# Association between Persistent *Staphylococcus aureus* Nasal Carriers with High Frequency of Skin Abscesses Among American Football Players

**SUZUKI Kotaro** [*] , SHIBA Hideki [*] , ARAI Nobunari [**] ,
GOCHI Kentaro [*] , MATSUMOTO Tsuyoshi [*] and TAGAMI Kazumi [*]

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**Appendix:** This project report submitted for publication in a scientific journal. Primary results were reported in this study.

## 1. Introduction

*Staphylococcus aureus* (*SA*) is a common bacterium that often resides harmlessly on the skin or in the nasal cavities of healthy individuals. It is both a human commensal and a frequent source of infections that can be mild or life-threatening. The primary reservoir of *SA* is thought to be the anterior nares (nostrils). About 25% to 50% of individuals carry nasal *SA* in at any given time[1], but this does not necessarily manifest as clinical infection. However, *SA* can cause deep skin infections[2].

Risk factors for *SA* that contribute to a high incidence of infection among athletes include close physical contact[3,4], skin damage[5] and sharing contaminated equipment[6]. Direct physical contact of athletes with exposed extremities during practice or competition can lead to minor abrasions and create portals of entry for *SA*. Most *SA* infections in athletes are caused by minor skin cuts and *SA* is one of the major sources of wound infections in American football players[7,8]. Contact sports provide players with more opportunities for skin infections due to skin trauma from turf burns, shaving and contact with secretions and body fluids[7]. Additionally, *SA* typically infects cutaneous skin and spreads to form clusters, although common-source exposure can contribute to transmission[8].

Nasal carriage of *SA* is an important risk factor for infection in both community and hospital settings[9]. Longitudinal studies have shown that healthy adults can be differentiated into the following types of *SA* carriers: 10% - 20% are persistent carriers (PC), 30% - 50% are intermittently colonized and are thus intermittent carriers (IC), and the remainder comprises non-carriers (NC), who apparently never carry nasal *SA*. The risk of SA infection among IC and occasional carriers (OC) is similar to that of NC, suggesting that only PC and OC actually exist[10]. Carriage rates are higher among medical patients[9]. Skin wounds have recently been identified as triggers of persistent nasal carriage of *SA* and PC are at higher risk of developing *SA* infections. The mechanisms involved in nasal *SA* colonization and growth at skin lesions are largely unknown.

Skin abscesses might also play a role in nasal *SA* carriage due to a difference in carriage within populations and nasal carriage as a source of *SA*. Skin abscess formation is a pathogen-driven process that usurps the default response of the host and enhances microbial replication and dissemination[11]. Also, the frequency of nasal colonization with *SA* in the general population has increased in parallel with an increase in that of *SA* skin infection[12]. Thus, skin abscesses are potential sources of nasal *SA* carriage.

Biofilms are bacterial communities that are resistant to host immune responses and antimicrobial agents[13] and whether they play roles in nasal colonization remains a matter of debate[14]. Colonization of the nasal epithelium[15] and the mucosa of patients with chronic rhinosinusitis[16] with *SA* strains has recently been associated with their ability to form biofilms *in vitro*. Thus, we investigated

[*] Faculty of Health and Sport Sciences, University of Tsukuba
[**] Faculty of Education, Hakuoh University

whether nasal biofilm formation is involved in persistent nasal *SA* colonization in a population of athletes involved in a contact sport.

We found an association between skin abscesses and persistent nasal *SA* carriage, including nasal *SA* density and biofilm formation. Furthermore, our results suggested that persistent *SA* carriers should be profiled among populations of athletes.

## 2. Materials and methods

### Subjects

Thirty-six male (age: 20.5 ± 1.2 years; height: 172.3 ± 5.4 cm; body mass: 81.3 ± 10.8 kg, BMI: 27.3) collegiate American football players participated in the present study. The same person collected nasal samples from the players between January and November 2012. All of the players completed a questionnaire regarding basic demographic information and clinical data about abscesses and skin lesions. The research protocol was approved by the institutional ethical review board, and volunteers signed informed consent forms before entering the study.

### Bacterial isolates

Samples collected from the right or left anterior nares using sterile swabs were mixed with 100 μL of phosphate-buffered saline (PBS) and then 10 μL portions were spread on compact Dry X-SA media (CD-XSA; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and incubated at 37ºC for 24 ± 2 h. Specific blue *SA* colonies[17] were counted and *SA* was extracted from the media to determine biofilm formation.

