

Treatment Outcomes of Secondarily Impetiginized Pediatric Atopic Dermatitis Lesions and the Role of Oral Antibiotics

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Abstract: Patients with atopic dermatitis (AD) are predisposed to infection with *Staphylococcus aureus*, which worsens their skin disease; it has been postulated that the lack of antimicrobial peptides due to aberrant allergic inflammation in skin with AD could mediate this enhanced bacterial susceptibility. We sought to characterize the amounts of *S. aureus* and biological products found in infected AD lesions and whether treatment with topical corticosteroids and oral cephalexin as the only antimicrobial improved outcomes. Fifty-nine children with clinically and *S. aureus*-positive impetiginized lesions of AD were enrolled in this study. A lesion was graded clinically using the Eczema Area and Severity Index, and wash fluid was obtained from the lesion for quantitative bacterial culture and antibiotic sensitivities and measurement of bacterial products and cytokines. Subjects were re-evaluated 2 weeks after treatment. Improvement in the clinical and inflammatory characteristics of impetiginized lesions were noted, even in the 15% of lesions infected with Methicillin-resistant *S. aureus* (MRSA). In a subgroup of subjects whose lesions did not contain *S. aureus* 2 weeks after initiating treatment, beta-defensin levels were higher at both visits than in normal skin. Treatment of uncomplicated impetiginized pediatric AD with topical corticosteroids and cephalexin results in significant clinical improvement, even in subjects infected with MRSA. We propose that the inhibition of abnormal inflammation by the treatment regimen, resulting in the high levels of defensins, is involved in the improvement of AD and that systemic antibiotics do not appear to be necessary in secondary impetiginized AD.

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Staphylococcus aureus infections are known triggers for skin inflammation and can modulate immune responses. Patients with atopic dermatitis (AD) are predisposed to staphylococcal colonization and infections that worsen their skin disease (1,2). The mechanisms by which individuals AD are susceptible to bacterial and viral infections are not completely known, but several lines of evidence suggest a role for cationic antimicrobial defensin and cathelicidin peptides (antimicrobial peptides (AMP)) (3,4). AMP levels are low in AD lesions (5). In addition, the T helper 2 (Th2) cytokines interleukin (IL)-4 and IL-13, which are high in AD skin lesions, have been reported to inhibit AMP expression in human keratinocytes cultures in vitro (6–8). Moreover, several agents that have been reported to be effective at diminishing staphylococcal colonization and infection in the setting of AD, including systemic vitamin D and topical pimecrolimus, can upregulate AMP production in keratinocytes (9,10). Finally, the inhibition of abnormal allergic (Th2) inflammation could explain the isolated reports that topical corticosteroids alone can decrease *S. aureus* numbers in AD lesions (11).

Methicillin-resistant *S. aureus* (MRSA) is emerging as an important health concern (8,12). Given that patients with AD are predisposed to staphylococcal infection, the incidence and clinical significance of MRSA in this special population is of importance.

Previously, we reported a group of 89 children with AD from whom we characterized the wash fluid obtained from a quantitative culture of a clinically impetiginized skin lesion for amounts of *S. aureus*; antibiotic susceptibilities; and quantified lipoteichoic acid (LTA), staphylococcal protein A (SPA), and a panel of cytokines (13,14). The present study examined a subpopulation of this group whose lesions were positive for *S. aureus* and were treated with a regimen of topical corticosteroids, oral antihistamines, and the oral antibiotic cephalexin as the only antimicrobial to assess the effectiveness of this therapy. This study indicates that treatment of children with AD using this regimen resulted in significant improvement of clinical and inflammatory parameters, even in subjects found to harbor cephalexin-resistant MRSA.

