal fluid	2
	Eye	2
Total		110
*not recorded		

Table 3. Non-*Staphylococci* obtained from the Microbiology Laboratory, School of Fundamental Science, Universiti Malaysia Terengganu (UMT).

Non-Staphylococci	Number of isolates	Source
Streptococcus uberis	1	UMT
Micrococcus sp.	1	UMT
Bacillus sp.	1	UMT
Escherichia coli	1	UMT
Pseudomonas aeruginosa	1	UMT
Klebsiella sp.	1	UMT
Salmonella sp.	1	UMT
Aeromonas sp.	1	UMT
Total	8	

modifications [34]. In brief, 1 - 2 bacterial colonies were suspended in sterile water and boiled for 10 minutes. The suspension was then spun down at 10,000× g for 5 min. The supernatant containing bacterial DNA was kept on ice and freshly used as a template for PCR amplification.

2.4. *vicK* Gene Amplification

PCR was performed in 25 µl reaction using an EppendorfMastercycler® Gradient. The PCR mixtures contained 5 µl 5X PCR buffer, 4 µl $MgCl_2$ (25 mM), 1 µl dNTPs (10 mM), 1 µl each reverse and forward *vicK* gene primers (10 µM), 1U Taq DNA polymerase, DNA template (1 µl) and sterile nuclease-free water to the final volume of 25 µl. The *vicK* primer sequences used: forward 5'-CTAATACTG AAAGTGAGAAACGTA-3' and reverse 5'-TCCTGCACAATCGTACTAAA-3', as established by Liu *et al.* (2007). Distilled water with no DNA template was used as a negative control. The thermal PCR cycling conditions consisted of initial denaturation at 95˚C for 5 min; and 30 cycles of denaturation at 95˚C for 1 min, annealing at 52.6˚C for 1 min, extension at 72˚C for 1 min, and a final extension at 72˚C for 5 min. Amplified PCR products were separated in 1.2% agarose gel stained with ethidium bromide (0.5 µg/ml), visualized under UV light and photographed using Image Master VDS was prepared by *boiling* method as previously described with minor modifications [34].

2.5. DNA Sequencing

In order to confirm that *vicK* gene had been successfully amplified, at least three *vicK* gene amplicons were sequenced-verified. The PCR products were excised from agarose gel and the DNA was purified using QIAquick Gel Extraction Kit according to the manufacturer's protocol. Purified DNA was eluted in 20 µl of nuclease free water and centrifuged at 12,000 × g. The purified fragments were sent to First BASE Laboratories Sdn. Bhd. for DNA sequencing using both forward and reverse vicK primers. Results obtained were analyzed using Chromas in order to assess the quality of chromatogram. Nucleotide sequence obtained from the chromatogram was exported to Entrez NCBI. The sequence was submitted for BLAST search against NCBI bacterial database.

2.6. Data Analysis

The specificity and sensitivity of PCR targeting *vicK* were calculated according to Kateete *et al.* (2010) as follows:

$$\text{Sensitivity (\%)} = [\text{True positive}/(\text{True Positive} + \text{False Negative})] \times 100$$

$$\text{Specificity (\%)} = [\text{True Negative}/(\text{True Negative} + \text{False Positive})] \times 100$$

3. Results

3.1. Identification of *S. aureus*

A total of one hundred *S. aureus* isolates collected from HSNZ, Kuala Terengganu were identified generally based on conventional phenotypic methods.

Upon growth on blood agar, the organisms appeared round golden-yellow colonies and resulted in haemolytic activity. Growth was visible on mannitol salt agar, changing the colour of the medium to yellow as a result of mannitol fermentation. The isolates were catalase-positive and coagulase-positive. Isolates with uncertain coagulase activity was further tested with tube coagulase test as previously recommended [24], which produced a clot for positive reaction. The bacteria cells appeared as Gram-positive cocci and retained purple upon gram stain.

