

Pulsed-Field Gel Electrophoresis of *Staphylococcus aureus* Isolates from Atopic Patients Revealing Presence of Similar Strains in Isolates from Children and Their Parents[▽]

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Skin colonization with *Staphylococcus aureus* is often associated with atopic dermatitis, and staphylococcal enterotoxins have been implicated in the etiology of atopic disease. In this study, the colonization of patients with atopic dermatitis and their parents was investigated in order to evaluate the possibility of intrafamilial transmission. *S. aureus* strains were isolated from 30 of 45 patients (66%). In 19 of 29 families (65%), at least one parent carried *S. aureus*, and the overall colonization rate of the parents was 48%. All strains were typed by pulsed-field gel electrophoresis (PFGE), and the presence of enterotoxin genes in the strains was assayed by multiplex PCR. A high percentage (84%) of the isolates present on the children and on at least one of their parents displayed identical PFGE and enterotoxin patterns as well as identical antibiotic resistance profiles, indicating intrafamilial transmission. Forty-five percent of the strains did not carry any enterotoxin gene. The most frequently found enterotoxin genes were *seg* and *sei*, which were present in 36% of the strains, and *seb*, which was found in 24% of the strains. The other toxin genes occurred only in low frequencies. Most strains were resistant to penicillin (82%), and 15% showed resistance to more than one antibiotic. Intermediately-vancomycin-resistant *S. aureus* or methicillin-resistant *S. aureus* strains were not detected. In conclusion, this study indicates that the colonization rate of parents of atopic children is rather high and may increase the risk of recolonization of the child.

Staphylococcus aureus is a gram-positive pathogen that has been implicated in the pathogenesis of atopic dermatitis (AD). AD is one of the most frequent chronic inflammatory skin diseases and is found in 10% of all German children starting school (35). It is characterized by a dysregulation of the immune system that involves an increased Th2 response with an enhanced level of Th2 cytokines in the acute phase, a decreased level of other cytokines (e.g., tumor necrosis factor alpha), and decreased production of antistaphylococcal peptides of the innate immune system, i.e., β -defensin 3 and cathelicidins (12, 30). This peptide deficiency, the impaired skin integrity, and the increased expression of fibrinogen (9) favor colonization with *S. aureus* in AD, which is found in a higher percentage on the skin of affected children than on the skin of a healthy control group (23). Many strains of *S. aureus* are able to secrete superantigens, i.e., enterotoxins and toxic shock syndrome toxin. The production of superantigens may lead to an aggravation of AD (3), and a reduction of colonization has been shown to be effective in reducing the severity of the disease (14). Furthermore, exposure to superantigens may lead to the sensitization of the patient and the production of specific immunoglobulin E antibodies against enterotoxin A and enterotoxin B. These antibody titers were found to be elevated in

patients with severe skin lesions (17, 25). Therefore, a reduction of *S. aureus* colonization in AD patients is desired. This study was conducted in order to assess the colonization of atopic patients and transmission of the strains between children and their parents. Moreover, whereas the roles of enterotoxins A, B, and C and toxic shock syndrome toxin in AD have been studied (7, 17), in the last years, a number of new enterotoxin genes have been detected in *S. aureus* (for a review, see references 21 and 40), and a further aim of this study was to determine the frequency of these genes in strains isolated from patients with AD. Besides, there is an increasing incidence of community-acquired methicillin-resistant *S. aureus* (34), and the first reports of infection of AD patients with methicillin-resistant strains in the far east (1, 16) and the United States (38) have been published, which led us to determine the antibiotic susceptibilities of our isolates.