METHODS

Participants with AD

Children with AD diagnosed using the criteria of Hanifin and Rajka (15) with clinical evidence of impetiginized skin lesions were enrolled in these studies, which the Indiana University Institutional Review Committee approved. As outlined in our previous study, subjects were not exposed to oral or topical antibiotics for a

period of 1 month before the study (13). None of the subjects had evidence of systemic involvement (e.g., fevers, chills) from their skin infection. Subjects enrolled in the study underwent a clinical assessment of a clinically infected lesion of dermatitis using the Eczema Area and Severity Index (EASI), as well as EASI scoring of their entire body (16). For consistency, both authors conducted all of the EASI scoring. Wash fluid was removed from the lesion and the subject treated with topical corticosteroids (daily desonide ointment mild areas of dermatitis on body, face, or intertriginous areas; triamcinolone 0.1% ointment for thicker areas of dermatitis on body), oral antihistamines (hydroxyzine 1–2 mg/kg or doxepin 0.5–1 mg/kg daily), and oral cephalexin (approximately 25–50 mg/kg per day divided into two or three times per day) for 2 weeks. After 2 weeks, subjects returned, and the procedures were repeated. None of the subjects in this cohort used topical antibiotics or bleach or other antiseptics in their bath water for 1 month before or during the 2-week treatment time.

Quantitative Analysis of Dermatitis Lesions

Wash fluid derived from lesions was removed from a 2.5-cm diameter polypropylene chamber as previously described (13,17) using the methodology established by Williamson and Kligman (18). Briefly, a sterile 2.5-cm-diameter ring of polyvinylchloride tubing (Nalgene Labware, Rochester, NY) was placed over the skin lesion of the patient, then 1 mL of sterile rinse solution (0.069M sodium pyrophosphate, 0.0064M sodium dihydrogen phosphate, and 0.1% Tx-100) was administered inside the ring chamber, which was held tightly on the skin to prevent leakage. The rinse solution was stirred around in the chamber using a sterile Teflon rod (Scientific Commodities, Inc., Lake Havasu City, AZ) 15–20 times and collected. This collection was repeated, and 2 mL of total rinse solution was obtained. This methodology has been shown to be 95% quantitative for aerobic surface bacteria (18). The wash solution was then aliquotted for immunoblotting analysis of LTA, enzyme-linked immunosorbent assay (ELISA) measurement of SPA and cytokines, and bacterial quantitation. *S. aureus* colonies were quantified in the microbiology laboratory in Indiana University Hospital using limiting dilution assay, and antibiotic susceptibilities were assessed using standard methodology.

Measurement of LTA, SPA, and Defensins in Wash Fluid Specimens

Quantitative measurements of LTA and SPA proteins were as previously described (13,14,17). Briefly, for LTA,

32 μL of rinse solution from each patient sample was separated on 18% Tris-hydrochloric acid gradient gel (Bio-Rad Laboratories, Hercules, CA), along with standards of 10, 5, 2.5, and 1 ng of LTA protein (Sigma-Aldrich, St. Louis, MO) dissolved in same rinse solution loaded on the same electrophoresis gel. LTA protein was determined by immunoblotting with LTA monoclonal antibody (QED Bioscience, San Diego, CA) and enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ). The arbitrary optical densities were measured using Image J Software (National Institutes of Health, Bethesda, MD). The quantification of LTA was determined according to the standard curve drawn. Quantitative measurement of SPA was performed using ELISA (Assay Designs Inc., Ann Arbor, MI) as previously described (13). Human beta-defensin (HBD)2 and HBD3 proteins were quantified using ELISA (Phoenix Pharmaceuticals, Inc., Burlingame, CA). Defensins and bacterial products LTA and SPA were quantified based upon area (ng/cm^2) of the chamber and then converted to volume (ng/cm^3) based upon estimation of 0.1-cm effective epidermal thickness. Defensin levels were also expressed relative to the total amount of protein measured in wash fluid. Total wash fluid protein was measured using the BCA Protein Assay (Thermo Scientific, Rockford, IL).

