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Association of adenoid hyperplasia and bacterial biofilm formation in children with adenoiditis in Taiwan

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Abstract The adenoid is a bacterial reservoir that contributes to chronic otolaryngologic infections. *Staphylococcus aureus* (*S. aureus*) is a common pathogen in the adenoid. The increase of antibiotic resistance in *S. aureus* has become an important issue in public health. The aim of this study was to compare adenoid hyperplasia and biofilm formation in children with *S. aureus* adenoiditis in Taiwan. The patients were divided into methicillin-resistant and methicillin-sensitive *S. aureus* groups according to the *S. aureus* obtained from adenoid tissue after antibiotic susceptibility testing. Adenoid hyperplasia was assessed by lateral cephalometry, and the severity of sinusitis was evaluated by Water's view. Microbiological investigation of available *S. aureus* isolates was performed by in vivo morphological observation and an in vitro bacterial biofilm assay. Sixty isolates of *S. aureus* were identified in 283 children (21.2%) after adenoidectomy, of which 21 (35%) were methicillin-resistant *S. aureus* (MRSA). The severity of adenoid hyperplasia and extensive biofilm formation were more prominent in patients infected with methicillin-resistant *S. aureus* than in those infected with methicillin-sensitive *S. aureus* (MSSA). The primary outcome of this study was to provide evidence that *S. aureus* constituted a significant portion of the adenoidal pathogens. The secondary outcome of this study was that MRSA adenoiditis may be associated with adenoid hyperplasia and biofilm formation.

Keywords Adenoid · Biofilm · *Staphylococcus aureus* · Antibiotic resistance

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Introduction

The adenoid is located in a pivotal position of the upper respiratory tract, in close proximity to the paranasal sinuses, adjacent to the middle ear cavity, and as the roof of the oropharynx. The adenoid can serve as a reservoir of pathogenic bacteria [1], and recurrent or persistent adenoiditis is associated with common diseases of neighboring structures including obstructive sleep apnea (OSA), otitis media, and sinusitis [2]. Removal of the adenoid can be effective in controlling pediatric sinusitis or otitis media [3]. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis,* and *Staphylococcus aureus* are common nasopharyngeal colonizations found in children [4]. *Staphylococcus aureus* is also a common nasopharyngeal pathogen in children with acute otitis media or

recurrent otitis media after amoxicillin therapy [5]. In addition, *S. aureus* is associated with respiratory diseases, chronic adenoiditis, and rhinosinusitis [6]. The emergence of methicillin-resistant *S. aureus* (MRSA) has become an important public health problem, both as a rising community pathogen and with respect to its potential impact on strategies for antibiotic therapy. The incidence of community-acquired MRSA infections has dramatically increased since 2002 [7]. An increased prevalence of MRSA has also been found in acute and chronic rhinosinusitis [8]. Little is known about the role of *S. aureus*, especially MRSA, in chronic adenoid infection.

Biofilm activity has been a topic of great interest in recent studies of infectious diseases. Bacterial biofilm formation may play an important role in many otolaryngologic infections, such as chronic otitis media, otitis media with effusion (OME), chronic rhinosinusitis, chronic adenoiditis, and chronic tonsillitis, and may result in their persistence and difficult eradication [9]. Biofilms are structured communities of bacterial cells embedded in an extracellular polymeric substance composed of nucleic acids, proteins, and polysaccharides. The biofilm architecture is highly resistant to host defense systems and can adhere to mucosal surfaces, leading to impaired host immune responses [10]. Additionally, the biofilm not only inhibits the transportation of antimicrobial agents, but also alters the physiology of bacteria within the biofilm [11]. Bacteria in the biofilm have a dramatically increased resistance to several types of antimicrobial agents [11].

Although much is known about the pathogenic mechanism of *S. aureus* biofilm, the association of biofilm formation in children with adenoiditis requires further investigation. In this study, we investigated the antibiotic resistance of *S. aureus* isolated from children with adenoid hyperplasia. Patients with adenoid hyperplasia were assessed by lateral cephalometry, and the severity of sinusitis was evaluated by Water's view. Microbiological investigation of available *S. aureus* isolates was performed by in vivo and in vitro morphological evaluation.

Patients and methods

Patient selection and sample collection

Adenoid tissues were obtained from 283 children in Taiwan with persistent or recurrent OME or OSA during routine adenoidectomy surgery by the authors at China Medical University Hospital (Taichung, Taiwan) from January 2001 through August 2010. The ages of the patients ranged from 1 to 18 years. There were 109 girls and 174 boys. Core tissues from adenoid specimens were streaked across Tryptic soy agar (Becton–Dickinson, Franklin Lakes, NJ, USA) containing 5% sheep blood and incubated at 37°C for 18–24 h. Organisms were identified as *S. aureus* using a BD Phoenix™ Automated Microbiology System (Becton–Dickinson) in 60 patients. The severity of maxillary sinusitis was analyzed, and lateral cephalometric analysis (described below) was carried out in 50 patients who were identified with *S. aureus* adenoiditis and who had available radiological images. Sixteen clinical isolates were selected by stratified random sampling to test the biofilm-forming activity.

The adenoidectomy tissues underwent routine pathologic examination. A portion of the adenoid tissue was cut and stored for Gram staining and scanning electron microscopy (SEM). The core adenoid tissue was aseptically collected for bacterial identification and the biofilm formation assay. Only patients whose adenoid tissue tested positive for *S. aureus* were enrolled in the study. This study was approved by the Ethics Committee of the China Medical University Hospital, Taichung, Taiwan.

