



Investigation of Nasal *Staphylococcus aureus* carriage in hospital employees and rapid detection of PVL and *mecA* genes by RT-PCR

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Abstract: *Staphylococcus aureus* (*S. aureus*) can cause serious fatal infections due to several enzymes and toxins that it secretes when it encounters favorable conditions. Thus, *S. aureus* is one of the most important pathogens currently causing hospital and community infections. Methicillin-resistant *S. aureus* (MRSA) strains are resistant to many antibiotics and have the ability to rapidly develop resistance to currently used antibiotics during treatment. MRSA origins cause serious infections with high mortality and also increase the rate of hospital infections that require difficult and longer hospitalization and the use of greater quantities of antibiotics in risk-prone units in hospitals. Another factor that is as important as MRSA in *S. aureus* pathogenicity is the Panton-Valentine leukocidin (PVL) toxin secreted by *S. aureus* strains, most commonly isolated from deadly necrotizing pneumonias and community-acquired soft skin tissue infections. PVL is especially observed in community-acquired *S. aureus* strains. However, studies showed that PVL-positive origins began to spread rapidly to hospital environments. In this study, methicillin-sensitive *S. aureus*, methicillin-resistant coagulase-negative *Staphylococcus* (MR-CNS), and Panton-Valentine leukocidine genes were identified by real-time polymerase chain reaction (RT-PCR) by investigating the rate of nasal *S. aureus* carriers (NSAC) in nasal swab samples collected from hospital staff belonging to the highest risk group for staphylococcal infections, at which screening is aimed. Classical NSAC scans were performed on nasal swab samples collected from 98 healthcare professionals in Hospital. Nasal *S. aureus* (4 MSSA, 13 MRSA) was detected in 17 samples (17.35%). During detection, 48 MR-CNS were detected. In addition, 61 *mecA* (+), 17 *nuc* (+), 1 *pvl* (+) genes were detected in RT-PCR samples directly from the nasal swabs collected in accordance with the culture method.

Key words: *mecA*, MRSA, *nuc*, *pvl*, *S. aureus*.

Hastane çalışanlarında Nazal *Staphylococcus aureus* taşıyıcılığı ve PVL ile *mecA* genlerinin RT-PCR ile hızlı tespiti

Özet: *Staphylococcus aureus* (*S. aureus*) kommensal bir bakteri olarak hem insanla beraber yaşamakta, hem de fırsatçı olarak uygun koşulları bulduğunda salgıladığı bazı enzim ve toksinler nedeniyle çok ciddi ölümcül enfeksiyonlara neden olabilmektedir. Bu nedenle günümüzde *S. aureus* hastane ve toplum kaynaklı enfeksiyonlara neden olan en önemli patojenlerden biridir. Metisilin dirençli *S. aureus* (MRSA) kökenleri birçok antibiyotiğe karşı dirençlidirler ve tedavi süresince kullanılan günümüz antibiyotiklerine karşı çok hızlı bir şekilde direnç geliştirme kabiliyetine sahiptirler. Bundan dolayıdır ki MRSA enfeksiyonları normal *S. aureus* enfeksiyonlarına oranla daha şiddetli ve inatçı enfeksiyonlara neden olmaktadır. *S. aureus* patojenitesinde MRSA kadar önemli olan diğer bir etken, özellikle ölümcül nekrotizan pnömonilerden ve toplum kökenli deri, yumuşak doku enfeksiyonlarından izole edilen *S. aureus* suşları tarafından salgılanan Panton-Valentine lökositidin (PVL) toksinidir. PVL özellikle toplum kökenli *S. aureus* suşlarında gözlenmektedir. Ancak yapılan çalışmalar PVL pozitif kökenlilerin hastane ortamlarına hızla yayılmaya başladığını göstermiştir. Bu çalışmada stafilocok enfeksiyonları için en yüksek risk grubunda yer alan hastane personelinin alınan nazal swap örneklerinde nazal *S. aureus* taşıyıcılığı (NSAT) oranının araştırılması ile rt-PCR ile Metisiline Duyarlı *S. aureus*, Metisiline Dirençli Koagülaz Negatif Stafilocok ve Panton-Valentine lökositidin virülans genlerinin taramasının yapılması amaçlanmıştır. Devlet hastanesinde çalışan personelden alınan nazal swap örneklerinden klasik NSAT taraması yapılmış 98 sağlık personelinin 17'sinde (%17,35) nazal *S. aureus* taşıyıcılığı (4'ü MSSA, 13'ü MRSA) saptanırken, 48'inde MR-KNS tespit edilmiştir. Ayrıca alınan nazal swablardan direk olarak yapılan RT-PCR örneklerinde kültür metodu ile birebir uyumlu olarak 61 *mecA*(+), 17 *nuc*(+), 1 *pvl*(+) genleri tespit edilmiştir.