Cytokine Multiplex Analysis of Wash Fluid Specimens

Patient wash fluids were collected and levels of IL-1 beta (β), IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, interferon gamma ($\text{IFN-}\gamma$), and tumor necrosis factor alpha ($\text{TNF-}\alpha$) were measured using the Multiplex Bead Immunoassays according to the manufacturer's protocol (Millipore, Billerica, MA). A Luminex 200 analyzer (Luminex Corporation, Austin, TX) was used for data acquisition and analysis. Cytokine concentrations were calculated using Bio-Plex Manager 2.3 software (Bio-Rad Laboratories) using a five-parameter curve-fitting algorithm applied for standard curve calculations. Cytokines in wash fluid were quantified based upon area (ng/cm^2) of the chamber and converted to volume (ng/cm^3) based upon estimation of 0.1-cm effective epidermal thickness.

Statistical Analysis

Spearman rank correlation coefficients were used for all correlation analyses. The Wilcoxon rank sum test was applied to compare clinical outcomes at visit 1 and changes in these outcomes from visit 1 to visit 2 between MRSA- and methicillin-susceptible *S. aureus* (MSSA)-positive skin lesions. The Wilcoxon signed rank test was

used to detect a significant change in outcomes of interest from visit 1 to visit 2. In computing changes in outcomes, we used value 0 whenever outcomes were not detectable.

RESULTS

Correlation Between Lesional Concentration of *S. aureus* Bacteria and Clinical Inflammation

Fifty-nine children (aged 4 mos to 6 yrs) with AD from our original cohort of 89 subjects (13) who fulfilled the study criteria (positive for *S. aureus*, followed the treatment regimen, returned to clinic in 2 weeks) outlined in the Methods section were enrolled in these studies to clinically define eczema severity and quantitatively determine the levels of lesional cytokines and bacterial products before and after defined treatment. The experimental procedure, including a photograph of a typical patient at visits 1 and 2 is depicted in Fig. 1. Lesion wash fluid was obtained and used for quantitative *S. aureus* culture or aliquotted and stored at -80°C before being used for measurement of other proteins and cytokines.

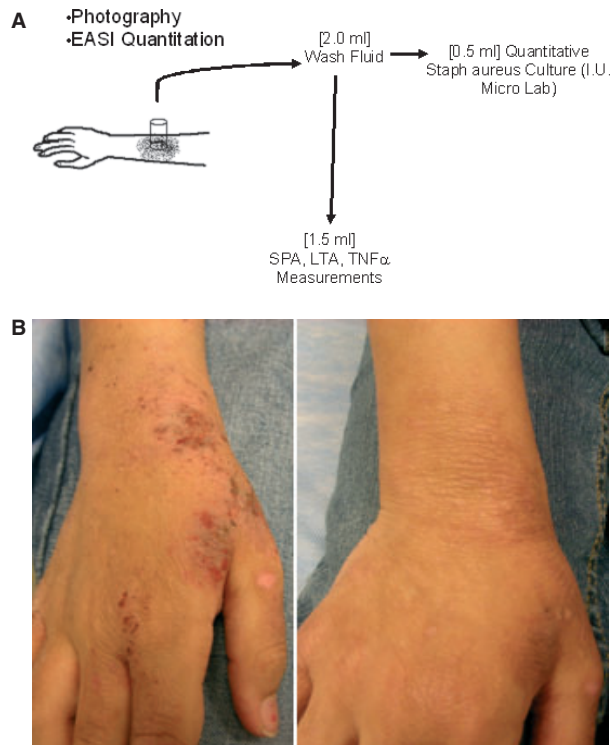


Figure 1. Outline of research protocol. (A) Outline of clinical protocol involving wash fluid derived from clinically impetiginized atopic dermatitis (AD) lesions. (B) Typical subject with clinically impetiginized (methicillin-resistant *Staphylococcus aureus* in this case) AD lesion at visit 1 and visit 2 2 weeks later after treatment with topical corticosteroid and oral cephalixin.

An isolate of the *S. aureus* was assessed for antibiotic sensitivities.