Bacterial culture and antimicrobial susceptibility

Staphylococcus aureus susceptibility to various antimicrobial agents and confirmation of MRSA were determined using the BD Phoenix™ Automated Microbiology System. *Staphylococcus aureus* samples were stored at −80°C in Tryptic soy broth containing 20% glycerol until use. The antimicrobial susceptibility of all isolates to oxacillin, erythromycin, penicillin, vancomycin, teicoplanin, tetracycline, clindamycin, linezolid, levofloxacin, and trimethoprim/sulfamethoxazole was confirmed using the disk diffusion method following guidelines and criteria from the Clinical Laboratory Standards Institute [12]. *Staphylococcus aureus* isolates showed resistance to oxacillin, erythromycin, penicillin, and clindamycin and sensitivity to vancomycin, teicoplanin, tetracycline, linezolid, levofloxacin, and trimethoprim/sulfamethoxazole, which were determined as MRSA. In contrast, MSSA isolates showed variable sensitivity to erythromycin and clindamycin, and sensitivity to oxacillin, vancomycin, teicoplanin, tetracycline, linezolid, levofloxacin, and trimethoprim/sulfamethoxazole.

Lateral cephalometric analysis and scoring of maxillary sinusitis severity

Lateral cephalograms were obtained with the patients in the erect position with a wall-mounted cephalostat and oriented with the Frankfort horizontal plane during film exposure. Adenoid size was assessed by adenoidal–nasopharyngeal ratios (ANRs), using previously described reference lines and points (Fig. 1a) [13, 14]. Basic reference points included the posterior-superior edge of the hard palate (P), the posterior edge of the sphenobasioccipital

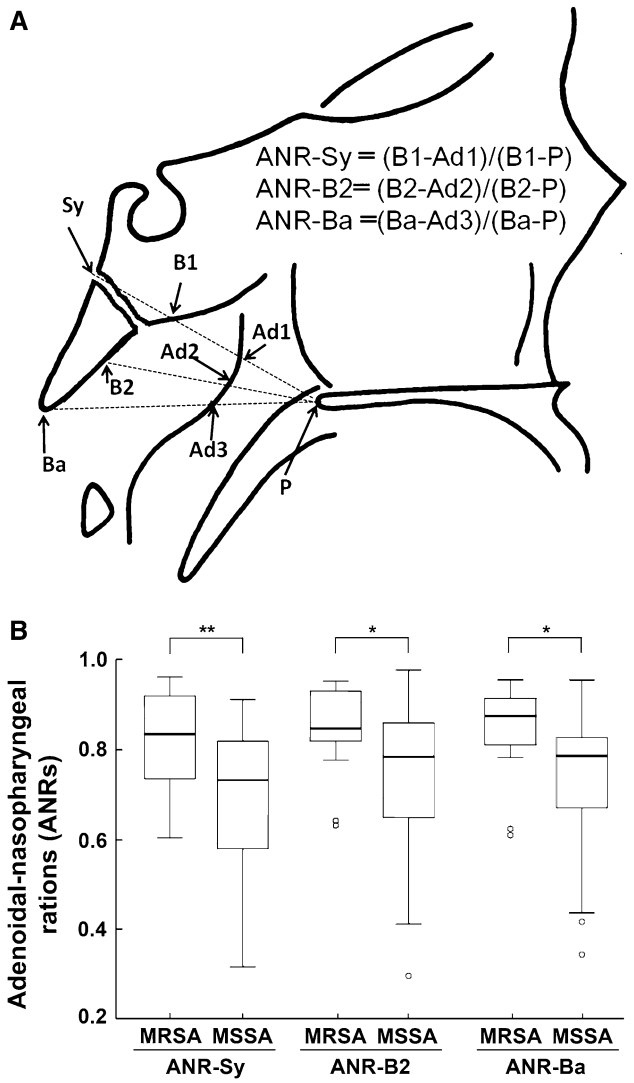

Fig. 1 **a** The reference points and lines on a lateral cephalogram, modified from previous studies [13, 14]. *P* posterior end of hard palate, *Sy* posterosuperior point of sphenobasioccipital synchondrosis, *Ba* basion, *Ad2* the nearest point of the adenoidal surface to point P. ANR-Sy is the ANR based on the P-Sy line, ANR-B2 is based on the P–Ad2 line, and ANR-Ba is based on the P–Ba line. Please refer to the text for detailed definitions and formulae. **b** Comparison of the differences in adenoid hyperplasia between MRSA- and MSSA-infected patients based on the ANRs. The *box plots* show the summary statistics for the distribution of the data. The ends of the *boxes* define the 25th and the 75th percentiles. *Lines* drawn from the ends of the box represent the largest and the smallest values. *Bold horizontal lines* in the boxes indicate the median value in each group. Outlier values are represented by *open circles*. Adenoid hyperplasia was more prominent in MRSA-infected patients than in MSSA-infected patients. Statistical analysis was determined using a Mann–Whitney *U* test. All values are presented as the mean ± S.D. (**$P < 0.01$; *$P < 0.05$)

synchondrosis (Sy), the basion (Ba, the most posteroinferior point on the anterior margin of the foramen magnum), and the nearest adenoidal point (Ad2) to P. Three lines were drawn thereafter: P–Sy line (PSyL), P–Ad2 line (PAd2L), and P–Ba line (PBaL). Secondary reference points were identified as Ad1 (intersection between adenoidal surface and PSyL), B1 (intersection between the nasopharyngeal surface of the spheno-occipital bone and PSyL), B2 (intersection between the nasopharyngeal surface of the spheno-occipital bone and PAd2L), and Ad3 (intersection between the adenoidal surface and PBaL). The ANRs include ANR-Sy [equal to (B1-Ad1)/(B1-P)], ANR-B2 [equal to (B2-Ad2)/(B2-P)], and ANR-Ba [equal to (Ba-Ad3)/(Ba–P)] (Fig. 1a).