### Definitions of carriers

The types of *SA* nasal carriage comprise persistent (PC), occasional (OC), intermittent (IC) and non- (NC) carriage. However, how to precisely identify these states has not reached consensus, but most studies have been based on cultures from 10 - 12 weekly nasal swabs[18]. We defined the carrier index as the number of positive/ number of total swabs for each player. We excluded those with < 4 swabs during the study period. We defined PC, IC, OC and NC as *SA* positivity in five of seven swabs, four swabs, three consecutive swabs or sporadic, and three or less and none, respectively.

### Biofilm formation assay

*Staphylococcus aureus* cells cultured in Luria-Bertani (LB) overnight were 100 fold diluted in Tryptic Soy Broth (Sigma-Aldrich, Tokyo, Japan), mixed with 3% NaCl and 0.5% glucose and then incubated at 96-well plates at 37ºC for 24 h. The cells were washed twice with distilled water and stained with 200 μL of 0.3% crystal violet (CV) as previously described[19], rinsed twice with 300 μL of double-distilled water (DDW) and thoroughly dried. The plates were shaken for 1 h incubation at room temperature with 200 μL of 95% ethanol per well. After incubation, the optical density (OD) at 595 nm was measured using a plate reader (BIO RAD, Hercules, CA, USA).

### Statistical data analysis

The statistical significance of *SA* colony counts and biofilm formation among carriers was determined using Student's t-test. Statistical significance was set at $P < 0.05$.

## 3. Results

We obtained seven swabs from each player for a total of 217 swabs. According to our definitions, 5 (13.8%), 4 (11.1%), 17 (47.2%) and 10 (27.8 %) were PC, IC, OC and NC (Fig. 1). None of the isolates obtained in the present study carried the *mecA* gene encoding methicillin resistance (data not shown).

We compared the frequency of skin abscesses in each *SA* nasal carrier (Table 1) based on responses to three questionnaires. An average of 3.60 of 5 PC had skin abscesses, most of which were located in the knees and elbows (Fig. 2), and in the armpit of one. During the study, three carriers each caused severe abscesses in 32, 34 and 36 players. Conversely, four OC and 18 IC had an average of 1.25 and 0.76 skin abscesses, respectively (Table 1), most of which were located at the knees and elbows (Fig. 2). Ten NC had an average of 1.20 skin abscesses, and these were sporadically located at the knees, shanks, fingers and ankles. Skin abscesses in PC who had been nasally infected with *SA* over the long term mostly presented in the knees and elbows.

Mean colony counts were significantly higher among PC than IC, and OC (109.9 ± 84.5 vs. 72.6 ± 82.7 CFU/mL, $P = 0.038$) (Fig. 3A). The density of *SA* colonies recovered from nasal swabs of samples from PC varied from one to a few hundred (Fig. 3B) and curves of kinetics differed among PC groups.

We compared relationships between amounts of nasal *SA* colonies and biofilm formation in six PC by