The location of the lesions varied between the subjects, with 47 lesions from extremities; eight from the trunk, back, and abdomen; and four from the face. No significant differences were seen in staphylococcal colony-forming units (CFU) or lesional EASI scores between samples taken from various locations (data not shown). Previous studies (21,22) have reported that the amount of skin inflammation tends to correlate with the numbers of *S. aureus* bacteria, with levels greater than 10^6 CFU/mL indicative of clinical infection. As depicted in Fig. 2, the *S. aureus* CFU correlated with the lesional EASI scores at visit 2 (Spearman correlation coefficient (r) = 0.37, p = 0.004). These studies indicate that the levels of staphylococcal CFU significantly correlate with clinical inflammation in this cohort of children with AD.

Cytokine and Bacterial Product Analysis of Wash Fluid Specimens from Impetiginized AD Lesions

Staphylococcal products, including the bacterial lipoprotein LTA and protein SPA, have potent immunomodulatory effects (13,17,21,22). Our previous studies have discovered that significant levels of these mediators are found in impetiginized AD skin lesions (13,14,17). We examined the amounts of LTA and SPA in the wash fluid derived from the skin lesions from our cohort of

subjects. In this cohort of children with AD, there was a significant positive correlation between amounts of LTA and staphylococcal bacteria (visit 1, r = 0.47, p < 0.001). Similarly, there was a strong positive correlation between SPA levels and staphylococcal CFU (visit 1, r = 0.68, p < 0.001; visit 2, r = 0.46, p < 0.001). The amounts of LTA and SPA also correlated with EASI lesional score at visit 1; because LTA and SPA levels were not detectable for more than 90% of visit 2 samples, further analysis was not performed.

Wash fluid samples were also subjected to multiplex analysis to measure protein levels of IL-1 β , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IFN- γ , and TNF- α . As outlined in Table 1, the cytokines found in the greatest abundance in visit 1 lesions were IL-6 and IL-8, with smaller amounts of TNF- α and IL-1 β . Higher levels of Th2 cytokines IL-4 and IL-13 were found in visit 1 wash fluid samples and essentially no Th1 cytokines IL-12 and IFN- γ . Significant Spearman correlation coefficients were found for the following cytokines versus EASI lesional scores at visit 1: IL-1 β (r = 0.33, p = 0.01); IL-6 (r = 0.34, p = 0.009); IL-8 (r = 0.38; p = 0.003); IL-13 (r = 0.29; p = 0.02); and TNF- α (r = 0.43; p < 0.001). Stronger correlation was observed at visit 2 for these same outcomes. These findings indicate that significant levels of LTA and SPA and pro-inflammatory cytokines are found in impetiginized AD lesions.

Treatment Outcomes in Subjects with MSSA Versus MRSA

Of the 59 staphylococcal isolates from our cohort of subjects, nine (15%) were MRSA. As shown in Table 1, there were no statistically significant differences in the various parameters except slightly but statistically significantly (p = 0.049) more MRSA than MSSA CFU at visit 1. Children with MRSA tended to have higher total EASI scores, although this was not statistically significant. Treatment with our regimen of topical corticosteroids and oral cephalexin resulted in significant improvement in EASI lesional and total scores, as well as fewer *S. aureus* CFU in the majority of impetiginized lesions. All nine subjects with MRSA exhibited improvement in EASI lesional and total scores, and in seven subjects, the number of staphylococcal CFU was lower (and in four of the seven, no *S. aureus* was measured at visit 2). In one of the nine subjects with MRSA, the number of staphylococcal CFU was almost unchanged, and in one, the number of staphylococcal CFU increased more than six times. Forty-eight of the 50 subjects with MSSA exhibited improvement in lesional EASI, with two subjects unchanged. Forty-nine of the 50