The severity of maxillary sinusitis was scored with a quantitative index of X ray opacity of the maxillary sinus in the Water's view [15]. In brief, "0" is a radiologically clear maxillary sinus, "1" is an identifiable bony margin with slight cloudiness in the sinus, "2" is an identifiable bony margin with moderate cloudiness in the sinus, "3" is a poorly identifiable bony margin with severe cloudiness in the sinus, and "4" is an unidentifiable bony margin with severe cloudiness in the sinus. Each side of the maxillary sinus was scored individually, and then both scores were summed to represent the severity of maxillary sinusitis in the patient.

Scanning electron microscopy (SEM)

The morphology of bacterial microcolonies on adenoid tissue surfaces was examined using SEM of bacterial biofilms. The specimens removed during routine surgical procedures were immediately fixed in 2.5% glutaraldehyde for 2 days at 4°C. The specimens were washed and stored in 0.1 M PBS (pH 7.4) at 4°C. Prior to SEM, specimens were washed three times with 0.1 M sodium cacodylate buffer (pH 7.4) for 10 min each, followed by post-fixing for 1 h using 1% osmium tetroxide dissolved in 0.1 M sodium cacodylate buffer (pH 7.4). The specimens were washed again and dehydrated with successive immersions in increasing concentrations of ethanol. The specimens were subsequently dried using the critical point drying method (Critical Point Dryer; Hitachi, Tokyo, Japan) with liquid carbon dioxide as a transitional fluid. The dried samples were adhered to aluminum stubs with carbon tape. The sample surface was sputtered and coated with gold (15-nm particles) with an ion coater (Giko Engineering Co., Tokyo, Japan). The samples were examined using a scanning electron microscope (Hitachi) equipped with a digital image processor (Mirero Inc., Seongnam-Si, Korea).

Biofilm formation assay

The biofilm formation assay using crystal violet staining was performed as described [16]. Briefly, stock bacterial isolates were inoculated on Tryptic soy agar supplemented with 5% sheep blood and cultured for 18 h at 37°C. Bacterial isolates were harvested from blood agar plates and

resuspended in 3 ml of PBS (pH 7.4). The optical density at 600 nm (OD_{600}) of the bacterial suspension was adjusted with PBS to 0.1 with a spectrophotometer (Biochrom Ltd., Cambridge, UK). Bacterial suspensions were diluted 1:1,000 in 96-well plates containing Tryptic soy broth with 0.5% glucose (Sigma-Aldrich, St. Louis, MO, USA). The plates were incubated at 37°C with agitated shaking (100 rpm). After 24 h, the supernatant was discarded, and bacteria were washed three times with PBS. The attached bacterial biofilm was stained with crystal violet (Sigma-Aldrich) for 10 min at room temperature. The plates were washed with PBS, and the stained crystal violet was eluted with methanol. The OD_{570} was determined using a spectrophotometer. Results were determined by averaging five independent experiments performed in duplicate.

Confocal laser scanning microscopy (CLSM)

Bacterial isolates were harvested from blood agar plates and resuspended in 3 ml of PBS (pH 7.4). Each *S. aureus* isolate was adjusted to an OD_{600} of 0.1 with Tryptic soy broth to reach a bacterial density of ca. 1×10^8 CFU/ml. To visualize the *S. aureus* biofilm, each diluted bacterial suspension (1×10^5 CFU/ml) was grown on coverslips (1.8 × 1.8 cm) that were vertically inserted in 12-well plates supplemented with Tryptic soy broth. The plates were incubated at 37°C with agitated shaking (100 rpm) for 24 h. The plates were washed with PBS and fixed for 1 h in 3.7% (wt/vol) paraformaldehyde (Sigma-Aldrich) at 4°C. The preparations were incubated for 5 min with 20 μmol/l propidium iodide (PI; Sigma-Aldrich) at room temperature to label *S. aureus*. The bacterial extracellular polysaccharide glycocalyx was stained for 5 min at room temperature with 100 mg/l fluorescein isothiocyanate–conjugated concanavalin A (ConA-FITC; Sigma-Aldrich). Samples were mounted and observed with a confocal laser scanning microscope (Zeiss LSM 510; Zeiss, Göttingen, Germany) with a 100× objective (oil immersion, aperture 1.3). The total depth of the bacterial biofilm was imaged using *z* stacks with slices taken at a thickness of 0.5 μm.

Image analysis of biofilm criteria

All tissue sections were evaluated for bacterial biofilm formation as determined by the presence of immobile, irreversibly attached clustered towers of microcolonies using SEM [17]. Tissue sections from each sample were performed by Gram stain to examine bacteria in biofilm [17]. In vitro biofilm formation was evaluated based on bacterial size, morphology, and extracellular glycocalyx using CLSM images [18]. Investigators assessing the samples were blinded to the disease state of the corresponding patient.

Statistical analysis

The sinusitis severity scores and ANRs of the MSSA and MRSA patients were compared with a non-parametric analysis by using the Mann–Whitney *U* test. The box plots show the summary statistics for the distribution of the data. The ends of the boxes define the 25th and the 75th percentiles. Lines drawn from the ends of the box represent the largest and the smallest values. Bold horizontal lines in the boxes indicate the median value in each group. Statistical analysis was performed by using SPSS (version 10.1; SPSS Inc., Chicago, IL, USA). *P* value less than 0.05 was considered statistically significant.