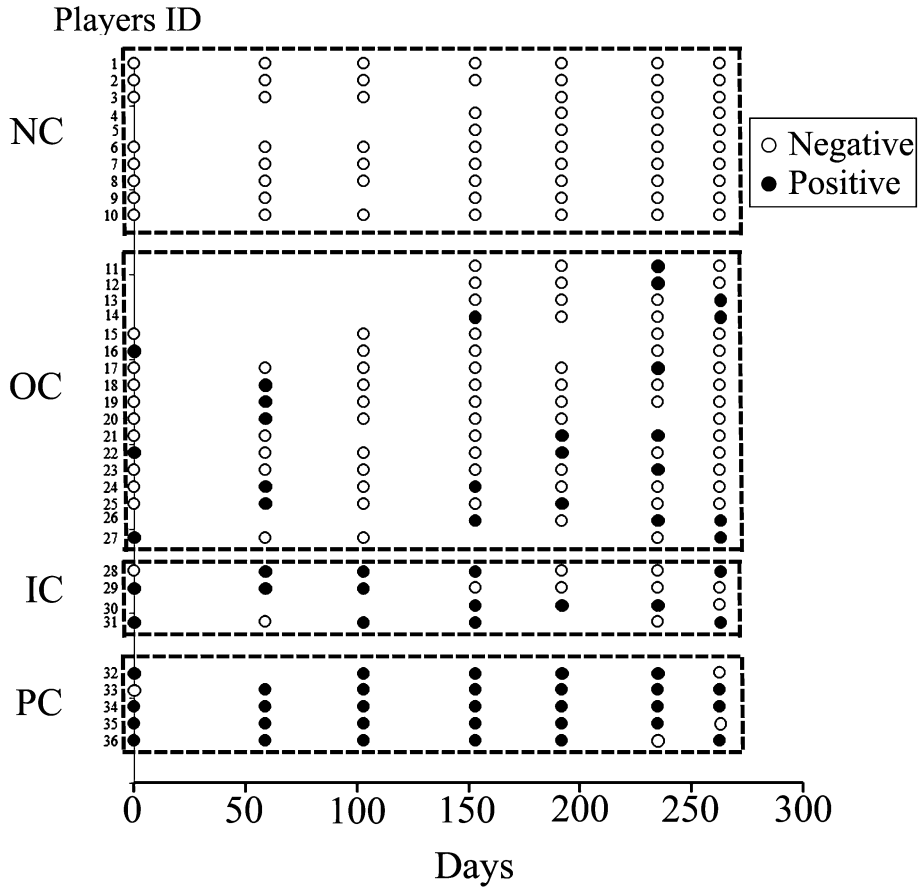


Fig. 1 Longitudinal distribution of *Staphylococcus aureus* nasal carrier indices among 36 collegiate American football players. Carrier indicator represents number of nasal swabs that were positive for *Staphylococcus aureus* among total number of swabs per player. Black and white circles, positive and negative samples respectively. IC, intermittent (n = 4); NC, Non (n = 10); OC, occasional (n = 17) and PC, persistent (n =5) carriers.

Table 1 Abscesses in carriers among American football players (n = 36).

| | [a]PC | [b]IC | [c]OC | [d]NC |
|---|---|---|---|---|
| Players (n) | 5 | 4 | 17 | 10 |
| Total abscess | 18 | 5 | 13 | 12 |
| [e]Average skin abscesses | 3.60 | 1.25 | 0.76 | 1.20 |

[a]PC, persistent carrier, [b]IC, intermittent carrier; [c]OC, occasional carrier; [d]NC, non-carrier. [e]Calculated

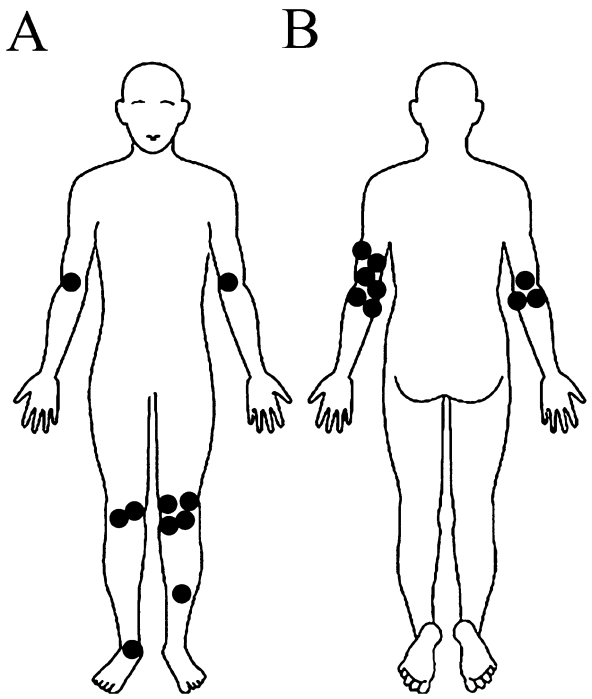


Fig. 2 Anatomical location of skin abscess in front (A) and on back (B) of players. Closed cycle ( ● ) indicates a site of skin lesions.

measuring adherence to a plastic membrane. The samples were categorized as high- ( ≥ 200), middle- (100−199) and low- (>99) CFU. This cutoff was selected based on the data shown in Fig. 3B. Fig. 4 shows the representative optical density (OD) of adherence. The OD of the biofilm formation was 2-fold higher in those with high-, than with middle- or low-CFU (0.497 ± 0.175 vs. 0.293 ± 0.261 and 0.268 ± 0.25; $P = 0.045$ and $P = 0.025$, respectively).