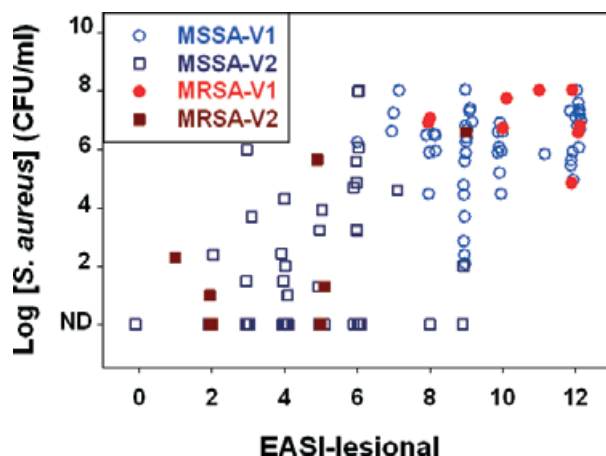


Figure 2. Amounts of *Staphylococcus aureus* bacteria versus clinical assessment of inflammation in individuals with atopic dermatitis (AD). Wash fluid obtained from 59 subjects at first visits (open circle for methicillin-susceptible *S. aureus* (MSSA) and filled circle for methicillin-resistant *S. aureus* (MRSA)) and at 2-week follow-up visits (open square for MSSA and filled square for MRSA) were assessed for levels of *S. aureus* bacteria, which was compared with lesional Eczema Area and Severity Index scores.

TABLE 1. Comparison of Clinical Assessment of Inflammation, Levels of *Staphylococcus aureus*, and Levels of Lipoteichoic Acid (LTA), Staphylococcal Protein A (SPA), and Cytokines in Methicillin-Resistant *Staphylococcus aureus* (MRSA)- Versus methicillin-susceptible *S. aureus* (MSSA)-Positive Atopic Dermatitis Lesions

Variable	Visit 1	Visit 2		
	MSSA (N = 50)	MRSA (N = 9)	MSSA (N = 50)	MRSA (N = 9)
	Mean $\pm$ Standard Deviation (Median)			
EASI-lesional	9.8 $\pm$ 1.7, 9.0	10.6 $\pm$ 1.7, 11	4.5 $\pm$ 1.8, 4.0*	3.7 $\pm$ 2.5, 2.0*
EASI-total	19 $\pm$ 11, 22	33.4 $\pm$ 24, 28	4.8 $\pm$ 4.4, 3.3*	6.3 $\pm$ 5.2, 5.0*
Log <i>S. aureus</i> CFU/mL	6.1 $\pm$ 1.4, 6.4	7.0 $\pm$ 1.0, 6.9†	1.7 $\pm$ 2.3, 0*	1.9 $\pm$ 2.5, 1.0*
Log LTA, pg/cm ³	1,024 $\pm$ 1,892, 274	1,466 $\pm$ 2,150, 624	ND: 92%*	ND: 78%*
Log SPA, pg/cm ³	17 $\pm$ 52, 1.1	15 $\pm$ 28, 8.4	1.5 $\pm$ 8, 0.02*	1.5 $\pm$ 3.4, 0*
Log IL-1 β , pg/cm ³	133 $\pm$ 457, 40	96 $\pm$ 126, 52	15 $\pm$ 33, 4.0*	18 $\pm$ 21, 16*
Log IL-4, pg/cm ³	21 $\pm$ 42, 3.8	9.8 $\pm$ 14, 0.8	ND: 88%*	ND: 56%
Log IL-5, pg/cm ³	58 $\pm$ 231, 2.9	1.8 $\pm$ 1.9, 1.7	ND: 84%*	ND: 100%
Log IL-6, pg/cm ³	338 $\pm$ 502, 131	582 $\pm$ 874, 338	23 $\pm$ 48, 3.4*	28 $\pm$ 55, 1.0*
Log IL-8, pg/cm ³	10,190 $\pm$ 15,034, 4,935	7,924 $\pm$ 10,826, 4,598	2,054 $\pm$ 6,364, 180*	678 $\pm$ 779, 545*
Log IL-10, pg/cm ³	2.2 $\pm$ 3.6, 0.1	1.6 $\pm$ 2.3, 0.3	ND: 96%*	ND: 89%
Log IL-12, pg/cm ³	ND: 92%	ND: 100%	ND: 96%	ND: 100%
Log IL-13, pg/cm ³	214 $\pm$ 861, 15	22 $\pm$ 23, 11	30 $\pm$ 101, 0.6*	2.3 $\pm$ 4.8, 0*
Log IL-17, pg/cm ³	23 $\pm$ 65, 2.5	0.5 $\pm$ 1.1, 0	ND: 60%*	ND: 67%
Log tumor necrosis factor alpha, pg/cm ³	47 $\pm$ 48, 29	47 $\pm$ 31, 47	8.2 $\pm$ 15.7, 3.0*	4.4 $\pm$ 5.9, 1.3*
Log interferon gamma, pg/cm ³	ND: 94%	ND: 96%	ND: 96%	ND: 100%