MATERIALS AND METHODS

Strains and media. A total of 67 isolates from patients and their parents who visited the outpatient clinic at the Department of Dermatology, University of Bonn, were characterized in this study. The samples were taken from lesional skin and the anterior nares (patients) or the anterior nares only (relatives) using Metaswab tubes (Mast, Brescia, Italy) and were cultured within 24 h on Columbia blood agar (Becton Dickinson, Heidelberg, Germany). The identification of *S. aureus* was carried out by testing the production of clumping factor and free coagulase. *S. aureus* strains were stored as frozen stocks in 50% glycerol at -70°C and were cultured in brain heart infusion medium (Oxoid, Wesel, Germany) at 37°C with aeration unless indicated otherwise.

PFGE. Chromosomal DNA for the SmaI restriction digest was purified from the strains as described previously (12). Pulsed-field gel electrophoresis (PFGE) was performed using the Chef DRIII system (Bio-Rad, Munich, Germany) by employing pulsed-field certified agarose (1%) (Bio-Rad) at 6 V/cm, a field angle

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of 120°, and switch times of 5 to 15 s for 7 h and 15 to 60 s for a further 19 h. A chromosomal DNA digest of *S. aureus* NCTC8325 served as the mass standard.

Antimicrobial susceptibility tests. Antimicrobial susceptibility tests against penicillin, methicillin, oxacillin, cefazolin, ceftiofur, imipenem, gentamicin, netilmicin, erythromycin, clindamycin, doxycycline, ciprofloxacin, levofloxacin, fosfomycin, teicoplanin, vancomycin, mupirocin, fusidic acid, and linezolid (Oxoid, Hampshire, England) were performed according to protocols recommended by the Clinical and Laboratory Standards Institute or DIN 58940-4 by employing the disk diffusion method or Etest (AB Biodisk, Solna, Sweden).

PCR of enterotoxin genes. Attempts to perform a PCR employing whole cells were unsuccessful. Therefore, chromosomal DNA was purified using Genomic-Tips 20/G (Qiagen, Hilden, Germany) after cell lysis in the presence of lyso-staphin (Sigma-Aldrich, Taufkirchen, Germany) or by employing the Instagene Matrix kit (Bio-Rad), both in accordance with the manufacturers' instructions. DNA was analyzed using a Nanodrop 1000 spectrophotometer (Peglab Biotechnologie, Erlangen, Germany).

The presence of enterotoxin genes was screened by two multiplex PCR assays covering the *sea*, *seb*, *sec*, *seh*, and *sej* (group 1) genes or the *sed*, *see*, *seg*, and *sei* (group 2) genes. The primers *seb*-sec forward, *seb* reverse, *sei* forward, and *sei* reverse were described previously by Løvseth et al. (26), whereas all other primers were designed as described previously by Monday and Bohach (28), using the 16S rRNA gene as an internal control for both groups. The PCR conditions were 95°C for 10 min for the initial denaturation step, followed by 20 cycles (denaturation step at 95°C for 1 min, annealing step at 68°C for 45 s, and primer elongation step at 72°C for 1 min), which were followed by 30 cycles with an annealing step at 62°C and a final elongation step at 72°C for 10 min. When only *sei* or *seg* had been detected in a strain, the presence of the second gene was probed using additional alternative primers for *seg* and *sei* that had been designed according to nucleotide exchanges described previously (6): *seghinmut* (5'-ATGCTCTCCACCTGTTGAAGG-3'), *seihinmut* (5'-CAACTTGAATTTTC AACCGGTACC-3'), and *seirueckmut* (5'-CAGGCAGACCATGTCTG-3').

These primers were employed in a conventional PCR (Go Taq polymerase; Promega GmbH, Mannheim, Germany) (annealing step at 60°C and 30 cycles). All positive multiplex PCR signals were verified by conventional PCR by employing the specific primers at an annealing temperature of 60°C for 30 cycles.

The following *S. aureus* reference strains were employed: *S. aureus* Mu50 (*sea*, *sei*, and *seg*) (20), *S. aureus* ATCC 14458/NCTC10654 (*seb*) (2), *S. aureus* LT 759/04 (Reference Centre for Staphylococci, Bonn, Germany) (*sea* and *sec*), *S. aureus* ATCC 23235/NCTC10656 (*sed* and *sej*) (8), and *S. aureus* ATCC 27664 (*see*) (5).

