Pharyngeal Carriage of Toxin-Producing *Staphylococcus aureus* in University Students

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Summary

Pharyngeal and nasal swabs taken from 233 university students in October 2010 proved positive for *S. aureus* in 80 (34%) and 65 (28%), respectively. Staphylococcal enterotoxin, toxic shock syndrome toxin and exfoliative toxin were produced by 31 (39%), 12 (15%) and 2 (3%) single isolates from the pharyngeal streaks of 80 students. In May and November 2011, 183 students underwent further throat examinations, and 101 (55%) were positive for *S. aureus* at least once. The carrier rates do not differ largely from those reported over the last 2 ~ 4 decades, and confirm that most healthy individuals can be sources of *S. aureus* infection and toxins. (*Bulletin of Nakamura Gakuen University* 47: 183-186, 2015)

Introduction

Carriage of *Staphylococcus aureus (S. aureus)* is a well-known risk factor for *S. aureus* infections [1, 2]. The bacteria can also be toxic, causing food poisoning, scalded skin syndrome, or toxic shock syndrome. *S. aureus* colonizes the nasal and pharyngeal mucosa, skin, and other parts of healthy individuals. The nasal carrier rates have been examined in many studies, and positivity rates obtained by mucosal swabbing vary from 23% to 43% at any time [3-7].

The throat is another major habitat of *S. aureus*, and positivity rates are equivalent to, or exceed those for the nares [8-14]. It has been suggested that *S. aureus* strains colonizing the nasal and pharyngeal mucosa are not always identical [15, 16]. Therefore, the rate of toxin-producing *S. aureus* in the throat may differ from that in the nares. Saliva is likely the most important vehicle for pharyngeal bacteria. Several studies have shown that the positivity rate of *S. aureus* in saliva or on the tongue is as high as 33 - 43% of healthy individuals [17, 18].

However, in contrast to many studies on the growing frequencies of methicillin-resistant *S. aureus*, only a few studies have reported the oro-pharyngeal carriage or presence in saliva of toxin-producing *S. aureus* in healthy adults [17, 19]. In order to know the trends in carrier rates in healthy adults, we examined the pharyngeal carriage of *S. aureus* and its toxin production in Japanese students.

Methods

The study subjects were Japanese students in the Faculty of Nutritional Sciences, Nakamura Gakuen University. They had not participated in any laboratory works or experienced clinical rotations. Two-hundred thirty-three students (6 males, 227 females, 21.2 ± 1.1 years old (mean ± SD)) were included in the study. The Ethics Committee at Nakamura Gakuen University approved the study and written informed consent was obtained from the subjects. Two-hundred and thirty-three students were screened in October 2010. These students were asked to be repeatedly sampled in May and November 2011, and of these 183 agreed and completed both additional

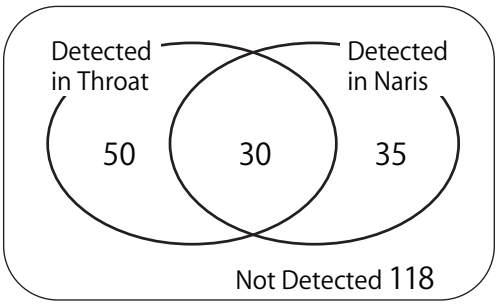

Figure 1. Isolation of *S. aureus* from the nares and throat of 233 university students in October 2010. Swab samples were streaked on mannitol salt agar with egg yolk, and then incubated. One of the grown colonies that were positive for both mannitol fermentation and egg yolk reaction were subcultured in brain heart infusion broth. Bacteria giving a positive result in the latex agglutination test were defined as *S. aureus*.

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Table 1. Toxins produced by a single isolate from 80 subjects.

SE	TSST	EXT	Number of student (%)	
+ (A and C)	+	-	1	(1.3)
+ (A)	+	-	3	(3.8)
+ (A)	-	-	10	(12.5)
+ (B)	-	-	5	(6.3)
+ (C)	+	-	6	(7.5)
+ (C)	-	-	5	(6.3)
+ (E)	-	-	1	(1.3)
-	+	-	2	(2.5)
-	-	+ (A)	2	(2.5)
-	-	-	45	(56.3)

SE: enterotoxin-A, B, C, D, E
TSST: toxic shock syndrome toxin
EXT: exfoliative toxin

examinations.