The Eczema Area and Severity Index (EASI) score of the tested lesion and total body EASI scores, concentration of *S. aureus* bacteria, LTA, SPA, and cytokine levels were compared in samples in which the bacterial isolate was sensitive (MSSA) or resistant (MRSA) to the antibiotic oxacillin.

*Reduction from visit 1 to visit 2 within each group was significant, $p < 0.05$.

IL, interleukin; ND, nondetectable.

†Number of colony-forming units (CFU) of MRSA was statistically significantly ($p = 0.049$) greater than MSSA at visit 1.

exhibited improved EASI total scores at visit 2, with one almost unchanged. The number of staphylococcal CFU decreased in 47 of the 50 subjects with MSSA (and in 27 of the 47, no *S. aureus* was measured at visit 2). In three of the 50 subjects with MSSA, the number of staphylococcal CFU was two to 10 times greater.

As outlined in Table 1, levels of bacterial products LTA and SPA and proinflammatory cytokines IL-1 β , IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, and TNF- α were also significantly lower after treatment in MSSA- and MRSA-infected skin lesions. These findings indicate that the treatment regimen of topical corticosteroids and the oral antibiotic cephalexin was effective, even in subjects with MRSA.

Defensin Analysis of Wash Fluid Specimens from Successfully Treated Impetiginized AD Lesions

To explore the mechanisms by which successful therapy was able to remove *S. aureus*, the visit 1 and 2 wash fluids from five subjects whose first visit demonstrated high levels ($> 10^6$ CFU) of *S. aureus* at the initial visit with no bacteria at follow-up visit were tested for the presence of HBD2 and HBD3. As shown in Table 2, HBD2 and HBD3 were detected in wash fluid specimens derived

from lesions at visits 1 and 2. Defensin levels are expressed as effective concentrations (ng/cm³), as well as amounts relative to total wash fluid protein (ng/mg). Wash fluids derived from clinically normal skin from five subjects with no history of skin disease revealed low levels of HBD2 (0.17–0.84 ng/cm³ and 3.7–12.4 ng/mg) and HBD3 (0–0.64 ng/cm³ and 0–3.9 ng/mg). In the children with AD, effective HBD2 concentrations were similar at visits 1 and 2, yet the amounts relative to total wash fluid protein were greater at visit 2. HBD3 effective concentrations were greater at visit 1 than visit 2, but the amounts relative to total wash fluid protein were similar and were clearly greater than those found in normal skin. Similarly, measurable amounts of the Th2 cytokines IL-4 and IL-13 were found in visit 1 lesions but not in treated skin lesions. The higher levels of HBD2 and HBD3 than in normal skin and the lack of detectable Th2 cytokines at visit 2 fit with the notion that removing the Th2-mediated inhibition (3,4) contributes to “normalization” of AMP levels and resolution of staphylococcal infection. These studies indicate that sustained levels of endogenous antimicrobial cationic peptides HBD2 and HBD3 and loss of Th2 cytokines are associated with successfully treated secondarily impetiginized AD lesions.