Results

Association of maxillary sinusitis severity and antibiotic resistance in *S. aureus*

There were 60 isolates (21.2%) identified as *S. aureus* from the adenoid specimens of 283 children with OME ($n = 202$) or OSA ($n = 81$). Of the patients with OME, 41 (20.3%) had adenoid specimens from which *S. aureus* was isolated; 17 (41.5%) of these isolates were MRSA. *S. aureus* was found in specimens from 19 patients (23.5%) with OSA, and 4 (21.1%) of these were MRSA.

In these 60 patients, a Waters' view and lateral cephalogram were available for 50 patients, including 15 patients infected with MRSA and 35 with MSSA. The ANR-Sy, ANR-B2, and ANR-Ba (mean ± SD) in MRSA-infected patients were 0.82 ± 0.11, 0.85 ± 0.10, and 0.85 ± 0.10, respectively. The ANR-Sy, ANR-B2, and ANR-Ba (mean ± SD) in MSSA-infected patients were 0.69 ± 0.17, 0.75 ± 0.16, and 0.75 ± 0.14, respectively. The degree of adenoid hyperplasia was significantly greater in MRSA-infected patients than in MSSA-infected patients (Fig. 1b). The scoring of maxillary sinusitis in MRSA- and MSSA-infected patients was 4.85 ± 2.23 and 4.89 ± 2.22, respectively. There was no significant difference in the severity of maxillary sinusitis between these two groups.

Because of these findings, we began to collect *S. aureus* isolates in recent years for further microbiological investigation. A total of 16 patients with adenoid hyperplasia who tested positive for *S. aureus* and available microorganisms underwent further microbiological investigation, including 8 OSA patients (mean age of 9.8 ± 3.6 years) and 8 OME patients (mean age of 7.5 ± 4.2 years). The antibiotic susceptibility of *S. aureus* isolates from these patients was determined. Of the 16 patients, 7 cultures were identified as MRSA, and 9 cultures were identified as MSSA (Table 1).

Table 1 Characteristics of patients with adenoid hyperplasia and their bacterial isolates with antibiotic susceptibility of *S. aureus* isolates

Subject no.[a]	Sex[b]	Age (years)	Clinical diagnosis	Isolated pathogen	Antimicrobial agent susceptibility (MIC, mg/L)										Biofilm formation[c]
					OXA	ERY	PEN	VAN	TEC	TET	CLI	LZD	LVX	SXT	
1	M	8	OSA	MRSA	>2	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	+
2	M	7	OSA	MRSA	>2	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	+
3	M	10	OME with AH	MRSA	>2	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	+
4	F	6	OME with AH	MRSA	>2	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	+
5	M	5	OME with AH	MRSA	>2	>4	>1	1	≦0.5	8	>2	2	1	≦0.5	+
6	M	7	OME with AH	MRSA	>2	>4	>1	1	1	4	>2	2	1	≦0.5	+
7	F	6	OME with AH	MRSA	>2	>4	>1	≦0.5	≦0.5	8	>2	2	1	≦0.5	+
8	F	5	OME with AH	MSSA	0.5	≦0.25	>1	1	≦0.5	>8	≦0.25	2	1	≦0.5	–
9	F	4	OME with AH	MSSA	0.5	≦0.25	≦0.12	1	≦0.5	≦0.5	≦0.25	2	1	≦0.5	+
10	M	10	OSA	MSSA	0.5	>4	>1	1	≦0.5	≦0.5	>2	1	1	≦0.5	–
11	M	8	OSA	MSSA	1	>4	>1	1	≦0.5	≦0.5	>2	1	1	≦0.5	–
12	M	10	OSA	MSSA	0.5	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	+
13	F	17	OME with AH	MSSA	0.5	>4	>1	1	≦0.5	≦0.5	>2	1	1	≦0.4	+
14	F	18	OSA	MSSA	1	>4	>1	1	≦0.5	8	>2	2	1	≦0.5	+
15	M	10	OSA	MSSA	0.5	>4	>1	1	≦0.5	≦0.5	>2	2	1	≦0.5	–
16	M	7	OSA	MSSA	1	0.5	>1	1	≦0.5	≦0.5	≦0.25	2	1	≦0.5	–

[a] Each subject number identifies a unique patient

[b] Sex: M, male; F, female

[c] Determination by both in vivo morphological observation and in vitro bacterial biofilm assay. +, presence; –, absence

OSA obstructive sleep apnea, *OME* otitis media with effusion, *AH* adenoid hyperplasia, *MIC* minimum inhibitory concentration, *OXA* oxacillin, *ERY* erythromycin, *PEN* penicillin, *VAN* vancomycin, *TEC* teicoplanin, *TET* tetracycline, *CLI* clindamycin, *LZD* linezolid, *LVX* levofloxacin, *SXT* trimethoprim/sulfamethoxazole

Biofilm formation on adenoid tissues

Morphological changes in bacterial colonies were visualized on the surface of adenoid tissues, and *S. aureus* biofilm formation activity was determined prior to tissue preparation for SEM observation. As shown in Fig. 2a and Table 1, some of MSSA bacterial colonies were not evenly dispersed on the adenoid tissue surface. The colonies appeared scattered across some regions only and showed no obvious biofilm structures. However, in adenoid tissue isolates from all seven patients with MRSA, SEM images revealed bacterial colony clusters and biofilm architecture in mucosal surface crypts (Fig. 2b and Table 1). The tissue sections from each tissue block were used for Gram staining and examined with a light microscope. The images showed that bacterial microcolonies were localized on the surface and crypts of the adenoids of all seven patients with MRSA (Fig. 3). Gram-positive cocci were also evidently