RESULTS

Colonization of atopic children and their families with *S. aureus*. One hundred fifty-six samples originating from 45 patients and their relatives were evaluated in this study. Representative skin lesions and the anterior nares of the patients were swabbed. Forty-three *S. aureus* isolates were obtained from 30 patients (66%). Fifteen children were not colonized. The 30 colonized children (17 patients 1 to 3 years of age, 6 patients 4 to 6 years of age, 3 patients 7 to 9 years of age, and four patients 9 to 17 years of age; mean age, 4.8 years) belonged to 29 different families. Relatives who accompanied these patients to the clinic were also probed for colonization with *S. aureus* using nose swabs. For 23 families, both father and mother were examined. Here, 13 mothers (57%) and 9 fathers (39%) were positive for *S. aureus*, and in 19 of the 23 families, at least one parent was colonized (Table 1). In total, the colonization rate of the parents was 48%. In six cases, it was not possible to obtain a sample from the father.

All 67 isolates were characterized by PFGE. The analyses showed that in 16 (84%) of the 19 families where an isolate had been obtained from at least one parent, isolates with an indistinguishable PFGE pattern were present on the patient and his (her) mother or father. In 10 cases, the patient strain colonized the mother, and in six cases, a similar strain was present on the father. In three cases, strains with related patterns were iso-

lated from the father. In one family (patient 16), all members were colonized by the same strain (Table 1). For 15 patients, isolates had been collected from infected skin and nose, and 11 patients (73%) were colonized with identical strains in both locations (Table 2). As a consequence, the *S. aureus* isolates obtained in this study could be grouped into 38 different strains.

Distribution of enterotoxin genes. All isolates were tested for the presence of enterotoxin genes by employing two multiplex PCRs. Although *sei* and *seg* are encoded on the same pathogenicity island, the enterotoxin gene cluster *egc*, frequently only *sei* or *seg* was detected using the primer pairs described previously (26, 28). This effect may be caused by the polymorphisms that were found in *egc* and that impede the annealing of some primers (6). Indeed, an analysis of the polymorphisms described and the primers used showed that some strains harbor a one-base exchange in the 5' terminus of primer "seg forward" as well as in the *sei* primers. The design of new primers led to a complete detection rate of *sei* and *seg* genes.

Seventeen strains (45%) did not carry any of the enterotoxin genes tested (Tables 1 and 2). For the enterotoxigenic strains, the *seg/sei* combination was found most frequently and was present in 14 strains (36%). Furthermore, nine strains (24%) were characterized by the enterotoxin B gene *seb*. The other enterotoxins were present in lower frequencies, ranging from 2.6% to 5.2% of the isolates; *see* and *seh* were not found (Fig. 1). If the enterotoxin gene cluster (*egc*) that carries *seg* and *sei* is counted as one enterotoxin locus, then 13 strains carried one enterotoxin-encoding locus, 6 strains were characterized by two loci, and 2 strains harbored three different enterotoxin loci.

Antibiotic resistance. The antibiotic resistances of all 67 isolates were tested. The isolates that belonged to single strains did not differ in their susceptibilities. Eighteen percent of the strains did not show any resistance, and the majority of the strains (31 strains [82%]) were resistant to penicillin (Tables 1 and 2). Only a few strains additionally showed resistance to other antibiotics, i.e., erythromycin, gentamicin, netilmicin, mupirocin, and fusidic acid. The percentage of multiply resistant strains was low (four strains were resistant to two substances, and two single strains were resistant to three and four agents, respectively). The strain that was resistant to four agents, penicillin, fusidic acid, mupirocin (MIC > 1,024 mg/liter), and erythromycin, colonized both parents and the child and carried the enterotoxin genes *seb* and *egc*. Methicillin-resistant *S. aureus* or strains showing intermediate vancomycin resistance were not detected.