TABLE 2. Comparison of Clinical Assessment of Inflammation, *Staphylococcus aureus* Colony-Forming Units (CFU), Levels of Defensins, Bacterial Products Lipoteichoic Acid (LTA) and Staphylococcal Protein A (SPA), and Cytokines in Visit 1 and 2 Samples from Participants with Impetiginized Atopic Dermatitis (AD)

Sample	EASI Lesional	Log SA	Total Protein, mg/mL	HBD2, ng/cm ³	HBD2, ng/mg	HBD3, ng/cm ³	HBD3, ng/mg	LTA, ng/cm ³	SPA, ng/cm ³	Interferon gamma, pg/cm ³	IL-12, pg/cm ³	IL-4, pg/cm ³	IL-5, pg/cm ³	IL-13, pg/cm ³	IL-1 β , pg/cm ³	Tumor Necrosis Factor alpha, pg/cm ³
Patient 1																
Visit 1	9	7.0	1.77	8.0	1.1	145	20	918	1.1	ND	ND	16	45	452	19	86
Visit 2	2	ND	0.026	7.8	75	3.5	34	ND	0.07	ND	ND	ND	ND	1.5	ND	ND
Patient 2																
Visit 1	9	6.7	2.41	7.4	0.8	104	11	1210	0.93	ND	ND	36	63	608	36	62
Visit 2	2	ND	0.068	2.2	8.1	2.0	7.1	ND	0.05	ND	ND	ND	ND	ND	ND	ND
Patient 3																
Visit 1	12	6.1	2.05	10	1.2	135	16	9804	2.4	ND	ND	3.8	6	11	122	36
Visit 2	4	ND	0.93	7.4	2.0	39	11	ND	0.03	ND	ND	ND	ND	1.4	4.1	ND
Patient 4																
Visit 1	12	6.7	1.59	9.0	1.4	86	13	6400	19	0.8	ND	0.8	27	98	25	34
Visit 2	4	ND	0.45	9.7	5.4	16	9.0	ND	0.07	ND	ND	ND	ND	ND	ND	ND
Patient 5																
Visit 1	9	7.4	0.64	7.1	2.8	90	36	720	22	ND	ND	3.0	ND	1.4	24	8
Visit 2	4	ND	0.43	8.7	5.1	55	32	ND	ND	ND	ND	ND	ND	ND	1.1	ND

The Eczema Area and Severity Index (EASI) score of the tested lesion, concentration of *S. aureus* bacteria, LTA, SPA, cytokine levels, and human beta defensin (HBD)2 and HBD3 levels of five subjects whose follow-up visits revealed no evidence of *S. aureus* were compared at visits 1 and 2 (after treatment). HBD levels are described as effective concentration (ng/cm³), as well as relative amounts per mg of total protein in the wash fluid samples. ND, nondetectable.

DISCUSSION

The present study confirms previous reports of an association between high amounts of *S. aureus* and clinically worse AD lesions (19,20). At visit 1, subjects with MRSA had slightly higher *S. aureus* levels than those with MSSA ($p = 0.049$). There were no other statistically significant associations between antibiotic susceptibility pattern (MRSA vs MSSA) and total or lesional EASI score or LTA, SPA, or cytokine levels in impetiginized AD lesions.

Fifteen percent of children with AD with clinically impetiginized lesions were found to have MRSA. These findings are similar to the 7.4% MRSA of positive *S. aureus* skin cultures of children with AD reported recently by Huang and colleagues (23), 14% by Matiz and colleagues (24), and 16% MRSA described by Suh and colleagues (27).