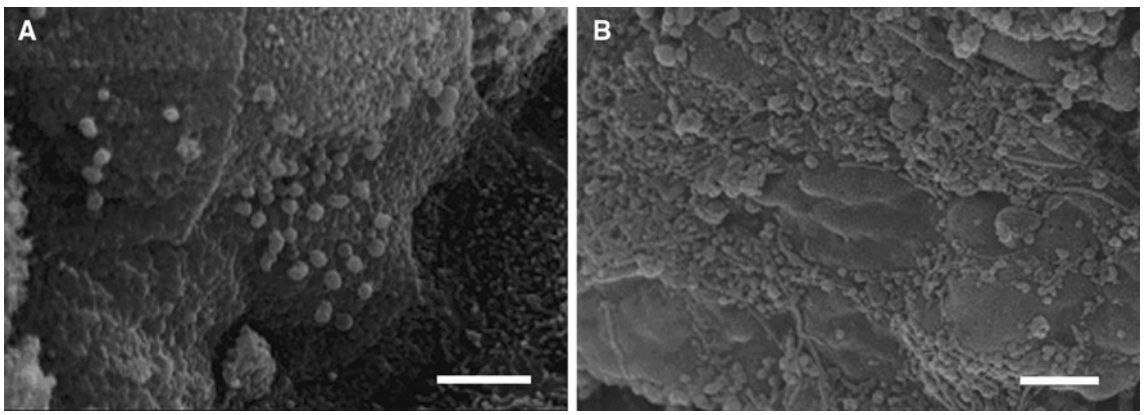

Fig. 2 SEM images of the mucosal surface of adenoid tissues. In patients with MSSA, bacteria were scattered on the adenoid tissue mucosal surface without significant network interconnections (**a** subject no. 10). In patients with MRSA, clusters of bacterial colonies and biofilm formation were delineated on the adenoid tissue surface (**b** subject no. 3). *Scale bar* 5 μm

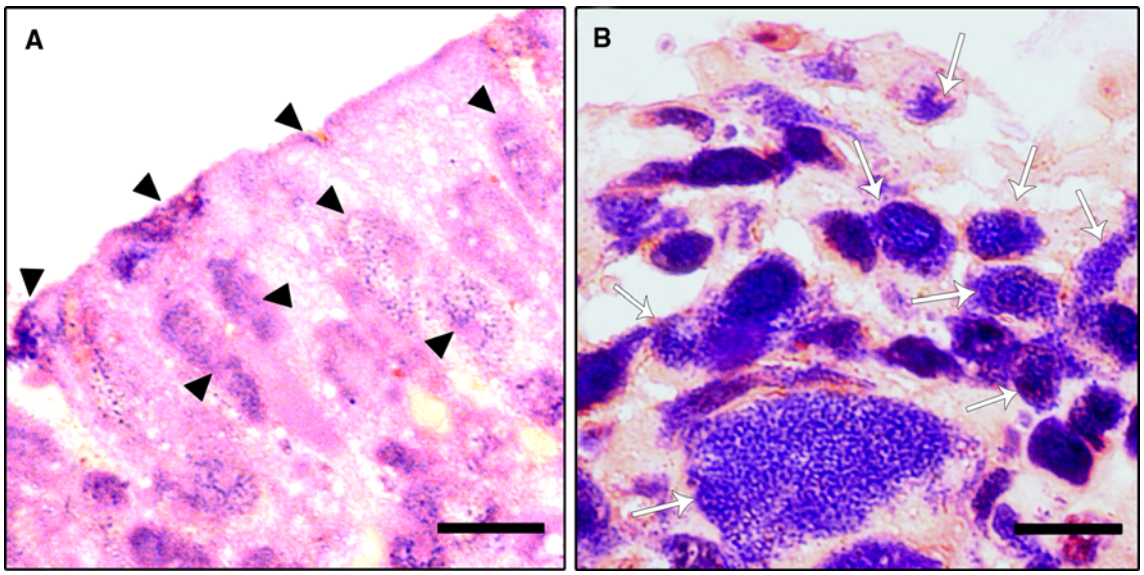

Fig. 3 Gram stain of tissue sections showed bacterial microcolonies localized on the surface of the adenoids. **a** Image showed that Gram-positive cocci (subject no. 10) were dispersed to the surface of the adenoids (*arrowheads*). **b** In patients with MRSA (subject no. 3), Gram-positive cocci were clustered in microcolonies, which were delineated on the adenoid tissue surface and crypts. *Arrows* indicated the formation of bacterial microcolonies. *Scale bar* 20 μm

