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Nasal Colonization of Serine Protease *esp*-Positive *Staphylococcus epidermidis* Affecting *Staphylococcus aureus*

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Abstract

Background: Staphylococcus aureus is a common colonizer of the nasal vestibule found in approximately 20%–30% of healthy adults, while *Staphylococcus epidermidis* appears to be the most frequent colonizer in all regions of the upper respiratory tract. Esp, a serine protease of *S. epidermidis*, was reported to inhibit *S. aureus* colonization. This study was performed to examine the nasal colonization of *S. aureus* and *S. epidermidis* and the presence of *esp* determinants. *Methods:* Nasal swab specimens from 54 patients were cultured on blood agar plates (BAP) and selective media for *S. aureus* for bacteria isolation. After 48 hours of incubation with BAP, three or four colonies suspected of being coagulase-negative staphylococci (CNS) were identified by mass spectrometry. Polymerase chain reaction (PCR) for *esp* was performed on all CNS isolates identified as *S. epidermidis*. *Results:* Forty-three *S. epidermidis* strains were isolated from 18 (33.3%) of the 54 patients. Nine (50.0%) of the 18 patients carried *S. aureus*, while the other nine did not. Of the 36 *S. epidermidis* non-carriers, 13 (36.1%) were colonized by *S. aureus*. All *S. epidermidis* strains were confirmed by PCR to have *esp* determinants. *Conclusion: S. epidermidis* colonization did not affect *S. aureus* colonization in the nasal cavity. All *S. epidermidis* strains harbored the *esp* gene. There were no differences in the nasal colonization rates of *S. aureus* according to the presence of *esp*-positive *S. epidermidis*. Further research on the characterization of *S. epidermidis* in Korea is needed to understand the association between *S. epidermidis* and *S. aureus* colonization.

Keywords

Colonization
esp
Serine protease
Staphylococcus aureus
Staphylococcus epidermidis

1. Introduction

Staphylococcus aureus colonizes the human nasal cavity, and 20%–30% of people carry *S. aureus* in their nasal cavity. Hospitalized patients with nasal carriage of *S. aureus* are at increased risk of opportunistic infections caused by *S. aureus*, sometimes leading to serious infections, including wound infections [1,2]. It has also been suggested that screening and treatment of *S. aureus* carriers on admission may reduce the risk of postoperative wound infection [3-5]. In general, the colonization of the nasal cavity by bacteria involves a complex interplay of factors such as the ability to adhere to nasal tissue, nutrients required for growth, production of antimicrobial substances, and interactions between strains and the body's immune system [6]. Some Staphylococcus species form biofilms composed of complexes of polysaccharides, proteins, teichoic acid, and extracellular DNA to protect themselves from human defense mechanisms, antibiotics, and disinfectants [7].

Staphylococcus epidermidis colonizes mainly on the skin and in the nasal cavity, and it has been reported that *S. epidermidis*, which produces Esp, a type of serine protease, inhibits the biofilm production of *S. aureus*, thereby inhibiting *S. aureus* from colonizing the nasal cavity [8,9]. Since Esp can degrade several proteins involved in biofilm formation, including extracellular adhesion protein, extracellular matrix protein-binding protein, fibronectin-binding protein A, and protein A [9], there are attempts to use the properties of Esp-producing *S. epidermidis* to prevent or treat *S. aureus* carriage.

Several existing studies have shown that Esp-producing *S. epidermidis* inhibits the formation of nasal colonies of *S. aureus*, but there are no reports on the possession of *esp* in clinical strains of *S. epidermidis* in Korea. This study was conducted to investigate the intranasal carriage of *S. aureus* and *S. epidermidis* in hospitalized patients and to determine the association between *S. aureus* carriage inhibition and *esp*-positive *S. epidermidis*.

2. Materials and methods

2.1. Subjects and specimens

Nasal specimens from 54 patients admitted to a general hospital in Seoul from July to September 2014 were inoculated on blood agar plates (BAP) and *S. aureus* ID (SAID; bioMerieux, Marcy-l'Etoile, France), a selection medium, and incubated at 35°C for 48 hours under aerobic condition. Green colonies suspected to be *S. aureus* were selected from the *S. aureus* selection medium, and 3–4 white colonies suspected to be coagulase-negative staphylococci were selected and sub-cultured on BAP and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, Bremen, Germany) to confirm that they were *S. epidermidis*. To investigate the presence of *esp* genes in *S. epidermidis* strains isolated from the nasal cavity, *S. epidermidis* 19 strains isolated from the blood of other patients during the same period were additionally included in the study (KD-2015-01).

2.2. *Staphylococcus epidermidis esp* gene detection using PCR

S. epidermidis esp (serine protease) gene detection was performed using polymerase chain reaction (PCR) as previously reported [8]. DNA extraction was performed using HiYield Genomic DNA Mini kit (RBC, New Taipei City, Taiwan), and PCR primers (Esp_F: TTTGGAGGTTATCATATGAAAAAGAG; Esp_R: CTGAATATTTATATCAGGTATATTGTTTC) were used with Solgent Taq polymerase kit (Solgent, Daejeon, Korea) at an initial stage of 95°C for 4 min followed by 30 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 40 s, and a final stage of 72°C for 4 min. A PCR product of 854 bp was identified after electrophoresis on a 2% agarose gel.

2.3. Statistical analysis

The chi-squared test was performed with Sigma plot software 13.0 (Systat Software, Ekrath, Germany) for *S.*

epidermidis carrier and *S. aureus* carrier data.