The clinical significance of MRSA in pediatric AD is unclear. The present study and previous studies (3,24,28) indicate that the incidence of MRSA in children with AD appears to be lower than that of the general population. The reasons for this are unclear but might be related to the fact that, with the immune and barrier abnormalities associated with AD, there is probably not a tremendous selective advantage to MRSA in this setting unless there is significant exposure to oral antibiotics, including cephalexin. Consistent with this notion, Huang et al (23) described clinical experiences in which patients with MRSA converted to MSSA and vice versa, but in children with severe or chronic AD and exposure to chronic antibiotics, MRSA is reported to be more problematic (25, 26,29).

MRSA isolates are reported to be resistant to cephalaxin; thus, the clinical and microbiologic improvement seen in this study in AD lesions impetiginized with MRSA cannot be attributed to antimicrobial therapy (30,31). Instead, we propose that improvement resulted from other components of the therapeutic regimen, including topical corticosteroids, oral antihistamines, and patient education regarding barrier care and daily baths with mild soap. The findings of the present studies using measurements of clinical, microbiologic, and inflammatory cytokines are in accord with the conclusions of a recently published Cochrane review regarding the lack of necessity of systemic antibiotics for the treatment of secondarily impetiginized AD (32).

Although this interpretation that the improvement of MRSA-infected AD lesions after administration of cephalaxin as the only antimicrobial indicated that the effects of topical care were adequate appears reasonable, there is not an absolute correlation between in vitro sensitivities and in vivo clinical improvement of

infections. It has been reported that, in clinical practice, approximately 90% of individuals treated with an antimicrobial drug that is active in vitro would be expected to improve, whereas 60% of individuals treated with antibiotics lacking in vitro activity against a causative pathogen will have a clinical response (33). This “90–60” rule described in immunocompetent individuals could be a partial explanation for why people with AD with MRSA improved with cephalexin.

We hypothesize that the inhibition of the abnormal Th2 immune response associated with flaring AD by this regimen allowed normalization of barrier function and reversed AMP deficiencies, both of which served to help improve, if not eliminate, the staphylococcal skin infection. A body of literature has been developing that demonstrates that certain Th2 cytokines (IL-4, IL-13) can not only inhibit the synthesis of proteins such as filaggrin involved in skin barrier formation (28,34), they can also serve to inhibit the production of endogenous antimicrobial peptides (6–8). Our preliminary data examining levels of defensins and cytokines that up-regulate (e.g., IL-1 β , TNF- α) or inhibit (IL-4, IL-13) their synthesis from five subjects with more than 10⁶ CFU *S. aureus* at visit 1 but no evidence of bacteria at visit 2 fit with this hypothesis.

The limitations of this study include the small numbers of subjects and the considerable variability in the amounts of bacteria and bacterial products found in the skin lesions. One possible reason for the variability of bacteria and bacterial products in wash fluid specimens could be the time since the lesion tested had been disturbed (e.g., washed) before being tested. Another variable could be the positive effects associated with being part of a clinical study, which resulted in better adherence to treatment than people seen in the “usual” clinical setting.

Limiting the use of systemic antibiotics in the setting of a chronic disease like AD could reduce the development of antibiotic resistance and the frequency of side effects from these drugs. Although our data suggest that oral antibiotics are not required to treat uncomplicated AD impetiginized with MRSA, this should be interpreted with caution until larger studies can test this hypothesis. Complicated staphylococcal skin infections, especially involving MRSA, are a significant source of morbidity and mortality and should be treated with appropriate systemic antibiotics (25,26).

In summary, the current study confirms the importance of *S. aureus* as a trigger for AD flares. Evaluation of wash fluid samples by quantitative bacterial culture from clinically impetiginized AD lesions before and after defined treatment revealed improvement in staphylococcal CFU and EASI scores and lower levels of staphylococcal products and inflammatory cytokines. A subset of

patients whose AD lesions were impetiginized with MRSA improved with a treatment regimen that included oral cephalexin as the only antimicrobial. The presence of high levels of defensins in wash fluid specimens of AD lesions after treatment provides a mechanism by which the *S. aureus* bacterial superinfection is resolved.

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