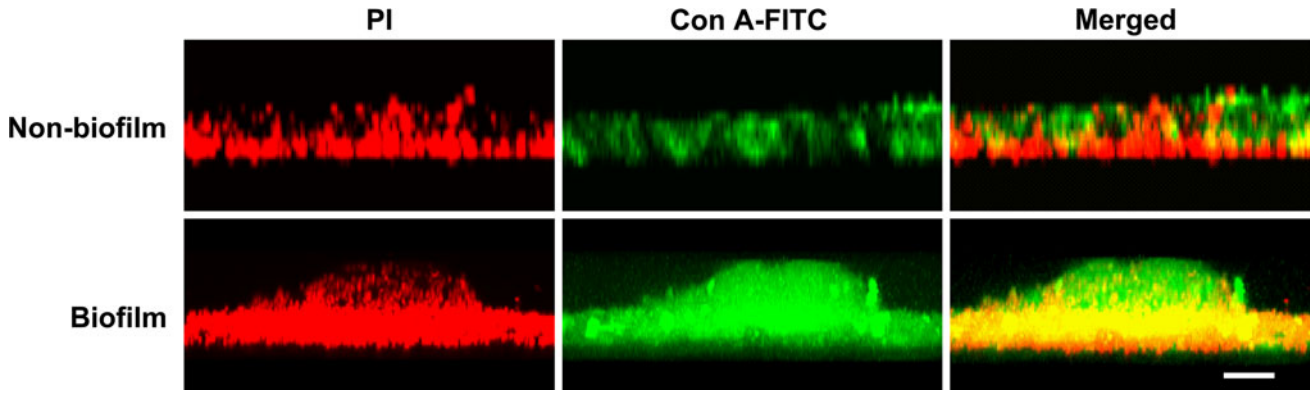

Fig. 4 CLSM shows biofilm formation activity in *S. aureus* isolates. Bacterial biofilm formation of *S. aureus* bacteria was stained using PI (*red*), and extracellular glycocalyx was stained using ConA-FITC (*green*). In the merged images, *yellow* indicates co-localization of bacteria and glycocalyx. *Upper panel* subject no. 10; *lower panel* subject no. 3. Biofilm formation in MRSA isolates showed a large amount of glycocalyx organized in clusters (merged, *lower panel*). *Scale bar* 50 μm. *PI* propidium iodide; *ConA-FITC* fluorescein isothiocyanate-conjugated concanavalin A

seen clustered in some compartments along the surface of the adenoids.

Quantification of bacterial biofilm formation

Biofilm formation activity was further visualized in all *S. aureus* isolates by staining the bacterial isolate with PI and ConA-FITC to distinguish between bacterial cells and extracellular polysaccharide glycocalyx within the biofilms. CLSM images revealed the accumulation of *S. aureus* bacterial cells (red) and extracellular polysaccharide glycocalyx (green) after 24 h in culture (Fig. 4). The biofilm formation activity of the MRSA isolates was much higher than that of the MSSA isolates (Fig. 4, ConA-FITC). The thickness of the biofilm in MRSA and MSSA isolates ranged from 100 to 130 μm and 40 to 50 μm, respectively. To further compare the activity of biofilm formation between the MRSA and MSSA isolates, biofilm-forming activity was determined in the 16 *S. aureus* isolates by using the crystal violet method. The activity of biofilm formation by the MRSA isolates was higher than that by the MSSA isolates (OD_{570} at 0.61 ± 0.09 vs. 0.45 ± 0.04 by using the Mann–Whitney *U* test; $P < 0.05$).

Using both in vivo morphological observation and in vitro bacterial biofilm assay, the biofilm colonies were

found in all seven patients infected with MRSA and, however, only in four patients with infection of MSSA (Table 1). These data suggest that interconnected bacteria become encapsulated in extracellular polysaccharide, which may lead to enhanced biofilm formation and persistent *S. aureus* infection, subsequently inducing more prominent clinical adenoid hyperplasia.

Discussion

MRSA is a hospital-acquired bacterium and is associated with invasive medical procedures such as indwelling vascular devices or catheters [19]. In 1999, 5% of patients who had received sinus surgery had MRSA growth in their sinus cultures, and the presence of these bacteria was believed to be nosocomial [20]. Community-acquired MRSA infections without an obvious connection to hospital facilities have increased noticeably since 2002 [7]. A nationwide assessment of common otolaryngologic infections in Japan in 2003 showed that MRSA comprised 15.6% of isolated *S. aureus* strains [21]. A comparative survey of *S. aureus* in acute and chronic rhinosinusitis between 2001–2003 and 2004–2006 found that colonization of *S. aureus* in acute/chronic rhinosinusitis significantly increased from 8–15% in 2001–2003 to 10–30% in 2004–2006. MRSA comprised 27–30% of *S. aureus* in 2001–2003 and dramatically increased to 61–69% in 2004–2006 [22]. In this study, *S. aureus* was found in 21.2% of specimens from chronic adenoiditis, of which 35% was MRSA. The increasing frequency of *S. aureus*, especially MRSA, in chronic otolaryngologic infections should not be ignored.

Although *S. aureus* is well known as a "persistent pathogen" in the human body [23], the exact role of MRSA in otolaryngologic infection is still unknown. Whether the presence of MRSA in the adenoid is indicative of its pathogenicity or whether MRSA in this environment acts as a commensal microorganism is still an issue of debate. Although computed tomography has been considered an important diagnostic and staging tool for evaluating chronic rhinosinusitis [24], it is usually recommended only in the case of persistent, recurrent, or complicated rhinosinusitis [25]. Comprehensive evaluation of paranasal sinuses by computed tomography is not considered a routine procedure for patients with OME or OSA, especially in the case of pediatric patients. A plain sinus radiograph such as the Water's view radiograph is a simple and inexpensive method that has sufficient sensitivity for evaluating the severity of inflammation in maxillary sinuses [26]. Using computed tomography results as the standard criterion, the diagnostic sensitivity of the Water's view radiograph for maxillary sinusitis is approximately 83.3% [27]. When the results of sinus puncture are considered as the diagnostic criterion, the diagnostic sensitivity of the Water's view radiograph for maxillary sinusitis is approximately 90% [28]. This study showed that the severity of maxillary sinusitis in patients with MSSA- or MRSA-infected adenoiditis was not significantly different, as assessed by the traditional Water's view. This is consistent with a previous finding that the presence of MRSA does not intrinsically imply a more virulent disease in rhinosinusitis [29].