Anahtar kelimeler: *mecA*, MRSA, *nuc*, *pvl*, *S. aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) is a commensal pathogen that lives in close proximity to humans and causes opportunistic serious infections when it finds suitable conditions (Tong et al. 2015). *S. aureus* colonization takes place in the normal flora in many body regions, especially the skin and mucous membranes in the mammalian (Haag et al. 2019). *S. aureus* is mostly observed in the nasal mucosa and reports suggest that it is transported in the nasal mucosa in 20%–30% of the population (Lee et al. 2018). When mucosal and cutaneous barriers break down in the normal nasal mucosa, *S. aureus* can enter the bloodstream and cause serious infections in the heart, skin, soft tissues, bones and joints (Peacock and Paterson 2015). The pathogenicity of *S. aureus* varies according to the fact that Panton-Valentine leukocidin (PVL) carry a toxin and originate from methicillin-resistant *S. aureus* (MRSA) (Gordon and Lowy 2008).

MRSA resistance, which is increasingly relevant worldwide, results from a modified protein called penicillin-binding protein 2a/2 (PBP2a/PBP2') encoded by the *mecA* gene (Sardan 2000, Zhu et al. 2006). MRSA strains with the *mecA* gene are resistant to all beta-lactams and beta-lactam-derived antibiotics (Ozel et al. 2011; Keyvan and Ozdemir, 2016).

The epidemiology and characteristics of MRSA differ in different geographic regions. With the increase in resistance problems in infections caused by MRSA, MRSA has become a serious health problem causing nosocomial epidemics worldwide (Bastidas et al. 2019). The origins of MRSA are categorized as hospital-associated (HA-MRSA) and community-associated (CA-MRSA). Compared to HA-MRSA strains, CA-MRSAs are generally sensitive to macrolides and fluoroquinolone antibiotics and are capable of producing PVL toxins (Deurenberg et al. 2004). PVL is a two-component cytotoxin that can render defense cells inactive by creating pores in immune system cells, including basophils, leukocytes, monocytes, and macrophages, in the human body (Meyer et al. 2009). The high virulence potential of CA-MRSA strains containing the PVL gene is due to the suppression of leukocytes during infection (Vandenesch et al. 2009). The PVL gene plays a critical role in the escape of *S. aureus* from the immune system and the clinical course is quite severe in the infections it causes (McDonald et al. 2005). Death is more common in CA-MRSA infections with PVL toxin, and the PVL gene is particularly observed

in CA-MRSA strains. However, studies have shown that PVL-positive strains begin to spread rapidly in hospital settings (Duman et al. 2013). Besides, it is reported that 20%–30% of hospitalized patients are colonized with the strain that is common in that hospital within the first 5–10 days (Uluğ 2012). Therefore, the rapid detection of microorganisms colonized in hospitals by molecular methods is a very important issue for both the treatment process and the implementation process of infection-control policies in the context of public health (Navarro et al. 2008). Further, although some CA-MRSA isolates contain the *mecA* gene, the methicillin minimum inhibitory concentration (MIC) values can be very low. In antibiotic susceptibility tests, these can result in mistaken identification as methicillin-sensitive *S. aureus* (MSSA). For this reason, it is recommended that the presence of the *mecA* gene be determined by PCR, considering that resistance cannot be detected in serious infections that do not respond to treatment (Gülay 2008). In addition, this method of determining the resistance of methicillin in MRSA strains, which are prominent with multiple resistances, is reliable, easy to apply, and gives quick results in routine laboratories. Therefore, in terms of infection-control policies, the method is very important in both treatment and application processes (Teng et al. 2009).

In this study, the *mecA* gene, which is the gold standard for determining the resistance of methicillin with both the classical culture and real-time polymerase chain reaction (RT-PCR), and the PVL virulence gene in MSSA and MRSA were scanned in nasal swab samples collected from health personnel working at 29 Mayıs State Hospital in Ankara.

Materials and Methods

Ethics committee approval was obtained from the Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Research Ethics Committee. With the approval of the ethics committee dated September 12, 2019 and numbered 77/18, 98 swab samples were collected from sterile swabs soaked in sterile saline from both nostrils of health personnel working in the 29 May State Hospital.

