Role of Nasal Staphylococcus aureus Carriage in Transmission among Contact Athletes

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Abstract
Among athletes, Staphylococcus aureus is thought to be transmitted by close physical contact with carriers. Nevertheless, evidence is limited with regard to both the tracking of individual strains and the role of S. aureus on the skin's surface. We investigated its transmission using molecular genotyping and the presence of S. aureus on the skin during exercise. In the first study, nasal samples were obtained from 172 athletes over a period of up to one year. The 200 strains of S. aureus collected from these athletes were genotyped, and transmission of S. aureus was detected by phage open reading frame typing (POT). In the second study, the presence of S. aureus on the skin's surface was compared between nasal carriers (n=9) and non-nasal carriers (n=9), who had participated in the first study. In the first study, 10 cases of transmission were confirmed. In the second study, exercise-induced sweating increased S. aureus isolates from the skin's surface (before vs. after exercise: 5.2 ± 5.4 vs. 41.7 ± 40.6 CFU/ml) in nasal carriers. In 5 of 9 nasal carriers, S. aureus isolates from the skin's surface were clonally identical to those from the nares. These results identify a major route of S. aureus transmission among athletes and provide insight into the role played by exercise-induced sweating in nasal carriers.

Introduction
Athletes who participate in contact sports frequently develop Staphylococcus aureus (SA) skin infections such as cellulitis, impetigo and abscesses that can disturb their sports activity [15]. These infections most commonly occur where the skin has been cut or scraped [1]. SA is highly contagious and a leading cause of disease outbreaks among athletes [3]. Factors associated with sports activity that are commonly implicated include compromised skin integrity [6], sharing items [8] and transmission by direct [3, 4] or indirect contact [9]. Outbreaks of SA infection can also disrupt or potentially eliminate opportunities for athletes and teams to compete at the highest level [3]. There has been little focus on the quality of care provided in the prevention of person-to-person SA transmission. Several studies reported SA transmission in athletes [10]. SA carrier status is usually determined using conventional culture media [2]. These methods do not provide reliable information regarding the transmission of individual strains. The primary reservoir of SA is the nasal cavity, and the nasal carriage is an established risk factor for transmission [16]. 2 factors may be associated with SA transmission in an athletic setting. First, nasal carriers also carry the organism on their hands, and contaminated hands are a likely means of transmission. The hands of carriers are often the primary vectors for nasal SA transmission [9]. Second, SA can live on the skin, and can easily be transmitted from one person to another via sweat. This route is likely the major mode of transmission. The high density of SA on the skin surface of nasal carriers is a result of sweating during exercise. Further investigations are needed on the relationship between nasal colonization and skin's surface SA during exercise in nasal carriers. In order to ensure appropriate study designs are selected to address specific research questions in SA transmission in athletes, an epidemiological study and an exercise experiment were employed. Recently, molecular typing methods have greatly improved our understanding of SA transmission and provide powerful tools for tracing the transmission of individual strains and revealing methicillin-resistant SA (MRSA) strains [7].
It is commonly agreed that there is a lack of data on the prevalence of SA transmission among athletes, which prevents effective surveillance and hence leads to failure in prevention. Samples were collected from athletes in 3 different sports popular in Japan. In this study, we investigated person-to-person SA transmission in athletes participating in contact sports, using modern molecular methods. We also compared the presence of SA on the skin's surface in nasal carriers and non-carriers during exercise. The results obtained may contribute to the development of a strategy for the prevention of SA transmission in an athletic setting.

Methods

We used 2 approaches to investigate SA transmission in athletes: molecular genotyping and testing for the presence of SA on the skin's surface during exercise. The study was conducted in accordance with international ethics standards as outlined by Harris and Atkinson [3] and approved by the ethics committee of the Institute of Health and Sport Sciences, University of Tsukuba (reference no. 23-11), in accordance with the declaration of Japan. Written informed consent was obtained from all participants before the start of the experiments.

Sampling for detection of person-to-person transmission of SA by molecular genotyping

In this study, performed between October 2011 and November 2012, swab samples were obtained from the anterior nares of a total of 172 athletes, and these subjects were composed of 3 individual sports groups (judo, n=53; rugby, n=69; American football, n=50). We selected 3 sports groups, in which athletes routinely make contact with each other. A questionnaire was administered to all players to determine their team positions, weight, height and training partners. To avoid cross-contamination, subjects were sampled by themselves. Nasal samples were collected on the following dates: for the judo team, in 2012 on 2/29, 4/25, 5/30, 6/27, 9/19 and 10/30; for the rugby team, in 2011 on 10/26, 11/16, 11/25, 2012 3/9, 4/21, 5/23, 6/27 and 9/1; and for the American football team, in 2011 on 12/3, 2/23, 4/21, 6/4, 7/25, 9/1, 10/14 and 11/11. Sampling time points differed among sports groups due to substantial seasonal differences among the sports competition, and the activity pattern varied across the seasons.