3. Results

S. epidermidis was detected in 18 (33.3%) of 54 subjects. *S. aureus* was detected in 22 of 54 (40.7%). Among the 18 *S. epidermidis* carriers, *S. aureus* was cultured in 9 (50.0%), compared to 13 (36.1%) of 36 *S. epidermidis* non-carriers (**Table 1**). The difference in *S. aureus* carriers according to *S. epidermidis* carriers was not statistically significant (P = 0.493). All *S. epidermidis* strains had the *esp* gene detected, so no differences were observed based on the *esp* gene carriage.

In total, 43 *S. epidermidis* strains were tested for *esp* PCR, including duplicate strains from 18 patients in whom *S. epidermidis* was isolated from the nasal cavity, and all strains were positive for *esp* PCR. For comparison, 19 strains of *S. epidermidis* isolated from the blood of other patients were also all *esp* PCR positive.

4. Discussion

S. aureus and *S. epidermidis* are commonly isolated from the nasal cavity, and *S. aureus* carriers are known to be at increased risk of opportunistic infections with *S. aureus*. *S. epidermidis* is an important cause of nosocomial infections and is a common cause of catheter-associated infections. *S. epidermidis* has few virulence factors and colonizes the human skin flora in balance with other bacteria [10]. The factors responsible for colonization are difficult to determine due to the complexity of the interactions between bacteria and their hosts, but several studies have attempted to inhibit pathogenic microorganisms with other microorganisms, and recently, *S. epidermidis* in the nasal cavity of mice has been shown to have antiviral effects against influenza viruses by inducing an immune response [11].

There is a study that Esp, a serine protease of *S. epidermidis*, can be used as a biofilm inhibitor of *S. aureus* because it has the property of degrading *S. aureus* biofilm [8]. In other words, some strains of *S. epidermidis* that inhibit the formation of *S. aureus* biofilm make it difficult for *S. aureus* to colonize the nasal cavity, so carriers of these strains have a lower rate of nasal carriage of *S. aureus*, and it is possible that Esp can be used to eliminate carriers of *S. aureus* and further use it for treatment. However, there are few investigations of the *esp* gene in clinically isolated *S. epidermidis* strains in Korea.

In this study, we cultured *S. aureus* and *S. epidermidis* from nasal specimens of patients to determine the effect of carriage of each strain and to investigate the presence of *esp* genes in *S. epidermidis* strains. However, all of the *S. epidermidis* strains were positive for the *esp* gene, and several *S. epidermidis* strains isolated from blood were also *esp*-positive, suggesting that the *esp* gene is present in most strains. Therefore, it was difficult to estimate the association between the presence or absence of *esp* in *S. epidermidis* strains and nasal colonization of *S. aureus* strains, and future studies should consider strain

Table 1. Nasal colonization of *Staphylococcus aureus* and *Staphylococcus epidermidis* for its presence of *esp* gene (n = 54)

Groups		*S. aureus* colonization	
		Carrier (n = 22)	Non-carrier (n = 32)
S. epidermidis colonization	Carrier (n = 18)	9	9
	Non-carrier (n = 36)	13	23
esp-positive *S. epidermidis* colonization*	Carrier (n = 18)	9	9
	Non-carrier (n = 36)	13	23

*All the *S. epidermidis* isolates showed *esp* gene positivity by polymerase chain reaction (PCR)

characteristics of *S. epidermidis* in Korea and abroad. However, this study did not examine the presence or absence of the *esp* gene to measure Esp production and serine protease activity, so direct comparisons are difficult.

The nasal cavity is home to a diverse microbiota [1,6,12], so it may be difficult to explain the colonization of other bacteria by a single bacterium with certain characteristics. Colonization of the nasal cavity by *S. aureus* is influenced by many factors, including bacterial factors such as *S. epidermidis*, *Streptococcus pneumoniae*, and *Corynebacterium spp*., genetic factors such as interleukin-4 (IL-4), immunological factors such as Toll-like receptor 2 (TLR2), antimicrobial peptides, various antibodies, age, sex, and smoking [2]. Therefore, it may be difficult to identify associations based on single gene characteristics of a single strain, as in this study.

The paper that first described Esp and its effect on *S. aureus* colonization found that *S. epidermidis* capable of inhibiting *S. aureus* biofilms comprised 51.2% of all *S. epidermidis* [8], but did not report the presence or absence of the *esp* gene in these strains. On the other hand, a study of healthy adolescents in Norway found no association between nasal carriage of *S. aureus* and the biofilm inhibitory ability of *S. epidermidis*, and *S. epidermidis* culture supernatants were unable to inhibit *S. aureus* carriage, even for strains that inhibit biofilm of *S. aureus* [12], and various clinical studies will be needed to prove the relationship between the two. In addition, a study of neonates reported in the United States in 2019 suggested that the presence of *S. epidermidis* in the nasal cavity had the effect of preventing the colonization of *S. aureus*, but there may be a protease with a mechanism other than Esp [13].

Inhibition of nasal colonization of *S. aureus* is expected to reduce endogenous *S. aureus* infections in patients, and it is known that Esp in *S. epidermidis* can inhibit biofilm formation of *S. aureus*, but the direct effect of Esp expression on biofilm inhibition of *S. aureus* is still unclear, and it has been suggested that about two-thirds of *S. epidermidis* strains does not inhibit *S. aureus* growth but does inhibit biofilm, which is also independent of Esp expression [14]. Therefore, the ability of *S. epidermidis* to inhibit *S. aureus* carriage in the intranasal cavity requires further study.

In this study, we investigated the carriage of *S. aureus* and *S. epidermidis* in the nasal cavity of hospitalized patients and examined the association between *S. epidermidis* and *S. aureus* with intranasal Esp positivity. The results showed that most of the *S. epidermidis* were *esp*-positive, and the presence or absence of *esp* in *S. epidermidis* did not seem to affect the nasal colonization of *S. aureus*.

Disclosure statement

The authors declare no conflict of interest.

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