Nasal and pharyngeal specimens were obtained from the students using dry cotton-rayon swabs (Nihon-Menbou, Saitama, Japan). After receiving a brief lecture on how to use the swabs, the students themselves wiped their nasal or pharyngeal mucosa with a swab. The swab was rotated gently in a nostril until it became wet, and another was used to obtain samples from both tonsils. Immediately, the self-taken samples were streaked on mannitol salt agar with egg yolk and incubated for 48 h at 36˚C. In October 2010, appropriate wiping of the nasal and pharyngeal mucosa was confirmed by placing each swab on sheep blood agar after inoculation on mannitol salt agar, and observing the growth of any microorganisms.

Grown colonies that were positive for both mannitol fermentation and egg yolk reaction were subcultured in brain heart infusion broth. Bacteria giving a positive result in the latex agglutination test StaphLA (Denka-Seiken Co., Niigata, Japan) were defined as *S. aureus*. Production of enterotoxin (SE)-serotype A, B, C, D, E, exfoliative toxin (EXT)-serotype A, B, and toxic shock syndrome toxin (TSST) were determined using Enterotox F Seiken, EXT-RPLA Seiken and TST-RPLA Seiken (Denka-Seiken), respectively [20, 21].

Pharyngeal and nasal prevalence of *S. aureus* was compared, and results were statistically analyzed using Excel Statistics (SSRI, Japan) at a significance level of $P < 0.05$ [22].

Results

At the examination conducted in October 2010, 115 (49%) of the 233 subjects were positive for *S. aureus*. *S. aureus* was present in the throat in 80 (34%) (95% confidence interval (CI): 28 - 40%) and in the anterior nares 65 (28%) (95% CI: 22 - 34%). Thirty subjects (13%) were positive for *S. aureus* in both the throat and nares (Fig. 1). The prevalence of *S. aureus* in the throat (34%) was more than that in anterior nares (28%), but the difference was considered to be not statistically significant ($P = 0.129$). Nasal carriers were more likely to be throat carriers than subjects lacking *S. aureus* in the nose (odds ratio = 2.02) (95% CI: 1.12-3.65, $P = 0.021$).

The isolates subcultured from a randomly selected single colony on culture dishes obtained from 80 pharyngeal specimen in October 2010 were examined for SE, EXT and TSST. A total of 35 (44%) produced toxins: enterotoxin was produced by 31 isolates (39%) (SEA 14 (18%), SEB 5 (6%), SEC 11 (14%), SEE 1 (1%)). TSST and EXT-A were produced by 12 (15%) and 2 (3%) isolates, respectively. Seven isolates produced 2 or 3 types of toxins concurrently (Table 1).

Pharyngeal carriage of *S. aureus* was further examined at 6-month intervals, and 183 subjects (6 males, 177 females) completed all of the examinations. *S. aureus* was isolated from 67 (37%), 47 (26%) and 38 (21%) subjects in October 2010, May 2011 and November 2011, respectively. Overall, 101 (55.2%) subjects were positive at least once among the 3 examinations (Table 2).

Discussion

Of the single isolates from 80 pharyngeal specimen, 35 (44%) produced toxins (Table 1). To clarify the extent of co-colonization of different strains, we randomly selected 21 of the 47 dishes positive for *S. aureus* in May 2011, picked up 10 independent colonies from every dish, and examined them for SE production. This revealed that 9 dishes had one or more enterotoxigenic colonies (data not shown), suggesting that single-colony examination underestimated the true proportion of carriers of enterotoxigenic *S. aureus*.

Regarding SE, a study of healthy Japanese food handlers in 1977 showed that pharyngeal *S. aureus* was present in 6-20% of 100 subjects during the four seasons of the year, and that 33-65% of the isolates were enterotoxigenic [19]. In a study conducted in 1990, *S. aureus* was isolated from the tongue in 33% of 307 children, and 40% of the isolates were enterotoxigenic [18]. In our present study, 34% of 233 students were pharyngeal carriers of *S. aureus*, and

Table 2. Consecutive and non-consecutive detection of *S. aureus* in throats of 183 subjects over time.

