

Staphylococcal Skin Colonization in Children with Atopic Dermatitis: Prevalence, Persistence, and Transmission of Toxigenic and Nontoxigenic Strains

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Staphylococcal skin colonization is a common feature of atopic dermatitis (AD) in adults. Little is known about prevalence and persistence of staphylococci in children. Forty-one AD children (mean age, 70 months) and 41 age-matched controls were studied. *S. aureus* was isolated from 38 AD patients (93%; 32% of controls, $P < .001$) and 37% of AD patients (5% of controls, $P < .001$) harbored toxigenic (enterotoxins, toxic shock syndrome toxin) *S. aureus* strains. No individual biotype prevailed. On follow-up (mean interval, 9 months), 70% of *S. aureus* strains were reisolated. Nasal and cutaneous *S. aureus* strains were identical in 73% of AD patients (7% of controls, $P < .001$), reflecting increased self-contamination. Identical staphylococcal strains in AD children and their mothers were observed in 38% (*S. aureus*) and 16% (coagulase-negative strains; $P < .001$). The prevalence of staphylococcal colonization in AD children is comparable to that in adults. High rates of self-contamination, transmission to contacts, and prevalence of toxigenic strains in AD children may have clinical and epidemiologic implications.

The association of staphylococcal colonization and atopic dermatitis (AD) in adults has been a focus of interest for many years [1]. *Staphylococcus aureus* can be isolated from 90%–100% of adults with AD. Bacterial colonization is favored by hyper- and dyskeratosis, increased transepidermal loss of water, and dysregulation of sweat and sebaceous glands [2, 3] as well as by local immunologic defects inherent to AD, such as depressed polymorphonuclear leukocyte (PMNL) chemotaxis [4]. Minor staphylococcal skin infections are often observed in AD patients [5–7]. Furthermore, chronic staphylococcal colonization may act as an unspecific stimulus to IgE synthesis [8] or may activate neutrophil oxidative metabolism [9]. Transmission of pathogenic *S. aureus* strains by *S. aureus*-carrying AD patients has been reported [10–12].

In the present study, we analyzed the staphylococcal colonization rate in AD children compared with unaffected age-matched controls. We were particularly interested in the prevalence of toxigenic *S. aureus* strains (staphylococcal enterotoxins [SE] A–D, toxic shock syndrome toxin [TSST] 1, exfoliative toxins [ET] A and B), which may cause severe systemic infections, and of individual capsular polysaccha-

ride types, which may act as virulence factors for *S. aureus* [13]. We also analyzed the transmission of staphylococci from AD patients to close contacts (mothers) and among different sites on the patient's own skin surface (self-contamination) [11].

Materials and Methods

Patients and controls. Forty-one children (caucasians; 25 boys and 16 girls) 3 months to 16 years old (mean, 70 months; median, 64 months) presenting with AD at the outpatient clinic of the Department of Pediatrics, University of Hamburg, were included in this study. AD was diagnosed according to standard criteria [14] and rated as to extent and severity of skin lesions on a 1–5 scale [4]. The mean score for extent of skin lesions was 2.4 (range, 1–5; median, 2.5) and for severity was 2.8 (range, 1–5; median, 3.0). Patients receiving topical or systemic immunosuppressive or antibiotic treatment within the 4 weeks (topical corticosteroids, 2 weeks) preceding the examination date were excluded. Of the 41 patients, 20 were followed for a mean of 9 months. Nose swabs and skin contact samples were obtained for culture from 28 mothers of AD patients. Forty-one age-matched children seen at our outpatient clinic for assessment of cardiac function or presumed growth disorders or for preoperative examination served as controls.