Sampling for detection of SA on skin of athletes during exercise

To investigate the relationship between nasal and skin's surface SA isolates, we selected 18 adult males (age 20.6±1.2 years; height 174.3±5.5 cm; body mass 84.8±14.0 kg) from the molecular genotyping study. 9 had positive SA cultures from the anterior nares (nasal carriers) and 9 had negative cultures (non-carriers). We compared these 2 groups for the presence of SA on the skin's surface before and after bicycle exercise.

Isolation and identification of SA from swabs

Nasal samples were obtained from the right and left anterior nares of all athletes using sterile swabs. Swabs were immersed in 1.0 ml of PBS, and samples (100 μl) were then inoculated onto Compact Dry CD-XSA medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) [14] and incubated at 37°C for 24±2 h. Blue colonies indicated the presence of SA. A small portion of a colony was picked up with a sterile pipette tip and transferred directly to 1.0 ml of PBS for a free coagulase test and DNA extraction. Specific blue SA colonies were confirmed by free coagulase production, detected by rabbit plasma agglutination (Eiken Chemical Co., Tokyo, Japan), using the tube method according to the manufacturer's instructions. In brief, SA colonies were cultured overnight in LB medium containing 7.5% NaCl and separated by centrifugation. Supernatants (100 μl) were mixed with 0.5 ml of 7% rabbit plasma in sterile saline and incubated at 37°C for 24 h. Plasma coagulation was visually judged 24 h later. Clot formation at either reading indicated that the sample was positive for SA.

Genotyping by phage open reading frame typing

Chromosomal DNA was isolated from specific SA colonies using the DNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of the extracted DNA was visually assessed by 2% agarose gel electrophoresis. 8 microliters of DNA per sample was used for phage open reading frame typing (POT) [13]. A commercially available POT kit (Cica Genesus Staph Pot Kit; Kanto, Tokyo, Japan) was used according to the manufacturer's instruction. In brief, the POT kit consisted of 3 multiplex PCRs. After PCR, PCR amplicon size, with either “1” for “+” or “0” for “-” (binary code), its output is a combination of the presence/absence of 22 targets, and the results are expressed as a 3-component POT score, calculated in a binary manner. These scores were then converted to decimal numbers, i.e., POT numbers. For example, the binary code of J003 (00100000000000000000000000) was converted to 16-0-0 as follows: 0×64+0×32 +1×16+0×128+0×64+0×32+0×16+0×8+0×4+0×2+0×1 +0×8+0×4+0×2+0×1+0×64+0×32+0×16+0×8+0×4+0×2 +0×1. Strains are classified according to their susceptibility to the set of phages selected. Clonal bands of the same size confirm clonal identity between the 2 tested strains. This method also detected the mecA gene associated with methicillin-resistant Staphylococcus aureus (MRSA). Strain genotyping contributes to a better understanding of SA transmission and could make it possible to track the dissemination of particular strains [7].

Exercise protocol

18 adult males were divided into 2 groups, based on nasal carriage status. There were 9 SA carriers (SAC) who remained nasally positive for SA throughout 3 sequential tests (These SA carriers were below 20% of healthy adult males in the pre-test phase) and 9 non-carriers (non-SAC). Exercise-induced sweating in healthy subjects was used as a model to predict an extra-nasal site of SA carriers. To quantify SA on the skin's surface before and after bicycle exercise, samples were obtained by swabbing the cervical region (5 cm² lateral from the jaw) and the cubital (medial approximately 5 cm²) and popliteal (medial approximately 5 cm²) fossae. Skin swabs were taken by rolling a sterile cotton-tipped swab stick on the skin for at least 5 s. Samples were placed in tubes containing 1.0 ml of PBS and vortexed for 30 s; then, SA was identified as described above. Bacterial colonies were quantified as the number of colony-forming units (CFU)/ml. SA colonization on the skin's surface was quantified as the sum of findings from the 3 swabbed areas. Participants cycled on a friction-braked Fitness Pro AF7600D ergometer (Alincor, Tokyo, Japan) for 15 min at 25°C (50% relative humidity) until moderate perspiration became evident as a sheen on the skin.
skin. The exercise was stopped before sweat droplets formed. The skin's surface was allowed to dry for 5 min and then a second set of samples was collected from the respective body sites.