DISCUSSION

Colonization of atopic patients with *S. aureus* has been well documented, and some older studies indicated colonization rates well over 90% (10, 24). In Bonn, only 66% of the patients were found to be colonized with *S. aureus*; however, this result corresponds to other studies performed in the last years that detected between 57% and 64.2% of colonized patients (16, 33, 39). It has been shown that colonization with *S. aureus* depends on the Th2 response and that even anti-inflammatory treatment will reduce skin colonization (13), indicating that colo-

TABLE 1. Comparison of isolates obtained from different family members^a

Source of isolate	Localization	PFGE	Strain	Enterotoxin gene(s)	Antibiotic resistance
Patient 2	Nose	Unrelated	D2		Pen
Patient 2, mother	Nose		D3		
Patient 3	Hand	Unrelated	D4		Pen, Fus
Patient 3, mother	Nose		D5		
Patient 8	Nose	Similar	D12	A, B, C	Pen
Patient 8, mother	Nose				
Patient 9	Nose	Similar	D13	A	Pen, Net
Patient 9, mother	Foot Nose				
Patient 10	Nose	Similar	D14	G, I	Pen
Patient 10, mother	Eyelid Nose				
Patient 11	Nose	Unrelated	D15	G, I	Pen
Patient 11, mother	Nose		D16		
Patient 12	Nose	Similar	D17	G, I	Pen
Patient 12, father	Nose				
Patient 13	Nose	Similar	D18	G, I	Pen
Patient 13, mother	Nose				
Patient 14	Hand	Similar	D19		Pen
Patient 14, father	Nose				
Patient 15	Nose	Similar	D20		Pen, Ery
Patient 15, mother	Nose				
Patient 16	Nose	Similar	D21	B, G, I	Pen, Mup, Ery, Fus
Patient 16, mother	Hand				
Patient 16, father	Nose Nose				
Patient 17	Cheek	Similar	D22	B, G, I	Pen
Patient 17, father	Nose				
Patient 18	Nose	Similar	D23	B	Pen
Patient 18, father	Forehead Nose				
Patient 19	Cheek	Similar	D24	G, I	Pen
Patient 19, mother	Nose				
Patient 20	Ear	Similar	D25	B	Pen
Patient 20, father	Nose				
Patient 23	Nose	Similar	D28	A	Pen
Patient 23, mother	Arm Nose				
Patient 24a	Hand	Similar	D29		Pen
Patient 24b	Face				
Patient 24b	Nose	Similar	D30		Pen
Patient 24, mother	Nose				
Patient 24, father	Nose	Related	D31		
Patient 26	Nose	Similar	D34	B, G, I	Pen
Patient 26, father	Hand Nose				
Patient 27	Nose	Related	D35	B, G, I	Pen
Patient 27, mother	Neck	Similar	D36		
Patient 27, father	Nose Nose	Related	D37		

^a Similar strains displayed identical PFGE patterns, whereas in related strains, at least one band shift had occurred. Abbreviations: Mup, mupirocin; Fus, fusidic acid; Pen, penicillin; Net, netilmicin; Ery, erythromycin.

TABLE 2. Comparison of isolates obtained from different locations

Source of isolate	Localization	PFGE	Strain	Enterotoxin gene(s)	Antibiotic resistance ^a
Patient 4	Nose Arm	Similar	D6	D, J	
Patient 5	Nose Knee	Related	D7 D8	B, G, I	Pen Pen
Patient 6	Nose Knee	Similar	D9		Pen
Patient 7	Nose Leg	Unrelated	D10 D11	G, I G, I	
Patient 9	Nose Foot	Similar	D13	A	Pen, Net
Patient 10	Nose Eyelid	Similar	D14	G, I	Pen
Patient 16	Nose Hand	Similar	D21	B, G, I	Pen, Mup, Ery, Fus
Patient 18	Nose Forehead	Similar	D23	B	Pen
Patient 21	Nose Arm	Similar	D26		Pen
Patient 22	Nose Foot	Similar	D27		Pen
Patient 23	Nose Arm	Similar	D28	A	Pen
Patient 24b	Face Nose	Related	D29 D30		Pen Pen
Patient 26	Nose Hand	Similar	D34	B, G, I	Pen
Patient 27	Nose Neck	Unrelated	D35 D36		Pen
Patient 28	Nose Perioral	Similar	D38	D, G, I, J	Pen, Net, Gen

