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Research paper



Prevalence of Panton-Valentine Leukocidin Staphylococcus aureus among Patients and Healthcare Workers in Malaysian Public Hospital

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Abstract

Staphylococcus aureus carrying Panton-Valentine leukocidin (PVL) has become a worldwide threat to public health. Recent reports showed an increasing numbers of *Staphylococcus aureus* infections caused by PVL-positive organisms worldwide which explain the need to carry out this study in Malaysia. The objective of this study was to determine the prevalence of PVL-positive *Staphylococcus aureus* among patients and healthcare workers in Malaysian Public Hospital. Collectively, 230 swabs were collected from the anterior nares of inpatients and healthcare workers at Sungai Buloh Hospital. The isolates were identified and characterized using conventional and molecular methods. From 230 samples collected, 23% (53/230) were detected as *Staphylococcus aureus* isolates. Out of the 53 *Staphylococcus aureus* isolates, 2 (3.8%) were found to be PVL-positive *Staphylococcus aureus* (from patient's sample) and the remaining 51 (96.2%) isolates were PVL negative *Staphylococcus aureus*. Out of 53 *Staphylococcus aureus* isolates, 40 (75.5%) were methicillin-sensitive and 13 (24.5%) were methicillin-resistance *Staphylococcus aureus*. The finding of the prevalence of PVL-positive *Staphylococcus aureus* among patients and healthcare workers will provide awareness to the hospital authority to take action for prevention and control.

Keywords: *methicillin-resistance S. aureus (MRSA); methicillin sensitive S. aureus (MSSA); polymerase chain reaction (PCR); PVL; S. aureus*

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the major human pathogen that often colonizes the host asymptomatically and lives as a commensal in the human skin and nose [1]. It is a common bacterium that colonizes approximately 30% of the general population [2]. The anterior nares are the major reservoir of *S. aureus*. However, *S. aureus* is an important pathogen causing a wide to life-threatening conditions [3]. The pathogenicity of staphylococcal infections depends on its virulence factors. The virulence factors of *S. aureus* include antigens, enzymes and toxins. Globally, the treatment of *S. aureus* infections has become more complicated after tremendous increase of antibiotics resistance of this bacterium [4]. Recently, several studies have shown the presence of Panton-Valentine leukocidin (PVL) gene within *S. aureus* strains. The first documented cases of PVL-positive *S. aureus* infection was reported in Israel by Amos et al. (2006) [5]. Also other two similar cases caused by methicillin-resistance *S. aureus* (MRSA) were reported among German tourists after visiting the Dead Sea in Jordan [6].

The PVL gene encodes for extracellular cytotoxin a bicomponents with a two non-associated classed of secretary proteins, Luk S-PV and Luk F-PV. These toxins disrupt the permeability barrier of leucocytes and erythrocytes by creating pores in their cell membranes. Thus, the PVL-positive *S. aureus* strains become a major risk factor especially in nosocomial and community acquired infections [7]. Previous studies demonstrated that PVL-positive *S. aureus* cases were associated with more severity, ranging from furuncles, cutaneous abscesses and severe necrotic skin infections requiring surgical drainage to severe chronic osteomyelitis and deadly staphylococcal necrotizing pneumonia. The current study aimed to identify the percentage of PVL-positive *S. aureus* nasal carriers among patients and healthcare workers in Sungai Buloh Hospital in Selangor.

2. Methods

2.1. Sample collection

This was a cross-sectional study that was carried out from 1 May to 31 November 2017 among patients and healthcare workers in four wards (medical, surgical, obstetrics and gynaecology, and paediatric) in Sungai Buloh Hospital. A total of 230 subjects were involved in the current study. All participants were agreed and signed consent form before sample collection. Epidemiological information was obtained by interviewing the subjects at the time that the nasal swabs were collected. Demographic (sex, race and occupation) and clinical conditions data (history of antibiotic usage in the past two weeks, duration of hospitalization and history of fever in the past 2 weeks) were included in this study. The samples were taken from the subjects by using a sterile cotton swab

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inserted into the both nostril, to a depth of approximately 1 cm and rotated a few times.

2.2. Conventional methods

For each subject, both nostrils were sampled using the same swab. Stuart Transport Medium was used for transportation and preservation of samples. The samples were immediately sent to the laboratory of Institute for Medical Molecular Biotechnology (IMMB), Faculty of Medicine, UiTM and inoculated onto blood agar plates. Then, the plates were incubated in 37℃ for 24 hours. The single colonies of the grown cultured samples were tested with staphylase test and tube coagulase test to confirm *S. aureus* species.