Statistics
Numbers of SA colonies were assessed by one-way analysis of variance (ANOVA) followed by post hoc Student's t-tests. P<0.05 was considered statistically significant.

Results
Person-to-person transmission was defined by detection of subjects with isolates having the same POT score and, based on the questionnaire, on the same sports team. 10 isolates from 21 athletes were found to have identical POT scores in 3 sports team. For example, in the judo group, 4 cases of transmission were confirmed (● Fig. 1a). An isolate with POT score 16-0-0 obtained from subject J003 was also detected in subject J009 on May 30, 2012, and a similar pattern was observed for POT score 0-2-2; in these cases, transmission occurred after a longer interval. In contrast, POT scores 64-0-24 and 0-1-1 confirmed person-to-person SA transmission at approximately the same time. 3 cases of transmission were confirmed in the rugby group (● Fig. 1b). Isolates with POT score 66-136-32 were obtained from 3 subjects (R003, R012, and R023). From these data, we confirmed person-to-person SA transmission at approximately the same time. An isolate with POT score 0-3-18 from subject R024 was detected in subject R008 at approximately the same time. An isolate with POT score 4-26-16 obtained from subject R007 was detected in subject R002 on Sept 1, 2012. 3 cases of transmission were confirmed in the American football group (● Fig. 1c). An isolate with POT score 2-7-32 obtained from subject A032 was detected in subject A001 on Sept 1, 2012. Isolates with POT scores 0-35-0 and 122-224-4 confirmed person-to-person SA transmission at approximately the same time. These data indicate that direct and indirect modes of SA transmissions occurred. No other POT score was common among other sports.

To compare skin's surface SA colonization during exercise in SA carrier and non-SA carrier groups, we swabbed their skin's surfaces before and after exercise. ● Fig. 2 shows SA colony counts on the skin's surface before and after exercise; the difference was significant (F=6.3, P=0.0016, one-way ANOVA for repeated measures). Skin's surface SA colony count was significantly higher after than before exercise in the SAC group (before vs. after exercise: 5.2±5.4 vs. 41.7±40.6 CFU/ml, P=0.025; post hoc Student's t-test). However, nasal colony counts did not significantly differ between samples taken before and after exercise in this group (before vs. after exercise: 53.9±37.6 vs. 47.3±37.4 CFU/ml). In 5 of 9 subjects, who had nasal colonization and in whom SA bacterial skin colonization was subsequently developed, the strains isolated from the anterior nares were clonally identical to those from the skin's surface (PI-1, 2, 5, 6, 8) (● Table 1). Clonally identical strains were recovered from the knee in 3 athletes, the forearm in one, and the neck in one. The resulting 4 isolates with SA on the skin's surface had nasal colonization with a clonally different strain (PI-3, 4, 7, 9). ● Fig. 3 shows the POT results for selected isolates. It can therefore be stated that for about half of the athletes the skin's surface was colonized during exercise with SA of a strain clonally identical to that present in the anterior nares.

Discussion
To assess nasal colonization status, longitudinal monitoring was performed, in which multiple nasal samples were obtained from 172 athletes for a period of up to one year (● Table 2). A total of 774 of nasal swabs were obtained from 172 athletes. 100 (58.1%) athletes were SA nasal carriers; these included members of all 3 sport groups. SA nasal colonization rates ranged from 20.1 to 33.3% of subjects at any one time (● Table 2), with stable frequency of colonization observed. POT scores were obtained for the isolated SA strains to assess genetic relatedness among them. A total of 200 strains were isolated from the 100 carriers. The POT kit detected the mecA gene in 33 (16.5%) of the 200 strains. We undertook systematic evaluation of person-to-person transmission of SA in persons who played a sport involving close physical contact. Previous studies have demonstrated the value of genetic typing of SA for detection of transmission by means of pulsed-field gel electrophoresis (PFGE) in an athletic setting [3]. In this study, we attempted to identify person-to-person SA transmission by the POT method in subjects participating in 3 sports (● Fig. 1). POT has been used to investigate SA outbreaks, and its discriminatory power has been shown to be excellent [7, 13]. Among a total of 172 athletes evaluated with repeat sampling, we identified 10 cases of person-to-person SA transmission, involving strains that were genetically highly related; this suggests that close physical contact can lead to SA infection in an athletic setting. We also showed that the amount of SA on the skin's surface increased 24-fold after exercise in nasal carriers, but not in non-carriers (● Fig. 2). In the present study, we found isolates from the nares that were clonally identical to those from the skin's surface (● Table 1). Identity was determined according to stringent criteria for evaluating the band patterns revealed by the POT method.