^a Abbreviations: Mup, mupirocin; Fus, fusidic acid; Pen, penicillin; Net, netilmicin; Ery, erythromycin.

nization is dependent on the severity of the disease and that pretreatment with steroids or antibiotics will inhibit the growth of *S. aureus*. In contrast to other studies (see, e.g., reference 27), the antibiotic and steroid treatments had not been suspended before the samples were taken, which may explain the rather low isolation rate.

The colonization rate for the parents (48%) was higher than that of the average population. For example, a study that elucidated the colonization rate of young menstruating women found only 26% colonization (31); in contrast, here, 57% of the mothers of atopic children were colonized. There may be multiple reasons for the high colonization rates of the parents: (i) the treatment of the infected lesions is normally carried out by the parents and offers excellent opportunities for transmission of the bacteria, (ii) there is close physical contact between parents and especially small children, and (iii) the parents of

atopic children are often characterized by susceptibility to atopic disease as well and therefore may be prone to colonization by *S. aureus*. In this context, it is interesting that one father of our group still suffered from atopic eczema.

In order to investigate whether transmission within the families had taken place, the bacteria were typed. The PFGE, enterotoxin, and antibiotic resistance patterns showed that in the large majority of the families (84%), identical strains had been isolated from the child and his or her parent(s), indicating transmission within the family. In one case, the strain isolated from the skin of the child was identical to the strain that was present in the nose of the mother, whereas a different strain colonized the nose of the child itself. Even if the strains were not identical, related strains were often present within one family, indicating that strain transmission had taken place some time ago. For 73% of the patients, identical strains were obtained from nose and skin, and in 13% of the cases, related strains were present. This high frequency of identical and related strains is best explained by the fact that nasal carriage of *S. aureus* has been shown to be associated with hand carriage (32, 37), and the children may transfer the nose strains to their lesions in this way.

The production of enterotoxins by strains that colonize atopic patients has been well studied, but most analyses examined only the excretion of enterotoxins A, B, and C and toxic shock syndrome toxin 1 (41, 42). In most of those studies, *seb* was the most commonly found enterotoxin gene (42), and likewise, *seb* was one of the most frequently encountered enterotoxin genes seen in our strain collection; it was found in 9 of the 38 strains in this study.

However, the most frequently isolated enterotoxin genes were *seg* and *sei*, indicating the presence of the *egc* enterotoxin gene cluster. In 14 of 38 strains, both genes were detectable, giving an isolation rate of 37% for all strains or of 66% with regard to 21 enterotoxin-positive strains. Recent studies indicated that *egc* seems to be rather frequently found in animal isolates (25%) (36) and even more so in human isolates (55%) (4). Mempel et al. (27) previously tested for the presence of *egc* in isolates from patients with AD and also found the gene cluster in 48% of the isolates. Therefore, the frequency of *egc* in our isolates is not surprising.