In addition, this study revealed that the degree of adenoid hyperplasia was more severe in MRSA-infected patients than in MSSA-infected patients, as shown by the lateral cephalometric analysis. The nasopharyngeal airway is a three-dimensional structure, and some information may be lost when three-dimensional data are compressed into two dimensions in methods like lateral cephalometry. Currently available data suggest that cephalometric imaging analysis of nasopharyngeal spaces should be used with caution [30]. The nasopharyngeal airway can be directly observed during nasoendoscopy; therefore, nasoendoscopy has been suggested as the gold standard diagnostic method for examining the nasopharyngeal airway [31]. A statistically significant correlation has been reported between ANR analysis of adenoid enlargement and nasoendoscopic examination findings [31], and lateral cephalometric analysis has been recommended as a reliable method for assessing the degree of adenoid enlargement [13].

Adenoid hyperplasia may cause eustachian tube dysfunction and may contribute to the development of otitis media in children [2]. However, our analysis only involved comparison of the degree of adenoid hyperplasia observed in MRSA- and MSSA-infected patients. Other factors also contribute to adenoid hyperplasia, such as gastroesophageal reflux disease [32], allergy [33], and tobacco exposure [34]. Because this is a retrospective study, it was not possible to obtain complete records regarding these potential confounding factors. The detailed interaction of these contributing factors could not be comprehensively and conclusively determined in this retrospective analysis. Although the results for the subgroup members (for biofilm analysis) may not be representative of the outcomes of the entire sample population, our results reflect the fact that adenoid hyperplasia was more severe and biofilm formation was more extensive in MRSA-infected patients than in MSSA-infected patients. Further prospective studies may be required to elucidate the relationship between the confounding factors and the development of antibiotic-resistant strains in patients with adenoid hyperplasia.

Bacterial biofilm formation has been implicated in both the pathogenesis of chronic diseases and the development of antimicrobial resistance [35]. An increase in biofilm density on the adenoid surface also occurs in children with recurrent or persistent otitis media [36]. Therefore, we further studied the biofilm activity of MRSA and MSSA in

vivo and in vitro. Direct morphological examination of infected adenoid specimens by traditional SEM was performed to determine the distribution of bacterial microcolonies that had adhered to the surface of adenoid tissues. The dynamic process of bacterial biofilm formation induces resistance to both the host immune attack and treatment with antimicrobial agents [9].

The biofilm may provide an environment for the transfer of DNA between bacteria (horizontal gene transfer), which could eventually lead to antimicrobial resistance [1]. This might support our finding of the association between antibiotic resistance and biofilm-forming tendencies of *S. aureus*, although there are also reports that suggest different mechanisms for the development of resistance in this bacteria [37, 38]. Here, we analyzed 16 bacterial isolates and showed that *S. aureus* bacterial biofilm formation on adenoidectomy tissues is associated with antibiotic resistance. The sample size for this study was, however, limited, and the results will have to be confirmed in a larger study. A greater number of bacterial isolates should clarify the linkage between antimicrobial resistance and the expression of genes that are required for biofilm formation.

Conclusion

This retrospective study demonstrated that *S. aureus* is a common pathogen in children with adenoid hyperplasia in Taiwan. Our data provide evidence for an association between persistent *S. aureus* infections and patients with adenoid hyperplasia. The degree of adenoid hyperplasia was significantly greater in MRSA-infected patients than in MSSA-infected patients. This suggests that clinical treatment of adenoid hyperplasia patients who harbor antimicrobial-resistant strains requires more extensive consideration of bacterial biofilm-forming activity, including surgical eradication.

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Conflict of interest None declared.

References

1. Post JC, Hiller NL, Nistico L, Stoodley P, Ehrlich GD (2007) The role of biofilms in otolaryngologic infections: update 2007. Curr Opin Otolaryngol Head Neck Surg 15:347–351
2. van Cauwenberge PB, Bellussi L, Maw AR, Paradise JL, Solow B (1995) The adenoid as a key factor in upper airway infections. Int J Pediatr Otorhinolaryngol 32 Suppl:S71–S80
3. Bernstein JM, Dryja D, Murphy TF (2001) Molecular typing of paired bacterial isolates from the adenoid and lateral wall of the nose in children undergoing adenoidectomy: implications in acute rhinosinusitis. Otolaryngol Head Neck Surg 125:593–597
4. Pettigrew MM, Gent JF, Revai K, Patel JA, Chonmaitree T (2008) Microbial interactions during upper respiratory tract infections. Emerg Infect Dis 14:1584–1591
5. Brook I, Gober AE (2005) Antimicrobial resistance in the nasopharyngeal flora of children with acute otitis media and otitis media recurring after amoxicillin therapy. J Med Microbiol 54:83–85
6. Bachert C, Zhang N, Patou J, van Zele T, Gevaert P (2008) Role of staphylococcal superantigens in upper airway disease. Curr Opin Allergy Clin Immunol 8:34–38
7. Crum NF, Lee RU, Thornton SA, Stine OC, Wallace MR, Barrozo C, Keefer-Norris A, Judd S, Russell KL (2006) Fifteen-year study of the changing epidemiology of methicillin-resistant *Staphylococcus aureus*. Am J Med 119:943–951
8. Becker SS, Russell PT, Duncavage JA, Creech CB (2009) Current issues in the management of sinonasal methicillin-resistant *Staphylococcus aureus*. Curr Opin Otolaryngol Head Neck Surg 17:2–5
9. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2:95–108
10. Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK (2005) The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN-gamma-mediated macrophage killing. J Immunol 175:7512–7518
11. Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34–39
12. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. 9th edn, 2006
13. Kemaloglu YK, Goksu N, Inal E, Akyildiz N (1999) Radiographic evaluation of children with nasopharyngeal obstruction due to the adenoid. Ann Otol Rhinol Laryngol 108:67–72
14. Major MP, Flores-Mir C, Major PW (2006) Assessment of lateral cephalometric diagnosis of adenoid hypertrophy and posterior upper airway obstruction: a systematic review. Am J Orthod Dentofacial Orthop 130:700–708
15. Saiki T, Yumoto E (1997) Quantification of X-ray opacity of the maxillary sinus in the Waters' view. Auris Nasus Larynx 24:289–297
16. O'Neill E, Pozzi C, Houston P, Smyth D, Humphreys H, Robinson DA, O'Gara JP (2007) Association between methicillin susceptibility and biofilm regulation in *Staphylococcus aureus* isolates from device-related infections. J Clin Microbiol 45:1379–1388
17. Kania RE, Lamers GE, Vonk MJ, Dorpmans E, Struik J, Tran Ba Huy P, Hiemstra P, Bloemberg GV, Grote JJ (2008) Characterization of mucosal biofilms on human adenoid tissues. Laryngoscope 118:128–134
18. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ (2007) Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. Laryngoscope 117:1302–1306
19. Steinberg JP, Clark CC, Hackman BO (1996) Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. Clin Infect Dis 23:255–259
20. Jiang RS, Jang JW, Hsu CY (1999) Post-functional endoscopic sinus surgery methicillin-resistant *Staphylococcus aureus* sinusitis. Am J Rhinol 13:273–277