Determination of Bacteria Isolation and Methicillin Resistance

Samples were inoculated on 5% sheep-blood agar by using single streak colony reduction method. Those samples were incubated at 35°C for another

24 h, in which more than 10 colonies considered to be significant grew on the plate, and samples those grown fewer colonies were not considered.

After the colony morphology was examined, Gram staining was performed, and those strains with gram-positive cocci morphology were included in the study, and catalase, coagulase tests were performed. Those strains with positive catalase and coagulase tests were classified as *S. aureus*. The cefoxitin disk was used to determine methicillin resistance. A suspension of 0.5 McFarland turbidity was prepared from the isolates and applied to Mueller Hinton agar (Merck, Germany). Thirty micrograms of cefoxitin disks (Oxoid) were placed on the medium and incubated at 35°C for 24 h. Isolates with zone sizes ≤ 21 mm were considered to be methicillin resistant (CLSI, 2015). The cefoxitin disk-diffusion (30 µg) method, which has higher sensi-

tivity and specificity than the oxacillin disk-diffusion test, was used to determine methicillin resistance in the CNS (Swenson et al. 2007). *S. aureus* ATCC 25923 and ATCC 43300 were used as control strains.

In-house Real-Time Polymerase Chain Reaction

In the triplex real-time PCR study, the primers to be used for the detection of *PVL*, *mecA* (for the detection of methicillin resistance), and *nuc* (for the identification of *S. aureus*) genes were synthesized by MacroGen Inc. considering the base pairs indicated by Galia et al. (Table 1).

Nasal swabs from ATCC 25923 (MSSA) and ATCC 43300 (MRSA) were used as a negative control with reference ATCC 25922 *E. coli* strain. DNA isolation with a Bacterial DNA isolation kit (AMBRD, Turkey) according to the kit protocol was performed by Soygen Biotechnology.

Table 1. Primers and probes used in the study (Galia et al. 2019).

| | GenBank® Access number | Primer/Probe | Position | Base pair | |
|-------------|------------------------|-------------------|---------------------------------|-----------|-----|
| <i>mecA</i> | KC243783.1 | <i>mecA</i> fw | CAATGCCAAAATCTCAGGTAAAGTG | 1931–1957 | 107 |
| | | <i>mecA</i> rev | AACCATCGTTACGGATTGCTTC | 2018–2018 | |
| | | <i>mecA</i> Probe | CY5–ATGAGCTATATGAGAACGG–BHQ2 | 1958–1979 | |
| <i>pvl</i> | X72700.1 | <i>pvl</i> fw | AAATGCTGGACAAAACCTTCTTGG | 606–624 | 108 |
| | | <i>pvl</i> rev | TTTGACGCTTTTGTTCG | 693–712 | |
| | | <i>pvl</i> Probe | HEX–AAATGCCAGTGTATCC–BHQ1 | 636–653 | |
| <i>nuc</i> | DQ50738 | <i>nuc</i> fw | GGCATATGTATGGCAATTGTTC | 25–48 | 73 |
| | | <i>nuc</i> rev | CGTATTGCCCTTCGAAACATT | 76–97 | |
| | | <i>nuc</i> Probe | FAM–ATTACTTATAGGGATGGCTATC–BHQ1 | 49–71 | |

An optimization study was performed with ATCC 25923 (MSSA) and ATCC 43300 reference strains to determine the optimal working concentration of synthesized primers and probes. According to the results of this study, 10 µl DNA, 2.5 µl primers (900 nM) and probe (250 nM), and 10 µl master mix were arranged in 25 µl reactions. The heat cycle was adjusted as 95°C pre-denaturation for 5 min, 40 cycles of two steps at 95°C for 15 s for denaturation, and 60°C for 30 s for binding elongation.

Results

In the current study, the classical culture and triplex real-time PCR methods were applied to the nasal swab samples of 98 healthcare workers. The results

obtained with the culture method and triplex real-time PCR method (Table 1) were 100% compatible.

In the triplex real-time PCR results from the 98 nasal swab samples included in the study, the *nuc* gene in 17 samples, the *mecA* gene in 61 samples, and the *pvl* gene in only one sample were determined in a short time. Among the results obtained with the classical culture and triplex real-time PCR methods, 13 MRSA, 4 MSSA, 48 MR-CN and 25 MS-CNS isolates were detected, while eight of them could not be detected. These results given in Table 2. Today, although PVL strains begin to spread rapidly in hospital settings, in this study, only 1 of 98 healthcare personnel was found to have a positive PVL gene.