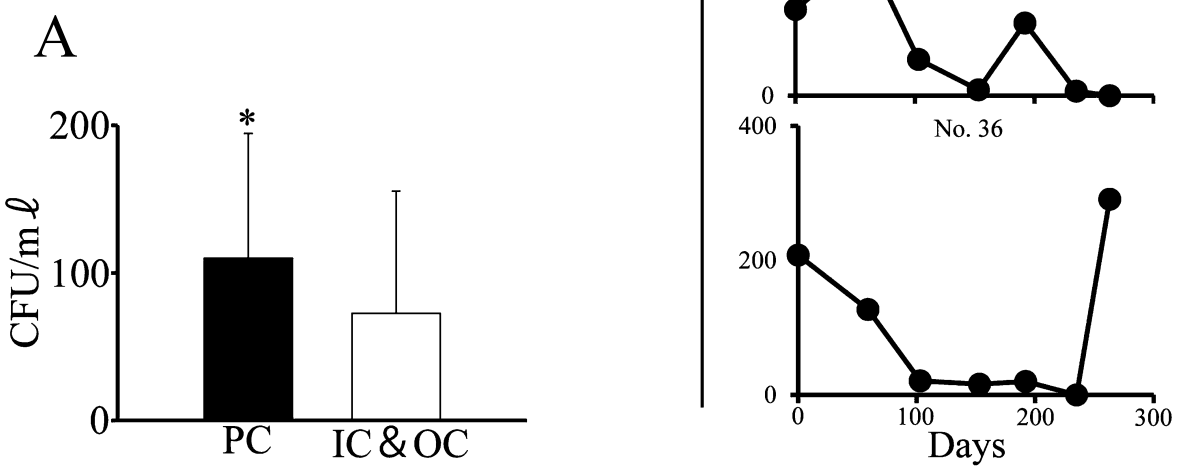


Fig. 3 Comparison of yield of *Staphylococcus aureus* colonies (A) and their kinetics (B) from nasal swabs of infected players. *Staphylococcus aureus* colony counts were visually determined. *Significantly different ($p < 0.05$) from intermittent and occasional carriers.

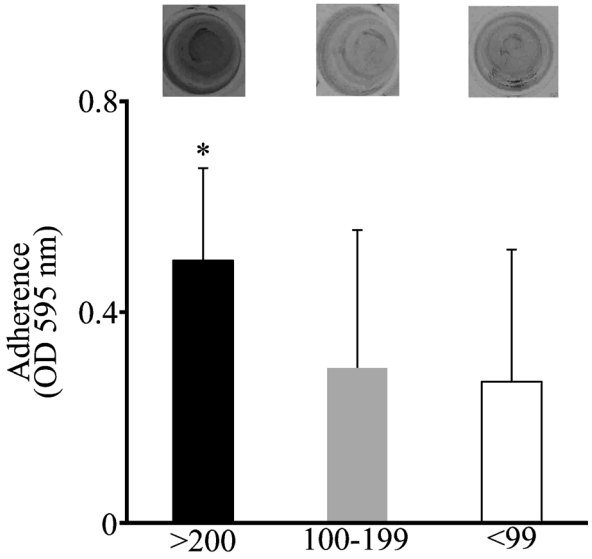


Fig. 4 Nasal colony counts are associated with *Staphylococcus aureus* biofilm formation in persistent carriers. Various concentrations of colony-forming units ( ≥ 200, 100 - 199, < 99) were generated from *Staphylococcus aureus* and biofilm formation was quantified using a microplate reader. Values are averages of quadruplicate readings from at least two separate experiments ± standard deviations. *$P < 0.05$.

## 4. Conclusion

1) We detected persistent *SA* nasal carriers (Fig. 1) in whom rates of rate of skin abscesses (Fig. 2) were higher than that in other carriers (Table 1).
2) The density of *SA* colonies that can be generated by nasal swabs obtained from carriers varies from single digits to a few hundred (Fig. 3B), and a high load of *SA* nasal colonization is closely associated with persistent carriage (Fig. 3A).
3) Biofilm formation depended on nasal *SA* density (Fig. 4).

## 5. Acknowledgments

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