Bacteriologic sampling and sample processing. Nose swabs were taken from both nares with sterile cotton tips. For assessment of cutaneous staphylococcal colonization, 8.5 × 5.5 cm² peptone-sheep blood agar slides (Biotest Hycon, Frankfurt/Main, Germany) were applied to eczematous and unaffected skin regions. Cultures of nose swabs (in nutrient broth) and contact slides were grown overnight (37°C in 5% CO₂). The strains were subcultured on Columbia blood agar (Biotest). Staphylococcal species were tested for coagulase activity using rabbit plasma (Difco, Detroit) and identified according to their fer-

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mentative characteristics by using a panel of carbohydrate and other substrates (API-STAPH, Montalieu-Vercieu, France). Antibiotic susceptibility was assessed with the Kirby-Bauer disk-diffusion test.

Phage-typing. *S. aureus* strains were phage typed using the International Phage Set and additional phages as described before [15]. Identity of *S. aureus* strains was assumed when they exhibited the same phage and capsular polysaccharide types and gave identical biochemical reactions and antibiotic susceptibility patterns. Coagulase-negative strains were identified by the latter two methods alone.

Capsular polysaccharide typing. *S. aureus* strains were analyzed for capsular polysaccharide (CP) types 5 and 8 in a two-step ELISA using type-specific monoclonal antibodies (clones J-1 and K-10/2) as described previously [16].

Detection of staphylococcal exotoxins. *S. aureus* strains were assayed for production of SEA-SED, ETA, ETB, and TSST-1 as described previously [17, 18]. The following toxigenic *S. aureus* strains were used as references: FRI-1169 for TSST-1, FRI-100 for SEA, S-6 for SEB, 1151-M for SED (all provided by M. S. Bergdoll, Food Research Institute, Madison, WI), PC7 for ETA, PC75 for ETB (both provided by P. Elsner, University of Würzburg, Germany), and 475 for SEC. Specific antibodies were raised by immunizing chinchilla rabbits with toxins, followed by affinity purification. Culture supernatants were screened for exotoxin production in a biotin-avidin ELISA system (for SEA-SED and TSST-1) or by double immunodiffusion (for ETA and ETB).

Statistical analysis. Statistical significance was analyzed with Student's *t* test and the χ^2 test with Yates's correction where indicated.

Results

Prevalence of staphylococcal species. The prevalences of *S. aureus* and of coagulase-negative staphylococci on the nasal mucosa and the unaffected and eczematous (respectively) skin of AD patients and controls are shown in figure 1. The overall prevalence rate (nose and skin) of *S. aureus* was 93% in AD patients and 32% in age-matched controls ($P < .001$). While *S. aureus* predominated in AD patients, coagulase-negative strains prevailed in healthy control children; the difference of nasal colonization between groups was statistically significant ($P < .05$). According to the results of phage typing, fermentative characteristics, CP type, and antibiotic sensitivity patterns, no individual *S. aureus* or coagulase-negative strain prevailed in AD patients. CP typing of *S. aureus* strains isolated from AD patients revealed that 15% (20% of controls) were CP type 5, 53% (67% of controls) were CP type 8, and 32% (13% of controls) were nontypeable, indicating they possessed other CP types or were CP-negative (differences not significant).

Correlation of nose and skin cultures. Identical *S. aureus* strains were isolated from nose swabs and skin contact specimens in 73% of AD patients and 7% of controls ($P < .001$; figure 2). For coagulase-negative staphylococci the nose-to-

skin correlation rate of strains was not different between patients and controls.

Persistence of staphylococci. On follow-up, 17 (57%) of 30 *S. aureus* strains persisted in 14 (70%) of 20 AD patients compared with 13 (18%) of 74 *S. epidermidis* strains ($P < .001$) in 10 (50%) of 20 AD patients (not significant). Persistence of *S. aureus* strains was independent of phage group or CP type. In 6 of 14 patients, spread of *S. aureus* from nose, eczematous skin regions, or both to unaffected or eczematous skin regions was observed, reflecting self-contamination [11]. During follow-up, afebrile, superficial skin infections (characterized by small pustules, crusting, and increased pruritus [5, 6, 8]) were observed in 7 (35%) of 20 AD patients and staphylococcal conjunctivitis in 1 (5%) of 20. One patient had a systemic staphylococcal infection (septicemia). Toxin-associated diseases (e.g., toxic shock syndrome, enterotoxin-associated diarrhea, or staphylococcal scalded skin syndrome) were not observed.