3.2. Amplification of *vicK* Gene

The specificity of PCR targeting *vicK* was evaluated in all bacteria isolates using *vicK* gene primers established previously [32]. Results of this study demonstrated amplification of a single fragment *vicK* (289 bp) in 98% of *S. aureus* isolates (**Figure 1**, **Table 4**). This gives 98% sensitivity level of using *vicK* as a target in identifying *S. aureus* from clinical isolates. In order to confirm the *vicK* sequence identity, randomly picked PCR amplicons were sequenced-verified. BLAST search analysis affirms the identification of *vicKS. aureus*, demonstrating 100% nucleotide sequence homology. In a parallel control experiments, there was no amplification of *vicK* in all ten isolates of *Staphylococcus* spp. and eight non-Staphylococci tested (**Table 4**). Hence, a 100% specificity level was achieved in discriminating *S. aureus* from other *Staphylococcus* spp. and non-Staphylococci.

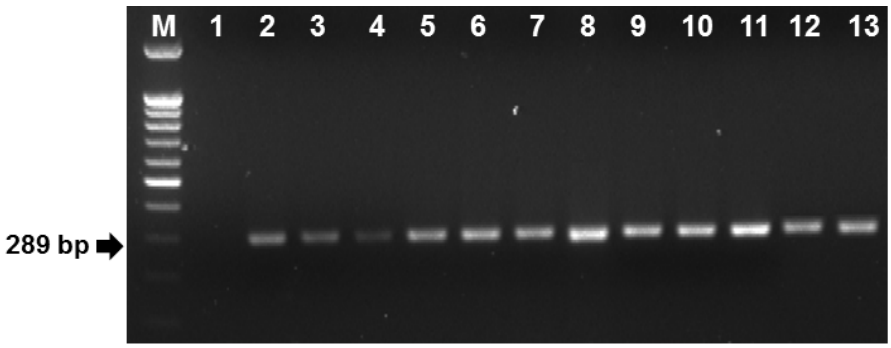

Figure 1. Amplification of *vicK* gene fragment of representative eleven *S. aureus* clinical isolates, resolved in 1.2% agarose gel and stained with ethidium bromide. Lane M is 100 bp marker; Lane 1 is PCR negative control (without DNA template); Lane 2 is *S. aureus* positive control (ATCC 29213); Lanes 3 to 13 are *S. aureus* clinical isolates, respectively. Arrow indicates *vicK* amplified fragments at 289 bp.

Table 4. Percentage of *Staphylococcus aureus* isolates identified by *vicK* amplification.

	***vicK* amplification**	
Bacteria isolates	**Positive**	**Negative**
Staphylococcus aureus (n = 100)	98% (98/100)	2% (2/100)
Staphylococcus spp. (n = 10)	0	100% (10/10)
Non-*Staphylococci* (n = 8)	0	100% (8/8)

4. Discussion

Staphylococci are colonizers of human skin and mucosal surfaces, frequently cause severe infection in humans. In the present study, 110 Staphylococci clinical isolates were obtained from HSNZ, Kuala Terengganu, Malaysia within five months (September 2008-January 2009). The highest percentage of organisms was found in pus (56.4%), followed by blood (18.2%), eye (10.9%) and cerebrospinal fluid (8.2%) (**Table 2**). The highly encounter of this organism in the pus is consistent with previous studies reported in many hospitals [35] [36] [37]. This could be due to the frequent association of Staphylococci with skin and soft tissue infections, which usually manifest abscesses [38] [39]. The exposure of skin wounds to Staphylococci particularly *S. aureus,* usually arises in patients with predisposing risk factors such as burns, chronic illness and the use of medical devices [40] [41]. *Staphylococcus aureus* may colonize almost 30% - 40% of individuals and can be easily spread through contact with an infected person or through personal belongings [1]. This colonization significantly increases the chances of infections by providing a reservoir of the pathogen. Once the bacteria penetrate through skin, they are potential of causing severe infections, such as skin infections, wound infections and bacteremia. The latter contributes to the greatest problem in medical field as *S. aureus* has been recognized the most common cause of nosocomial bloodstream infections [6]. This has also been a challenge due to the presence of heterogenous population of *S. aureus* colonizing a human body which severely complicate antibiotic therapy [42] [43].

