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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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 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>
<thead>
<tr>
<th>SE</th>
<th>TSST</th>
<th>EXT</th>
<th>Number of student (%)</th>
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<tr>
<td>+ (A and C)</td>
<td>+</td>
<td>-</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>+ (A)</td>
<td>+</td>
<td>-</td>
<td>3 (3.8)</td>
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<tr>
<td>+ (A)</td>
<td>-</td>
<td>-</td>
<td>10 (12.5)</td>
</tr>
<tr>
<td>+ (B)</td>
<td>-</td>
<td>-</td>
<td>5 (6.3)</td>
</tr>
<tr>
<td>+ (C)</td>
<td>+</td>
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<tr>
<td>+ (C)</td>
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<td>5 (6.3)</td>
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<tr>
<td>+ (E)</td>
<td>-</td>
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</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+ (A)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45 (56.3)</td>
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SE: enterotoxin-A, B, C, D, E
TSST: toxic shock syndrome toxin
EXT: exfoliative toxin

<table>
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<tr>
<th>Time of examination</th>
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<th>2011 May</th>
<th>2011 Nov</th>
<th>Number of subjects (%)</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12 (6.6)</td>
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<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>9 (4.9)</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>10 (5.5)</td>
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</tr>
<tr>
<td>+</td>
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<td>+</td>
<td>8 (4.4)</td>
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<td>-</td>
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<td>-</td>
<td>18 (9.8)</td>
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<td>-</td>
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<td>+</td>
<td>8 (4.4)</td>
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<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>82 (44.8)</td>
<td></td>
</tr>
</tbody>
</table>

+: detected, -: not detected

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

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

Acknowledgements

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Conflict of Interest

None.

References

15. Uemura E, Kakinohana S, Higa N, Toma C, Nakasone N. Comparative characterization of Staphylococcus aureus