In addition to enterotoxin I and enterotoxin G, *egc* often encodes the enterotoxin-like superantigens M, N, and O (*sem-seo*) (19) and sometimes enterotoxin U (22). It has been demonstrated that the *egc* enterotoxins are able to stimulate T-cell proliferation, and some cases of *egc*-mediated staphylococcal toxemias have been observed (18). However, although the en-

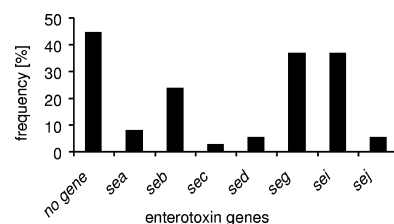


FIG. 1. Percent frequency of enterotoxin genes in 40 genetically distinct strains of *S. aureus*. The first bar shows the frequency of strains that carried none of the genes tested.

terotoxins of the *egc* gene cluster seem to be the most common staphylococcal enterotoxins, the serum levels of neutralizing antibodies against these toxins are lower than those against the classical enterotoxins (*sea* to *sed*) (15), and the toxins seem to be produced in lower quantities (29). The impact of these enterotoxins on AD remains to be evaluated.

It has been demonstrated that skin colonization with *S. aureus* is associated with an exacerbation of eczema in atopic disease, and therefore, current therapeutic approaches include anti-inflammatory and antimicrobial treatment in order to reduce or eliminate colonization (13, 14). Failure of treatment and recolonization of the skin may result from nasal carriage, antibiotic resistance of the strains, and contamination during treatment (11). The results demonstrate that the noses and/or skin of many children and their parents were colonized and indicate that intrafamilial spread had occurred. In conclusion, it should be determined whether the children might benefit from a surveillance of the carrier status of their own noses and those of their family members in order to avoid intrafamilial "ping pong" infections.