Due to their public importance, rapid detection and identification of *S. aureus* from clinical isolates is crucial. Accurate identification of *S. aureus* from the less pathogenic CoNS and non-Staphylococci is essential for the appropriate therapeutic use of antibiotics and timely intervention for infection control. In this study, *S. aureus* isolates collected from HSNZ were identified according to the standard protocols by significant growth on culture media and biochemical tests [12]. While single phenotypic test is inefficient for identification of *S. aureus* [15], morphological observation on blood agar, haemolytic activity, growth on mannitol salt agar, Gram staining, catalase test and coagulase test were performed. Although *S. aureus* can be easily grown on standard culture media and identified based on several biochemical tests, there is a need for rapid and sensitive DNA-based assay for the discrimination of *S. aureus* from other CoNS and non-Staphylococci. Rapid identification of this bacterium with high accuracy is important for early diagnosis of *S. aureus* infections which leads to treatment of patients with appropriate antibiotic therapy. Conventional PCR assays are highly versatile and commonly used for identification of pathogens within hours. It is recognized that PCR is highly sensitive and accurate, allowing for the detection of a very low copy number of genetic material [44] [45].

Most PCR methods target highly conserved genes within the species to identify *S. aureus* at the species level [46]. In the present study, PCR targeting a signal transduction gene, *vicK* of *S. aureus* was evaluated as a potential marker for

rapid identification of the bacteria. *vicK* is a unique gene that encodes the two-component signal transduction system, responsible for regulating bacterial responses to extracellular signals [33]. The gene is highly conserved and has been shown to be species-specific [32]. Following PCR amplification of HSNZ isolates, the *vicK* gene fragment has been successfully detected in 98% of *S. aureus* (**Figure 1**, **Table 4**). This correlates well with previous findings demonstrating high sensitivity and specificity of PCR targeting *vicK* [31] [32]. It was also evident that *vicK* was undetectable in other staphylococci (*S. epidermidis, S. haemolyticus, S. hominis, S. saphrophyticus* and *S. cohnii*) and non-staphylococci tested (*Streptococcus uberis*, *Micrococcus* sp., *Bacillus* sp., *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella* sp., *Salmonella* sp. and *Aeromonas* sp.). This gives a 100% specificity of *vicK* amplification by PCR in discriminating *S. aureus* from other bacteria species. Although one isolate represented each species in this study, many representative isolates of other types of bacteria have been previously tested for *vicK* detection, demonstrating similar observations [31] [32]. The high percentage of *S. aureus* identified by *vicK* amplification indicates the gene is species-specific, thus offering a useful target for specific and rapid identification of *S. aureus.*

It is noteworthy that a small percentage (2%) of *S. aureus* was unable to be recognized by *vicK* amplification (**Table 4**). This may be contributed by several factors. One factor might be due to a very low concentration of DNA harvested from boiling of the bacterial colonies. Although this method was rapid and easy to perform, the resulted DNA varies among samples [47]. The true limit of detecting *vicK* by PCR was reported above 5500 copies of plasmid DNA [31], thus the efficiency of harvesting DNA target and the amount of DNA yield can limit the sensitivity of PCR assay. We believe the use of appropriate DNA extraction protocols incorporating efficient cell lysis and purification steps may greatly improve the quality of harvested DNA.

Another factor that may contribute to the lack of *vicK* detection could be due to misidentification of *S. aureus.* False identification occasionally occurs by misinterpretation of results derived from phenotypic methods [48]. A high rate of false identification by phenotypic methods has been reported in Nigeria contributing to 85% misidentification of *S. aureus*, whereas 49% false identification has been documented in Libya [49] [50]. Although coagulase test represents the standard method for identification of *S. aureus*, a few CoNS produce clumping factor which can be erroneously interpreted as coagulase-positive *S. aureus* [51] [52]. Due to budgetary and time constraints, further experiments were not carried out to verify the identity of these isolates. We believe that identification of the bacteria through 16S ribosomal DNA sequencing will be able to precisely determine the identity of these organisms to the species level.

The advantage of PCR assay described in this study is the use of only one primer set targeting *vicK* for specific detection of *S. aureus.* An increasing number of bacterial isolates collected from various clinical and geographical sources may be performed in future to support findings of this study. We also believe that rapid

detection of *S. aureus* directly from clinical specimens is important for timely intervention and infection control. Thus, the potential use of PCR assay described here using *S. aureus* clinical sources requires further investigation.

5. Conclusion

PCR targeting the *vicK* gene had successfully identified a high percentage of *S. aureus* clinical isolates obtained from HSNZ. The gene is species-specific and highly discriminative of *S. aureus* from other organisms, thus potential to be used as a rapid diagnostic marker for identification of *S. aureus.*

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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