Intrafamilial transmission of staphylococci. Thirty-eight percent of the *S. aureus* strains but only 16% of the coagulase-negative strains were transmitted between AD patients and their mothers ($P < .001$; table 1). In most cases, isolation of the strains from AD patients preceded the isolation from their mothers. According to phage and CP typing results, no individual biotype prevailed among the transmitted strains.

Prevalence of toxigenic strains. Toxigenic *S. aureus* strains were found significantly more frequently in AD patients than in controls ($P < .001$; table 2). Taking into account the higher overall carrier rate of *S. aureus* in AD patients, the proportion of toxigenic strains among all *S. aureus* isolates in the AD patient group was not increased, however.

Discussion

Staphylococcal colonization is a constant phenomenon in AD and is independent of age. The *S. aureus* carriage rate of 93% we observed in children with AD lies within the range reported for adult AD patients [1, 12, 19]. While *S. aureus* colonization is found only transiently in healthy controls [20], 70% of our patients were persistently colonized with individual *S. aureus* strains. Eczematous hyperkeratinization seems to be an important cofactor for the high rate of staphylococcal colonization [20–22], but it does not explain the high prevalence of *S. aureus* on the unaffected skin and nasal mucosa of our AD patients (figure 1A). The prevalence of particular (and possibly particularly adapted) *S. aureus* strains in AD [23] and increased expression of adhesins such as lipoteichoic acid [20, 24, 25] and protein A [22] by *S. aureus* have been suggested. While we did not specifically address the question of adhesins, our phage typing results do not support the existence of any predominant biotype, nor did a previous report [26]. The questions of a specific epithelial

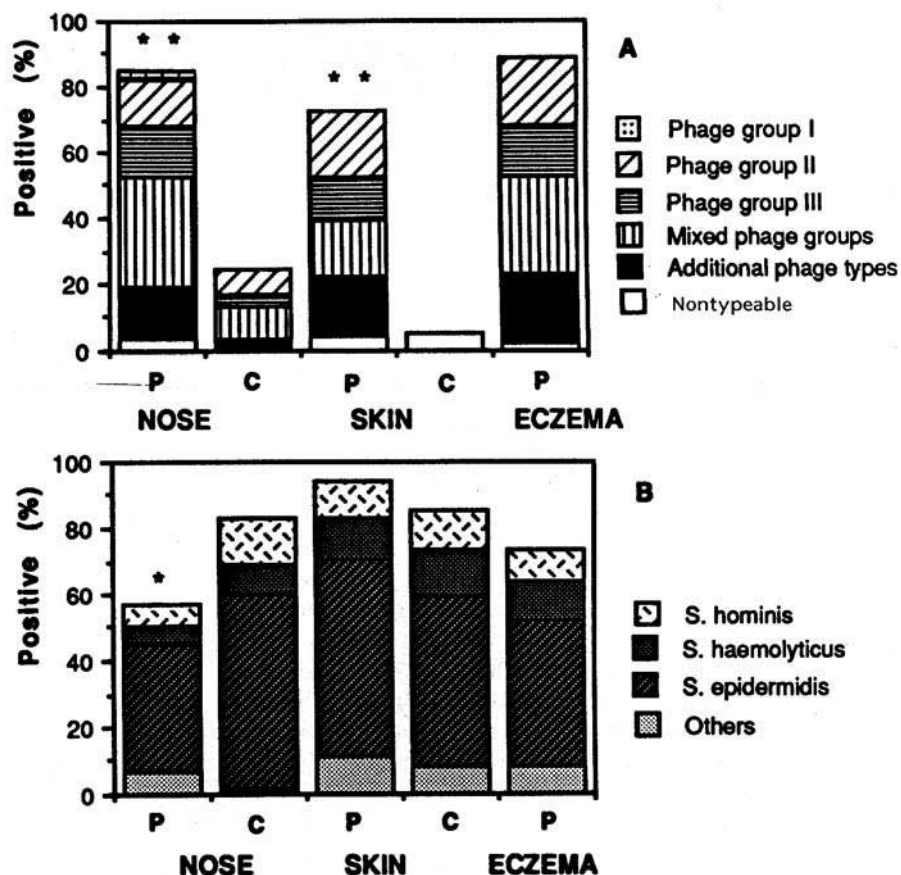


Figure 1. Prevalence of *Staphylococcus aureus* (A) and coagulase-negative staphylococci (B) in atopic dermatitis patients (P) and controls (C). *, $P < .05$; **, $P < .001$.