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REFERENCES

- Akiyama, H., O. Yamasaki, J. Tada, and J. Arata. 2000. Adherence characteristics and susceptibility to antimicrobial agents of *Staphylococcus aureus* strains isolated from skin infections and atopic dermatitis. *J. Dermatol. Sci.* **23**:155–160.
- Altner, R. A. 1975. Membrane mutations and production of enterotoxin B and alpha hemolysin in *Staphylococcus aureus*. *Can. J. Microbiol.* **21**:275–280.
- Ardern-Jones, M. R., A. P. Black, E. A. Bateman, and G. S. Ogg. 2007. Bacterial superantigen facilitates epithelial presentation of allergen to T helper 2 cells. *Proc. Natl. Acad. Sci. USA* **104**:5557–5562.
- Becker, K., A. W. Friedrich, G. Lubritz, M. Weiler, G. Peters, and C. von Eiff. 2003. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J. Clin. Microbiol.* **41**:1434–1439.
- Bergdoll, M. S., C. R. Borja, R. N. Robbins, and K. F. Weiss. 1971. Identification of enterotoxin E. *Infect. Immun.* **4**:593–595.
- Blaiotta, G., V. Fusco, C. von Eiff, F. Villani, and K. Becker. 2006. Biotyping of enterotoxigenic *Staphylococcus aureus* by enterotoxin gene cluster (*egc*) polymorphism and *spa* typing analyses. *Appl. Environ. Microbiol.* **72**:6117–6123.
- Bunikowski, R., M. E. Mielke, H. Skarabis, M. Worm, I. Anagnostopoulos, G. Kolde, U. Wahn, and H. Renz. 2000. Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J. Allergy Clin. Immunol.* **105**:814–819.
- Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. A. Issa. 1967. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. *J. Bacteriol.* **94**:1875–1882.
- Cho, S. H., I. Strickland, A. Tomkinson, A. P. Fehring, E. W. Gelfand, and D. Y. Leung. 2001. Preferential binding of *Staphylococcus aureus* to skin sites of Th2-mediated inflammation in a murine model. *J. Invest. Dermatol.* **116**:658–663.
- Christophers, E., and T. Henseler. 1987. Contrasting disease patterns in psoriasis and atopic dermatitis. *Arch. Dermatol. Res.* **279**:S48–S51.
- Gilani, S. J., M. Gonzalez, I. Hussain, A. Y. Finlay, and G. K. Patel. 2005. *Staphylococcus aureus* re-colonization in atopic dermatitis: beyond the skin. *Clin. Exp. Dermatol.* **30**:10–13.
- Goering, R. V., and T. D. Duensing. 1990. Rapid field inversion gel electrophoresis in combination with an rRNA gene probe in the epidemiological evaluation of staphylococci. *J. Clin. Microbiol.* **28**:426–429.
- Gong, J. Q., L. Lin, T. Lin, F. Hao, F. Q. Zeng, Z. G. Bi, D. Yi, and B. Zhao. 2006. Skin colonization by *Staphylococcus aureus* in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. *Br. J. Dermatol.* **155**:680–687.
- Guzik, T. J., M. Bzowska, A. Kasprzowicz, G. Czerniawska-Mysik, K. Wojcik, D. Smyd, T. Adamek-Guzik, and J. Pryjma. 2005. Persistent skin colonization with *Staphylococcus aureus* in atopic dermatitis: relationship to clinical and immunological parameters. *Clin. Exp. Allergy* **35**:448–455.
- Holtfrete, S., K. Bauer, D. Thomas, C. Feig, V. Lorenz, K. Roschack, E. Friebe, K. Selleng, S. Lovenich, T. Greve, A. Greinacher, B. Panzig, S. Engelmann, G. Lina, and B. M. Broker. 2004. *egc*-encoded superantigens from *Staphylococcus aureus* are neutralized by human sera much less efficiently than are classical staphylococcal enterotoxins or toxic shock syndrome toxin. *Infect. Immun.* **72**:4061–4071.
- Hon, K. L., M. C. Lam, T. F. Leung, W. Y. Kam, M. C. Li, M. Ip, and T. F. Fok. 2005. Clinical features associated with nasal *Staphylococcus aureus* colonisation in Chinese children with moderate-to-severe atopic dermatitis. *Ann. Acad. Med. Singapore* **34**:602–605.
- Ide, F., T. Matsubara, M. Kaneko, T. Ichijima, T. Mukoyama, and S. Furukawa. 2004. Staphylococcal enterotoxin-specific IgE antibodies in atopic dermatitis. *Pediatr. Int.* **46**:337–341.
- Jarraud, S., G. Cozon, F. Vandenesch, M. Bes, J. Etienne, and G. Lina. 1999. Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. *J. Clin. Microbiol.* **37**:2446–2449.
- Jarraud, S., M. A. Peyrat, A. Lim, A. Tristan, M. Bes, C. Mougel, J. Etienne, F. Vandenesch, M. Bonneville, and G. Lina. 2001. *egc*, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. *J. Immunol.* **166**:669–677.
- Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, K. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu. 2001. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* **357**:1225–1240.
- Le Loir, Y., F. Baron, and M. Gautier. 2003. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* **2**:63–76.
- Letertre, C., S. Perelle, F. Dilasser, and P. Fach. 2003. Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. *J. Appl. Microbiol.* **95**:38–43.
- Leung, D. Y. 2003. Infection in atopic dermatitis. *Curr. Opin. Pediatr.* **15**:399–404.
- Leyden, J. J., R. R. Marples, and A. M. Kligman. 1974. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br. J. Dermatol.* **90**:525–530.
- Lin, Y. T., W. Y. Shau, L. F. Wang, Y. H. Yang, Y. W. Hwang, M. J. Tsai, P. N. Tsao, and B. L. Chiang. 2000. Comparison of serum specific IgE antibodies to staphylococcal enterotoxins between atopic children with and without atopic dermatitis. *Allergy* **55**:641–646.
- Løvseth, A., S. Loncarevic, and K. G. Berdal. 2004. Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. *J. Clin. Microbiol.* **42**:3869–3872.
- Mempel, M., G. Lina, M. Højka, C. Schnopp, H. P. Seidl, T. Schäfer, J. Ring, F. Vandenesch, and D. Abeck. 2003. High prevalence of superantigens associated with the *egc* locus in *Staphylococcus aureus* isolates from patients with atopic eczema. *Eur. J. Clin. Microbiol. Infect. Dis.* **22**:306–309.
- Monday, S. R., and G. A. Bohach. 1999. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *J. Clin. Microbiol.* **37**:3411–3414.
- Omoe, K., M. Ishikawa, Y. Shimoda, D. L. Hu, S. Ueda, and K. Shinagawa. 2002. Detection of *seg*, *seh*, and *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring *seg*, *seh*, or *sei* genes. *J. Clin. Microbiol.* **40**:857–862.
- Ong, P. Y., T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R. L. Gallo, and D. Y. Leung. 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.* **347**:1151–1160.
- Parsonnet, J., M. A. Hansmann, M. L. Delaney, P. A. Modern, A. M. Dubois, W. Wieland-Alter, K. W. Wisemann, J. E. Wild, M. B. Jones, J. L. Seymour, and A. B. Onderdonk. 2005. Prevalence of toxic shock syndrome toxin 1-producing *Staphylococcus aureus* and the presence of antibodies to this superantigen in menstruating women. *J. Clin. Microbiol.* **43**:4628–4634.
- Reagan, D. R., B. N. Doebbeling, M. A. Pfaller, C. T. Sheetz, A. K. Houston, R. J. Hollis, and R. P. Wenzel. 1991. Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin ointment. *Ann. Intern. Med.* **114**:101–106.
- Ricci, G., A. Patrizi, I. Neri, B. Bendandi, and M. Masi. 2003. Frequency and clinical role of *Staphylococcus aureus* overinfection in atopic dermatitis in children. *Pediatr. Dermatol.* **20**:389–392.
- Said-Salim, B., B. Mathema, and B. N. Kreiswirth. 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. *Infect. Control Hosp. Epidemiol.* **24**:451–455.
- Schäfer, T., U. Krämer, D. Vieluf, D. Abeck, H. Behrendt, and J. Ring. 2000.