2.2.1. Antibiotic susceptibility testing

Disc diffusion method by (Kirby-Bauer) was used to determine antibiotic susceptibility of *S. aureus* isolates based on the Clinical Laboratory Standards Institute (CLSI) in Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline (CLSI MM3-A2). The antibiotics panel used in sensitivity tests included: oxacillin (1 µg), ciprofloxacin (5 µg), tigecycline (15 µg), linezolid (30 µg), mupirocin (5 µg), rifampin (5 µg), gentamicin (10 µg), teicoplanin (30 µg), telithromycin (15 µg), trimethoprim-sulphamethazole (25 µg) and vancomycin (30 µg), fusidic acid (10 µg). The antibiotic discs were purchased from Oxoid (Basingstoke, Hants, UK).

2.3. Molecular methods

2.3.1. Designing primers for multiplex PCR assay

According to sequence data gotten from GenBank databases, we design a three various primer sets [8]. The ClustalW platform in Vector NTI software was used to align the DNA sequences. GeneDoc software was used to conceive the conserved and non-conserved areas of the DNA sequence alignments [9]. Then a specific primer pairs were designed based on the conserved regions of the alignment to amplify the *S. aureus* for *fem*A, MRSA for *mec*A and Panton Valentine leukocidin genes for PVL. A primer pair based on the *fem*A (350 bp), *mec*A (200 bp) and PVL (600 bp) gene was designed. The three primer pairs (First BASE Laboratories Sdn Bhd, Selangor, Malaysia) were designed to give PCR bands ranged from 200 to 600 bp. The characteristics of the designed primers were checked using BLAST, from GenBank website [10].

2.3.2. Optimisation

The optimal concentrations of primers for each gene (0.3 pmol femA, 0.3 pmol mecA, 0.3 pmol PVL) were used in the multiplex PCR. The other PCR components used included 25 mM MgCl2, 10× PCR buffer, 5 U Taq DNA polymerase and 10 µM dNTPs.
The PCR was accomplished using a Mastercycler Gradient Eppendorf, Hamburg, and Germany. The initial cycle of denaturation at 94°C for 3 min was followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 57.7°C, extension at 72°C for 1 min and a final extension at 72°C for 5 min.

2.3.3. Visualization of PCR

The PCR outcomes were parsed by electrophoresis on 1.5% low electroendosmosis (EEO) agarose gels (Vivantis, Selangor, Malaysia) by SYBR Safe at 90 V for 75 min. PCR outcomes were visualized under UV illumination and photographed using an image analyzer (Bio-Rad, Hemel Hempstead, United Kingdom).

3. Result and discussion

In this study, 230 nasal samples were collected from May to November 2017. The study samples were collected from different wards in Sungai Buloh Hospital. Fifty three out of 230 nasal samples (23%) were found to be *S. aureus* by conventional and molecular methods figure 1. Out of the 230 nasal swab only 2 (0.87%) were found to be positive for PVL gene. The percentage of PVL positive *S. aureus* from the total *S. aureus* nasal carrier was 3.8 %.

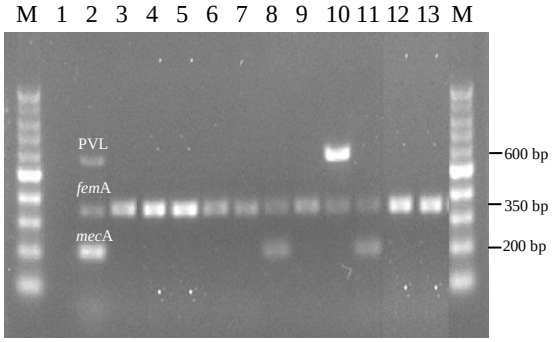


Fig. 1: Multiplex PCR assay portrait with reference strains. M, 100-bp marker; lane 1, negative control; lane 2, Staphylococcal positive control; lane 3-7, sample (*S. aureus*); lane 8, sample (MRSA); lane 9, sample (*S. aureus*); lane 10, sample (PVL); lane 11, sample (MRSA); lane 12-13, sample (*S.aureus*); M, 100-bp marker.