Transmission of SA is thought to require close physical contact with the carrier in an athletic setting. A retrospective analysis indicated that infection rates tended to be higher among rugby forwards [12], American football linemen [3, 11], cornerbacks and wide receivers [1], all of whom frequently engage in close contact with other players. Nevertheless, previous laboratory studies did not investigate both the tracking of individual strains and the role played by SA on the skin's surface. In a physical contact transmission, presence of SA on the skin during exercise implies a key role in transmission by close physical contact. In this study, we show that the potential for SA transmission is higher in nasal carriers than in non-nasal carriers (● Fig. 2). Further evidence, in support of our study, has been found that several sites on the bodies of nasal carriers could become colonized [16]. In addition, the greatest densities of SA have been found in sweat glands [5]. As shown by these studies, sweating during exercise may contribute to the presence of SA on the skin's surface. Hence SA nasal carriers may be reservoirs that facilitate SA transmission, and SA on their skin's surface may be a source of infection through dermal contact among athletes during practice and games.

Although nasal carriage of SA has been suggested as the source of subsequent SA transmission and infection [9], previous studies have not investigated whether the SA on the skin's surface originated from the athletes own flora. We systematically used POT typing in athletes in nasal SA carriers to determine whether there was identity between SA isolates obtained from sweaty skin and those isolated from the nose. Strains isolated from the skin and nares were clonally identical in 5 out of 9 athletes, as
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Fig. 1 POT results for SA isolates involved in sports activity-related events. a judo, b rugby, and c American football. In each sports group (rugby, judo, and American football), strains within teams are shown. Person-to-person transmission was defined as different subjects having the same POT score (†). Each POT score was composed of 3 POT numbers (POT 1, POT 2 and POT 3, i.e., 16-0-0), representing a combination of the presence or absence of 22 genetic elements (see Materials and Methods for details). 3 groups of participants are identified by an ID number (i.e., J003).
shown by POT score (Table 1). As a result, a substantial proportion of cases of transmission were through close physical contact with nasal carriers. We propose a mechanism of SA skin infection for a number of endogenous infections in which the skin is colonized with SA from the anterior nares, and this causes subsequent infection in athletes with areas of impaired skin such as cuts and scrapes.

Our study has some limitations. The nasal swab data we examined comprises a major determinant of the source SA of among athletes. Nevertheless, these data are obtained from healthy male adults, and may not be applicable to a population with poor health. The study was also conducted on subjects participating in only 3 sports teams with few cases of SA reported. However, our findings should be generally applicable to athletes, where similar infection control measures are in place. In addition, further investigations are still needed in order to improve our understanding of SA transmission through strenuous exercise and physical contact. Moreover, social contacts and propagation in communities were not excluded from this study.

In conclusion, these data identify a major route of SA transmission among athletes participating in events that involve close physical contact, and they provide insight into the role played by exercise-induced sweating in SA nasal carriers. Nasal SA carriers may be a reservoir for transmission in athletes and teams. The accurate determination of SA nasal carriage status will allow better targeting of prophylactic strategies to reduce infection while playing contact sports. Athletes involved in physical contact sports should shower immediately after training. Avoid

Table 1  Distribution of identical and non-identical pairs of *Staphylococcus aureus* isolates from anterior nares and subsequent exercise-induced sweaty skin in 7 healthy adults.

| PI code | Site of isolate | POT type | Clonal identity |
|---------|----------------|----------|-----------------|
|         | POT1 | POT2 | POT3 | YES | NO |
| PI-1    | Nasal | 20  | 48 | 112 | YES |
| PI-2    | Nasal | 20  | 48 | 112 | YES |
| PI-3    | Nasal | 40  | 32 | 0   | NO  |
| PI-4    | Nasal | 40  | 32 | 0   | NO  |
| PI-5    | Nasal | 0   | 0  | 0   | NO  |
| PI-6    | Nasal | 92  | 128| 0   | NO  |
| PI-7    | Nasal | 26  | 48 | 66  | YES |
| PI-8    | Nasal | 42  | 0  | 0   | NO  |
| PI-9    | Nasal | 78  | 0  | 0   | NO  |
|         | Neck | 74  | 0  | 16  | YES |
|         | Neck | 104 | 0  | 8   | NO  |

*Participant code
*Open reading frame typing
*These values indicate whether SA strains derived from the anterior nares were identical to those subsequently derived from skin, as analyzed by POT assays.
sharing personal items such as uniforms, personal protective equipment, clothing, towels, washcloths or razors, which may have come into contact with the infected wound or bandage. Additionally, infection control measures should be emphasized to reduce nasal SA and eliminate SA nasal colonization.