Time of examination			Number of subjects (%)	
2010 Oct	2011 May	2011 Nov		
+	+	+	12	(6.6)
+	+	-	9	(4.9)
+	-	+	10	(5.5)
+	-	-	36	(19.7)
-	+	+	8	(4.4)
-	+	-	18	(9.8)
-	-	+	8	(4.4)
-	-	-	82	(44.8)

+: detected, -: not detected

enterotoxigenic *S. aureus* accounted for 31 (39%) of 80 isolates in 2010. The proportions of toxin producers in these studies were comparable with each other, and also comparable with those of nasal isolates [4-6].

Concerning TSST, we have found only a limited amount of published data pertaining to healthy pharyngeal carriage rates of TSST-producing *S. aureus*. In a Japanese study conducted in 2008, 70 (34%) of 209 healthy female volunteers carried *S. aureus* in their throat, and 6 (9%) of these 70 isolates produced TSST [23]. In our present study, 12 (15%) of 80 pharyngeal isolates from healthy students produced TSST, the frequency being slightly higher than that in the former study.

Concerning EXT, few published data pertaining to the healthy carrier rates of EXT-producing *S. aureus* are available. In a previous study of 307 children visiting a dental clinic, *S. aureus* was isolated from the tongues of 100 (33%), and 19 (19%) of the 100 isolates produced EXT. From clinical samples including oral discharge, sputum, otorrhea, purulent materials and blisters (excluding patients with staphylococcal scaled skin syndrome), 3-12% of isolated strains synthesized EXT [24, 25]. In our study, 2 (3%) of 80 isolates from healthy adults produced EXT, the frequency being similar to those in the previous reports.

In overtime examination of pharyngeal carriage of *S. aureus* for three times at 6-month intervals on 183 subjects, 12 subjects were consistently positive, 89 transiently positive and 82 consistently negative (Table 2). The result suggests that *S. aureus* carriage could be dynamic: there may be persistent carriers, transient carriers and persistent non-carriers. Further gene analysis will reveal if the persistent carriers carry the same clones, as well as if the transient carriers obtain and harbor the same clones or different ones at different periods.

In conclusion, our results confirm that many healthy adults harbour toxin-producing and non-producing *S. aureus* at any given time, and that the carrier rates have not differed largely over the last 2 - 4 decades.

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Conflict of Interest

None.