Among 53 *S. aureus* 13 (24.5%) was MRSA while, 40 (75.5%) was methicillin-sensitive *S. aureus* (MSSA). Our developed multiplex PCR assay was successfully detected the study genes efficiently with fine and matched bands to product size (Fig. 1). The *fem*A gene was present in all *S. aureus* reference strains by multiplex PCR, while other Coagulase-negative staphylococci (CoNS) species were negative.
The *fem*A, is crucial for the expression of methicillin resistance in *S. aureus* and is particularly present only in *S. aureus* isolates. Specified primers for *fem*A were designed and used in the multiplex PCR to screen different staphylococcal swabs. All 53 *S. aureus* nasal samples examined, regardless of the presence or absence of PVL, showed a clear band for *fem*A gene by PCR (Fig. 1). The *mec*A gene is distinctive to methicillin-resistant staphylococci. Therefore, the *mec*A gene acts as a useful molecular component for rapid identification of MRSA by PCR.
About 5% of hospital acquired *S. aureus* isolates contain the PVL gene. The PVL-encoding gene permits the production of a necrotizing cytotoxin, which may be responsible for staphylococcal invasiveness and virulence [11]. We used this gene in the multiplex PCR assay to classify our isolates based on the presence or absence of PVL gene.
This study showed that the prevalence of *S. aureus* nasal carriage was 23% (53/230) that is similar to previous study which showed that the prevalence of *S. aureus* nasal carriage in Malaysia ranging between 20–25% [12].
All the *S. aureus* isolates were sensitive to gentamicin, tigecyline and vancomycin. The susceptibility of *S. aureus* strains to these three antibiotics were 100% (Table 1). Vancomycin was the main antibiotic for treating *S. aureus* especially for serious MRSA infections [13] and tigecycline also was effective against all of the oxacillin-sensitive or resistant *S. aureus* [14]. However, a reduction in susceptibility of MRSA to vancomycin has been reported lately from various countries including Japan, Brazil, United States, Scotland, France, South Africa and Korea [15] but in Malaysia there is no reporting on that.
The sensitivity of MRSA towards fusidic acid was low about 38.5% which mean 61.5% are resistance, PVL positive *S. aureus* was 100% susceptible, and MSSA was 68.4% susceptible and 31.6% resistance. Sofie et. al (2017) reported that fusidic acid was the most prevalence type of resistance *towards S. aureus* which is

about 41% [16]. The increasing of resistance's percentage due to widely use of this kind of antibiotic.

Table 1: Antibiotic susceptibility pattern of 53 Staphylococcus aureus isolates from clinical nasal swabs specimens

Antibiotics	Number (Percent of isolates sensitive)		
	PVL positive MSSA (n = 2)	MSSA (n = 38)	MRSA (n = 13)
Ciprofloxacin	2 (100%)	30 (78.9%)	4 (30.8%)
Fusidic acid	2 (100%)	26 (68.4%)	5 (38.5%)
Gentamicin	2 (100%)	38 (100%)	0 (0%)
Linezolid	2 (100%)	33 (86.8%)	13 (100%)
Mupirocin	2 (100%)	38 (100%)	9 (69.2%)
Oxacillin	2 (100%)	38 (100%)	0(0%)
Rifampin	2 (100%)	33 (86.8%)	13 (100%)
Teicoplanin	2 (100%)	38 (100%)	13 (100%)
Telithromycin	2 (100%)	29 (76.3%)	12 (92.3%)
Tigecycline	2 (100%)	38 (100%)	13 (100%)
Trimethoprim-sulphamethazole	2 (100%)	37 (97.4%)	2 (15.3%)
Vancomycin	2 (100%)	38 (100%)	13 (100%)

Increasing cases of MRSA infections among healthy individuals have inclined concerns worldwide. MRSA strains are resistant to nearly the entire family of β-lactam antibiotics due to the possession of an extra penicillin-binding protein PBP2a [17]. In this study shown all MRSA are resistant to oxacillin that stay resistant based on the previous study [18].

This result proves a few studies that have defined a low prevalence of PVL genes in *S. aureus* (<5%) and a particularly low prevalence in HA-MRSA [19]. Generally, PVL is used as a marker for community acquired MRSA that important for soft-tissue and deep dermal infections [20]. Even though in this study revealed that there is very low percentage of PVL positive *S. aureus* among patients and healthcare professionals, prevention actions must be taken by the authorities as those people will interact with outside community which may be vulnerable.

4. Conclusion

The reported prevalence of the PVL among the patient and healthcare workers in this study was found relatively low. There were no significant differences in the susceptibility pattern of PVL positive and PVL negative isolates indicating that PVL is not associated with drug resistance mechanisms. In our view, the presence of PVL can be used as a potential marker for hospital acquired due to possible interaction with community. This study will help to incorporate the DNA amplification technology into the diagnostic laboratory to detect the PVL-positive *S. aureus* isolates.

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