lial receptor [21] or the expression of particular staphylococcal adhesins in AD deserve further study [25].

The relative distribution of CP types in our patients (68% of *S. aureus* strains positive for CP types 5 or 8) is similar to that for other clinical isolates [13]. By inhibiting phagocytosis, CPs are important virulence factors for *S. aureus* [13]. On

the epithelial surfaces of AD patients, they might interfere with normal PMNL activity and thus facilitate staphylococcal persistence.

In contrast to the proliferation of *S. aureus*, there seems to be no predilection for coagulase-negative staphylococci in AD. The coagulase-negative staphylococci isolated from our patients (figure 1B) and those reported in previous studies [19, 23, 26] form part of the normal skin flora [27]. Their colonization density ($\sim 10^4/\text{cm}^2$, data not shown) equals that of healthy controls [19, 23, 26], whereas *S. aureus* exhibits average colonization densities of $>10^6 \text{ cfu}/\text{cm}^2$ in AD [8, 12, 19]. Its higher colonization density may be an important cofactor for the increased rates of persistence and transmission to contacts (table 1) and the more generalized colonization (figure 2) of *S. aureus* compared with coagulase-negative staphylococci. Furthermore, colonization density of *S. aureus*, but not of coagulase-negative staphylococci, has been found to correlate with clinical severity of AD [28].

The phenomenon of staphylococcal colonization in AD has important implications both for the patient and for his or her environment: For the patient, chronic colonization with *S. aureus* represents a risk factor for the development of purulent skin or even systemic infections [5–7]. Although overt bacterial infections are rarely observed in AD patients, studies in adults [5, 8, 12] and children [6, 7] revealed minor

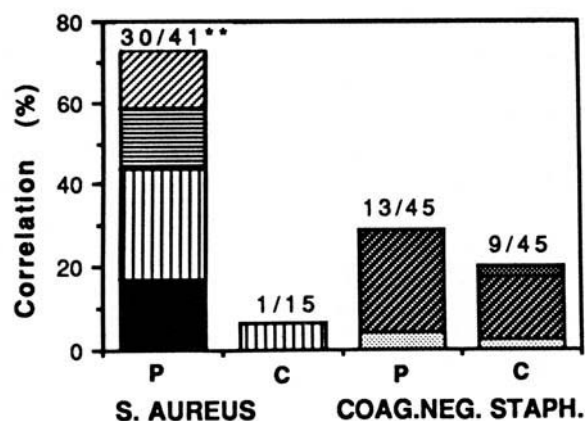


Figure 2. Correlation of staphylococcal nose and skin colonization in atopic dermatitis patients (P) and controls (C) ($P < .001$). Symbols for individual *Staphylococcus aureus* and coagulase-negative staphylococcal strains are same as in figure 1.

staphylococcal skin infections in about 30%–40%. This is in accordance with our own observations. The absence of severe or systemic infections in most AD patients may be related to enhanced PMNL oxidative metabolism [9] or increased cell-mediated immune responses to staphylococcal antigens [29] in AD.

Similarly, the absence of toxin-associated symptoms despite the high carriage rate of toxigenic strains could be explained by the high rate of preformed antitoxic antibodies observed in the population [30, 31]. It remains to be established whether staphylococcal toxins may indirectly contribute to skin damage in AD through their major histocompatibility complex-mediated stimulatory effect on T cells [32].