- The excess of atopic eczema in East Germany is related to the intrinsic type. *Br. J. Dermatol.* **143**:992–998.
36. **Smyth, D. S., P. J. Hartigan, W. J. Meaney, J. R. Fitzgerald, C. F. Deobald, G. A. Bohach, and C. J. Smyth.** 2005. Superantigen genes encoded by the *egc* cluster and *SaPIbov* are predominant among *Staphylococcus aureus* isolates from cows, goats, sheep, rabbits and poultry. *J. Med. Microbiol.* **54**:401–411.
 37. **Solberg, C. O.** 1965. A study of carriers of *Staphylococcus aureus* with special regard to quantitative bacterial estimations. *Acta Med. Scand. Suppl.* **436**: 1–96.
 38. **Suh, L. M., P. J. Honig, and A. C. Yan.** 2006. Methicillin-resistant *Staphylococcus aureus* skin abscesses in a pediatric patient with atopic dermatitis: a case report. *Cutis* **78**:113–116.
 39. **Szakos, E., G. Lakos, M. Aleksza, J. Hunyadi, M. Farkas, E. Solyom, and S. Sipka.** 2004. Relationship between skin bacterial colonization and the occurrence of allergen-specific and non-allergen-specific antibodies in sera of children with atopic eczema/dermatitis syndrome. *Acta Derm. Venereol.* **84**:32–36.
 40. **Thomas, D., S. Chou, O. Dauwalder, and G. Lina.** 2007. Diversity in *Staphylococcus aureus* enterotoxins. *Chem. Immunol. Allergy* **93**:24–41.
 41. **Tomi, N. S., B. Kranke, and E. Aberer.** 2005. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. *J. Am. Acad. Dermatol.* **53**:67–72.
 42. **Yagi, S., N. Wakaki, N. Ikeda, Y. Takagi, H. Uchida, Y. Kato, and M. Minamino.** 2004. Presence of staphylococcal exfoliative toxin A in sera of patients with atopic dermatitis. *Clin. Exp. Allergy* **34**:984–993.