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References

1 Begier EM, Frenette K, Barrett NL, Mshar P, Petit S, Boxrud DJ, Watkins-Colwell K, Wheeler S, Cebelinski EA, Glennen A, Nguyen D, Hadler JL. A high-morbidity outbreak of Methicillin-resistant Staphylococcus aureus among players on a college football team, facilitated by cosmetic body shaving and turf burns. Clin Infect Dis 2004; 39: 1446–1453
2 Creech CB, Saye E, McKenna BD, Johnson BG, Jimenez N, Talbot TR, Bossung T, Gregory A, Edwards KM. One-year surveillance of Methicillin-resistant Staphylococcus aureus nasal colonization and skin and soft tissue infections in collegiate athletes. Arch Pediatr Adolesc Med 2010; 164: 615–620
3 Harris DJ, Atkinson G. Ethical standards in sports and exercise science research. Int J Sports Med 2013; 34: 1025–1028
4 Kazakova SV, Hageman JC, Matava M, Srinivasan A, Phelan L, Garfinkel B, Boo T, McAllister S, Anderson J, Jensen B, Dodson D, Lonsway D, McDougal LK, Arduino M, Fraser VJ, Killgore G, Tenover FC, Cody S, Jernigan DR. A clone of Methicillin-resistant Staphylococcus aureus among professional football players. N Engl J Med 2005; 352: 468–475
5 Kong HH, Segre JA. Skin microbiome: looking back to move forward. J Invest Dermatol 2012; 132: 933–939
6 Larkin-Thier SM, Barber VA, Harvey P, Livdans-Forret AB. Community-acquired methicillin-resistant Staphylococcus aureus: a potential diagnosis for a 16-year-old athlete with knee pain. J Chiropr Med 2010; 9: 32–37
7 Maeda T, Suga T, Miyazaki T, Koyama Y, Harada S, Iwata M, Yoshizawa S, Kimura S, Ishii Y, Urita Y, Sugimoto M, Yamaguchi K, Tateda K. Genotyping of skin and soft tissue infection (SSTI)-associated methicillin-resistant Staphylococcus aureus (MRSA) strains among outpatients in a teaching hospital in Japan: application of a phage-open reading frame typing (POT) kit. J Infect Chemother 2012; 18: 906–914
8 Oller AR, Mitchell A. Staphylococcus aureus aureus recovery from cotton towels. J Infect Dev Ctries 2009; 3: 224–228
9 Oller AR, Province L, Curless B. Staphylococcus aureus aureus recovery from environmental and human locations in 2 collegiate athletic teams. J Athl Train 2010; 45: 222–229
10 Redziniak DE, Diduch DR, Turman K, Hart J, Grindstaff TL, MacKnight JM, Mistry DJ. Methicillin-resistant Staphylococcus aureus (MRSA) in the Athlete. Int J Sports Med 2009; 30: 557–562
11 Romano R, Lu D, Holtom P. Outbreak of community-acquired Methicillin-resistant Staphylococcus aureus skin infections among a collegiate football team. J Athl Train 2006; 41: 141–145
12 Stacey AR, Endersby KE, Chan PC, Marples RR. An outbreak of Methicillin-resistant Staphylococcus aureus infection in a rugby football team. Br J Sports Med 1998; 32: 153–154
13 Suzuki M, Tawada Y, Kato M, Hori H, Mamiya N, Hayashi Y, Nakano M, Fukushima R, Kato A, Tanaka T, Hata M, Matsumoto M, Takahashi M, Sakae K. Development of a rapid strain differentiation method for methicillin-resistant Staphylococcus aureus isolated in Japan by detecting phase-derived open-reading frames. J Appl Microbiol 2006; 101: 938–947
14 Teramura H, Mizuochi S, Kodaka H. Evaluation of the compact dry X-SA method for enumerating Staphylococcus aureus in artificially contaminated food samples. Biocontrol Science 2010; 15: 149–154
15 Turbeville SD, Cowan LD, Greenfield RA. Infectious disease outbreaks in competitive sports: a review of the literature. Am J Sports Med 2006; 34: 1860–1865
16 Wertheim HF, Melles DC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 2005; 5: 751–762