For the environment, AD patients are sources of potentially pathogenic microorganisms. Colonizing staphylococci (mainly *S. aureus*) tend to spread from eczematous skin regions all over the body surface (self-contamination) [11]. In contrast to previously held views [8], our results (figure 2) demonstrate that generalized skin colonization with *S. aureus* is common in AD children too. Since nearly 100% of AD patients are *S. aureus* carriers, with a high colonization density, it is not surprising that these microorganisms are often transmitted to contacts or to the inanimate environment [10–12]. Our data suggest that at least 38% of our AD patients transmitted *S. aureus* strains to their mothers (table 1).

The high prevalence of toxigenic *S. aureus* strains in our AD patients (37% vs. 5% of controls) is of particular importance (table 2). Outbreaks of staphylococcal toxin disease (such as enterotoxin-induced diarrhea or exfoliatin-induced

Table 2. Prevalence of toxigenic staphylococci in atopic dermatitis patients and controls.

	No. (%)		P
	Patients (n = 41)	Controls (n = 41)	
Persons with <i>Staphylococcus aureus</i>	38 (93)	13 (32)	<.001
Persons with toxigenic <i>S. aureus</i>	15 (37)	2 (5)	<.001
Absolute no. of toxigenic <i>S. aureus</i> strains	18*/61 (30)	2†/15 (13)	NS

NOTE. NS, not significant.

* Staphylococcal enterotoxin (SE) A (7); SEC (4); SEA + SEB, SEA + SEB + SEC + SED, SEB + SEC (1 each); SEC + exfoliative toxin A (1); toxic shock syndrome toxin 1 (3).

† SEA + SEB (1); SEC (1).

staphylococcal scalded skin syndrome) have been traced to *S. aureus*-carrying AD patients [10, 12]. In contrast to a previous report from Utah [31], where TSST-1 was the most commonly identified toxin among asymptomatic children, we observed a predominance of staphylococcal enterotoxins. The overall carrier rates of toxigenic *S. aureus* strains in healthy children (9.5% in [32]) was comparable to our results (13%). However, the increased rate of carriers of toxigenic *S. aureus* strains among AD patients does not represent a specific selection of toxigenic strains in AD but reflects the higher total number of *S. aureus* strains isolated (table 2).

To eliminate staphylococcal colonization, systemic [1, 6, 7] or topical [12, 33] antibiotic therapy has been attempted in cases of AD. In some it was temporarily accompanied by clinical improvement of the atopic eczema [6, 7]. Recently Lever et al. [33] reported significant reduction of the *S. aureus* bacterial count in AD after 14 days of topical treatment with mupirocin, but recolonization occurred immediately after termination of therapy. Hence, apart from side effects such as cutaneous sensitization or development of resistance, the efficacy of antibiotic therapy for the elimination of chronic cutaneous staphylococcal colonization is questionable. Since dryness of the skin and hyperkeratinization are important cofactors of staphylococcal adherence, future studies should address the effect of consequent skin care on the staphylococcal flora in AD. Another approach could be the blockade of epidermal staphylococcal receptors.

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Table 1. Transmission of staphylococci between atopic dermatitis patients and their mothers.

Strains	No. of strains in mothers/ no. of strains in children (n = 28 pairs)	Correlation rate
<i>Staphylococcus aureus</i>		
Phage group II*	4/6	22†/58‡ (38%)
Phage group III*	6/9	
Mixed phage groups*	5/20	
Additional phage types†	5/15	
Nontypeable	2/8	
Coagulase-negative staphylococci		
<i>Staphylococcus epidermidis</i>	31/111	32/204 (16%)
<i>Staphylococcus haemolyticus</i>	0/34	
<i>Staphylococcus hominis</i>	0/36	
Others	1/23	

* Typing with the International Phage Set (IPS).

† Phages not included in IPS.

‡ Of 22 strains, 4 were toxigenic (staphylococcal enterotoxin C) (non-transmitted strains, 10/36; difference not significant).

* $P < .001$ (for difference of correlation rates between *S. aureus* and coagulase-negative staphylococci).

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