Table 2. Result of Triplex Real-time PCR test of 98 nasal swab samples

| nuc (+) | | nuc (-) | |
|--------------|--------------|---------------|---------------------------------|
| 17 | | 81 | |
| mecA(+) MRSA | mecA(-) MSSA | mecA(+)MR-KNS | mecA(-) MS-KNS or Stafilokok(-) |
| 13 | 4 | 48 | 33 |

Discussion

S. aureus is one of the pathogenic microorganisms, which can be found in the mucous membranes of the skin, upper respiratory system, lower urogenital system and digestive system of animals and humans (Ozel et al. 2011; Keyvan and Özdemir, 2016). Also, *S. aureus* is one of the leading pathogens causing subclinical mastitis in dairy animals. Therefore, it causes contamination in milk and dairy products. They cause food poisoning with the different types of heat resistant enterotoxins produced. *S. aureus* is one of the most important factors of nosocomial infections. *S. aureus* quickly gains multiple antibiotic resistance, causing nosocomial and community related infections (Keyvan and Özdemir, 2016).

In terms of community health, rapid and accurate detection of hospital-borne MRSA isolates is crucial for the control of infection and the prevention of the nosocomial spread of bacteria. It has been determined that the spread of staphylococcal infections in the hospital environment occurs predominantly through the hands and clothes of the healthcare personnel. For this reason, healthcare personnel identified as nasal *S.aureus* carriers (NSAC) are an important source of transmission for MRSA (Uluğ 2012). In this study, the *mecA*, *nuc*, and PVL genes were scanned in nasal swab samples collected from health personnel working at the 29 Mayıs State Hospital using both the classical culture and triplex real-time PCR methods. While 13 MRSA, 4 MSSA, 48 methicillin-resistant coagulase-negative *Staphylococcus* (MR-CNS), and 25 MS-CNS isolates were detected in the 98 nasal swab samples included in the study, in eight of them *Staphylococcus* spp. could not be detected. *Staphylococcus* spp. was detected in 90 (91.83%) of the 98 nasal swab samples included in the study, and the NSAC rate of healthcare professionals working at 29 Mayıs State Hospital was determined as 17.34%. Furthermore, MR-CNS was detected in 48.97% of hospital staff. It is thought that the high rate of NSAC and MR-CNS in hospital personnel is due to the collection of nasal swabs during the winter period when the rate of upper respiratory tract infections is highest.

It is known that the determination of methicillin resistance is very important for the course and treatment of *S. aureus* infections. False-negative results can cause inadequate treatment during infection as well as the spread of MRSA origin in the community and transmission even to healthy individuals without any risk factors. Treatment options are restricted in infections caused by MRSA, and mortality and morbidity increase. Furthermore, false-positive

results lead to unnecessary use of antibiotics in patients (Telli et al. 2006). Therefore, the rapid and reliable detection of MRSA, which can rapidly develop resistance to currently used antibiotics, is very important for public health. Furthermore, the method chosen for determining resistance must be able to be used in routine laboratories and to be easily applied.

Although there are multiple methods to identify and confirm methicillin resistance in *S. aureus* strains, the time required to obtain results with conventional methods varies between 2–4 days (Özen et al. 2011). Currently, thanks to technological progress, MRSA verification can be performed in 3 h using molecular methods such as PCR (Kuzucu et al. 2002; Cesur et al. 2010; Okolie et al. 2015).

In a study by Okolie et al. (2015), *nuc* and *spa* genes were detected using pentaplex PCR for the detection of *S. aureus* in clinical samples. In this study, PCR analysis was performed after pre-enrichment of the samples. Galia et al. (2019), on the other hand, determined the rate of MSSA, MRSA, and MR-KNS in conventional rectal and pharyngeal swabs using triplex real-time PCR. In the study, the authors differentiated MRSA, MSSA, and MR-KNS through the direct detection of *nuc* and *mecA* genes in a short time (3 h) using triplex real-time PCR without any enrichment of the swab samples. Furthermore, Galia et al. (2019) detected the PVL gene in their study. In the current study, both classical and molecular methods were compared and one-to-one compatibility was found between the methods. J.R.Perez et al. found that all MRSA isolates carry the PVL gene in their study. When we compared our results with the literature, we found that PVL gene positivity in *S. aureus* carriers in our hospital was very low. In our study, only one of the 13 MRSA positive cases was positive (Pérez et al. 2011).

Even though PVL gene positivity, which has an important role in bacterial virulence in our study, is very low, it is an issue to be kept in mind in common and persistent MRSA (+) hospital infections considering its role in infectiousness. Triplex real-time PCR is very important in the implementation of treatment and infection-control strategies for rapid and reliable results that can be obtained in a short time (3–4 h) for MRSA confirmations, which are of great importance for public health.

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