21. Suzuki K, Nishimura T, Baba S (2003) Current status of bacterial resistance in the otolaryngology field: results from the Second Nationwide Survey in Japan. J Infect Chemother 9:46–52
22. Brook I, Foote PA, Hausfeld JN (2008) Increase in the frequency of recovery of meticillin-resistant *Staphylococcus aureus* in acute and chronic maxillary sinusitis. J Med Microbiol 57:1015–1017
23. Sheagren JN (1984) *Staphylococcus aureus*. The persistent pathogen (first of two parts). N Engl J Med 310:1368–1373
24. Bhattacharyya N, Fried MP (2003) The accuracy of computed tomography in the diagnosis of chronic rhinosinusitis. Laryngoscope 113:125–129
25. Esposito S, Bosis S, Bellasio M, Principi N (2007) From clinical practice to guidelines: how to recognize rhinosinusitis in children. Pediatr Allergy Immunol 18(Suppl 18):53–55
26. De Sutter A, Spee R, Peersman W, De Meyere M, Van Cauwenberge P, Verstraete K, De Maeseneer J (2005) Study on the reproducibility of the Waters' views of the maxillary sinuses. Rhinology 43:55–60
27. Timmenga N, Stegenga B, Raghoebar G, van Hoogstraten J, van Weissenbruch R, Vissink A (2002) The value of Waters' projection for assessing maxillary sinus inflammatory disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 93:103–109
28. Engels EA, Terrin N, Barza M, Lau J (2000) Meta-analysis of diagnostic tests for acute sinusitis. J Clin Epidemiol 53:852–862
29. Gerencer RZ (2005) Successful outpatient treatment of sinusitis exacerbations caused by community-acquired methicillin-resistant *Staphylococcus aureus*. Otolaryngol Head Neck Surg 132:828–833
30. Aboudara CA, Hatcher D, Nielsen IL, Miller A (2003) A three-dimensional evaluation of the upper airway in adolescents. Orthod Craniofac Res 6(Suppl 1):173–175
31. Caylakli F, Hizal E, Yilmaz I, Yilmazer C (2009) Correlation between adenoid–nasopharynx ratio and endoscopic examination of adenoid hypertrophy: a blind, prospective clinical study. Int J Pediatr Otorhinolaryngol 73:1532–1535
32. Yousef E, Kung S-J, Malloy C (2009) Risk factors for adenoidal regrowth among patients in a pediatric allergy practice. Pediatr Asthma Allergy Immunol 22:169–173
33. Sadeghi-Shabestari M, Jabbari Moghaddam Y, Ghaharri H (2011) Is there any correlation between allergy and adenotonsillar tissue hypertrophy? Int J Pediatr Otorhinolaryngol 75:589–591
34. Finkelstein Y, Malik Z, Kopolovic J, Bernheim J, Djaldetti M, Ophir D (1997) Characterization of smoking-induced nasopharyngeal lymphoid hyperplasia. Laryngoscope 107:1635–1642
35. Hunsaker DH, Leid JG (2008) The relationship of biofilms to chronic rhinosinusitis. Curr Opin Otolaryngol Head Neck Surg 16:237–241
36. Zuliani G, Carlisle M, Duberstein A, Haupert M, Syamal M, Berk R, Du W, Coticchia J (2009) Biofilm density in the pediatric nasopharynx: recurrent acute otitis media versus obstructive sleep apnea. Ann Otol Rhinol Laryngol 118:519–524
37. Vaudaux PE, Monzillo V, Francois P, Lew DP, Foster TJ, Berger-Bachi B (1998) Introduction of the mec element (methicillin resistance) into *Staphylococcus aureus* alters in vitro functional activities of fibrinogen and fibronectin adhesins. Antimicrob Agents Chemother 42:564–570
38. Seaman PF, Ochs D, Day MJ (2007) Small-colony variants: a novel mechanism for triclosan resistance in methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 59:43–50