References

1. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriological Review* 27: 56-71, 1963.
2. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology* Review 10: 505-520, 1997.
3. Moritz ED, Smith TC. Risk of *Staphylococcus aureus* carriage in childcare employees. *Epidemiology and Infection* 141: 1975-1982, 2013.
4. Stark L, Olofsson M, Löfgren S, Mölstad S, Lindgren PE, Matussek A. Prevalence and molecular epidemiology of *Staphylococcus aureus* in Swedish nursing homes - as revealed in the SHADES study. *Epidemiology and Infection* 142: 1310-1316, 2014.
5. Mukherjee DV, Herzig CT, Jeon CY, Lee CJ, Apa ZL, Genovese M, Gage D, Koenigsmann CJ, Lowy FD, Larson EL. Prevalence and risk factors for *Staphylococcus aureus* colonization in individuals entering maximum-security prisons. *Epidemiology and Infection* 142: 484-493, 2014.
6. Gamblin J, Jefferies JM, Harris S, Ahmad N, Marsh P, Faust SN, Fraser S, Moore M, Roderick P, Blair I, Clarke SC. Nasal self-swabbing for estimating the prevalence of *Staphylococcus aureus* in the community. *Journal of Medical Microbiology* 62: 437-440, 2013.
7. Meno Y, Yoshitake M, Fujimoto S. Epidemiological study of *Staphylococcus aureus* isolated from healthy student-nurses: production of enterotoxins and toxic shock syndrome toxin 1 and coagulase gene typing. *Bulletin of Seinan Jo Gakuin University* 9: 9-14, 2005.
8. Packalen T, Bergqvist S. Staphyloccci in throat and nose and antistaphylolysin titer. *Acta Medica Scandinavica* 127: 291-312, 1947.
9. Vogelsang TM. The incidence of penicillin-resistant pathogenic staphylococci isolated from the upper respiratory tract of young healthy persons. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica* 29: 363-367, 1951.
10. Nilsson P, Ripa T. *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. *Journal of Clinical Microbiology* 44: 3334-3339, 2006.
11. Lee CJ, Sankaran S, Mukherjee DV, Apa ZL, Hafer CA, Wright L, Larson EL, Lowy FD. *Staphylococcus aureus* oropharyngeal carriage in a prison population. *Clinical Infectious Diseases* 52: 775-778, 2011.
12. de Carvalho MJ, Pimenta FC, Hayashida M, Gir E, da Silva AM, Barbosa CP, Canini SR, Santiago S. Prevalence of methicillin-resistant and methicillin-susceptible *S. aureus* in the saliva of health professionals. *Clinics (Sao Paulo)* 64: 295-302, 2009.
13. Cruz ED, Pimenta FC, Hayashida M, Eidt M, Gir E. *Staphylococcus aureus* detection in the mouth of housekeepers. *Revista Latino-Americana de Enfermagem* 19: 90-96, 2011.
14. de Moura JP, Pimenta FC, Hayashida M, Cruz ED, Canini SR, Gir E. Colonization of nursing professionals by *Staphylococcus aureus*. *Revista Latino-Americana de Enfermagem* 19: 325-331, 2011.
15. Uemura E, Kakinohana S, Higa N, Toma C, Nakasone N. Comparative chracterization of *Staphylococcus aureus*

isolates from throats and noses of healthy volunteers. *Jpnanese Journal of Infectious Diseases* 57: 21-24, 2004.
16. Hamdan-Partida A, Sainz-Espuñes T, Bustos-Martínez J. Characterization and persistence of *Staphylococcus aureus* strains isolated from the anterior nares and throats of healthy carriers in a Mexican community. *Journal of Clinical Microbiology* 48: 1701-1705, 2010.
17. de Carvalho MJ, Pimenta FC, Hayashida M, Gir E, da Silva AM, Barbosa CP, Canini SR, Santiago S. Prevalence of methicillin-resistant and methicillin-susceptible *S. aureus* in the saliva of health professionals. *Clinics (Sao Paulo)* 64: 295-302, 2009.
18. Miyake Y, Iwai T, Sugai M, Miura K, Suginaka H, Nagasaka N. Incidence and characterization of *Staphylococcus aureus* from the tongues of children. *Journal of Dental Research* 70: 1045-1047, 1991.
19. Mori M, Kato E, Hamada S. Distribution of enterotoxigenic staphylococci in healthy food handlers and biological properties of the isolates. *Nihon Saikingaku Zasshi* 32: 501-508, 1977.
20. Oda T. Plate punch method as a simplified detection method for staphylococcal enterotoxins. *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)* 26: 29-32, 1985.
21. Oda T. Rapid and simplified detection method for enterotoxin-producing *Staphylococcus aureus* in food. *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)* 26: 405-410, 1985.
22. McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika* 12: 153-157, 1947.
23. Parsonnet J, Goering RV, Hansmann MA, Jones MB, Ohtagaki K, Davis CC, Totsuka K. Prevalence of toxic shock syndrome toxin 1 (TSST-1)-producing strains of *Staphylococcus aureus* and antibody to TSST-1 among healthy Japanese women. *Journal of Clinical Microbiology* 46: 2731-2738, 2008.
24. Machida K, Sakura S, Kurosaka K, Ikawa S. Epidemiological studies of exfoliative toxin producing strains of *Staphylococcus aureus* in various clinical samples. *Jikei Medical Journal* 35: 595-601, 1988.
25. Piemont Y, Rasoamananjara D, Fouace JM, Bruce T. Epidemiological investigation of exfoliative toxin-producing *Staphylococcus aureus* strains in hospitalized patients. *Journal of Clinical Microbiology* 19: 417-420, 1984.