

Characteristics of *Staphylococcus aureus* colonization in patients with atopic dermatitis in Sri Lanka

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Summary

Background. Colonization of the skin of patients with atopic dermatitis (AD) by *Staphylococcus aureus* (SA) is associated with more severe disease.

Aim. To determine the association of SA colonization patterns and densities in lesional and nonlesional skin in patients with varying severities of AD, and to determine the antibiotic sensitivity patterns of SA isolates from Sri Lanka.

Methods. Skin and nasal swabs collected from 100 patients with AD and 120 controls were used to investigate the presence of SA. Severity of AD was graded using the Nottingham Eczema Severity Score. Colony counts were obtained for skin samples, and antibiotic sensitivity testing was performed in cases positive for SA.

Results. Skin colonization was seen in 57 patients (57%) but in only 10 controls (8%). Lesional skin of most patients (52/57; 91%) had SA densities of > 300 colony-forming units/cm². Colonization rates with SA significantly increased with increasing age (Spearman correlation coefficient $R = 0.9$, $P < 0.05$) and increasing duration of lesions in patients with AD (Spearman $R = 0.87$, $P < 0.05$). Isolates from eight patients (13.5%) were found to be methicillin-resistant *S. aureus* (MRSA). Only 6 isolates (10%) were susceptible to penicillin and 22 (37%) to erythromycin, while 28 (47%) isolates had erythromycin-induced resistance to clindamycin.

Conclusions. SA colonization rates were significantly associated with increasing age and severity of AD, and particularly with duration of lesions. Patients with severe disease were also more likely to be colonized with SA strains resistant to conventional antibiotics.

Introduction

Atopic dermatitis (AD) is a common chronic, pruritic inflammatory skin condition, affecting 10–30% of children and 2–3% of adults in European countries.^{1–3} In Sri Lanka, the epidemiology is less well known, but the International Study of Asthma and Allergies in

Childhood (ISAAC) suggested a prevalence of 7.2% of 6–7-year-olds, ascertained using an eczema questionnaire.⁴ The reasons underlying such potential regional differences are unclear, but differential carriage of *Staphylococcus aureus* (SA) isolates may be one such contributory factor. The bacterial skin flora of patients with AD is strikingly different from that of healthy people. SA is seen in > 90% of patients with AD,^{5–7} and densities have been shown to be higher in lesional than in nonlesional areas. In addition, SA densities can reach over 10⁶ colony-forming units (CFU) per cm² in patients with AD.⁷ By contrast, colonization by SA is seen in only 2–40% of healthy people.^{8,9}

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Once the skin is colonized, SA can aggravate AD by various mechanisms, for example through bacterial superantigens and other exotoxins. Superantigens of SA activate various cells including T cells, eosinophils, Langerhans cells, macrophages, mast cells and keratinocytes, which are important in the pathogenesis of AD.^{3,10} Up to 65% of SA strains isolated from patients with AD colonized with this microorganism express superantigens,^{11,12} which polyclonally activate T cells, resulting in increased cytokine production,^{3,5} which can enhance presentation of allergen to T helper (Th)2 cells by keratinocytes.⁵ Furthermore superantigens induce the production of superantigen-specific IgE, which contributes to inflammation in AD through mast cell degranulation¹³ and is also thought to activate mechanisms of pruritus.¹⁴ In addition, it has also been found that SA superantigens can inhibit the suppressive activity of regulatory T cells.¹⁵

Although SA clearly plays an important role in the pathogenesis of AD, it is not clear whether the SA colonization rates and colonization densities correlate with the severity of AD in Sri Lankan patients. Furthermore, little is known of the SA antibiotic sensitivities in healthy donors or in patients with AD in Sri Lanka.

The aim of this study was to determine SA colonization patterns in AD in different patient groups and the density of colonization in lesional and nonlesional skin, and to compare these results with those of healthy controls. We also aimed to identify the relationship between AD severity and the colonization and antibiotic sensitivity patterns of SA isolates in Sri Lanka.

Methods

The study was approved by the local ethics committee, and all participants gave informed written consent.

Participants

In total, 100 patients with AD (40 male, 60 female patients; mean $\pm$ SD age 6 ± 9.6 years, range 0.1–46.8) were recruited from a tertiary care hospital in Sri Lanka from a group of patients attending the hospital as part of their routine care for management of their AD. AD was defined according to the UK Working Party's Diagnostic Criteria.¹⁶ AD severity was graded by a dermatologist according to the Nottingham Eczema Severity Score.¹⁷ Of the 100 patients, AD was graded as mild in 61 (61%), moderate in 30 (30%) and severe in 9 (9%). Most of the patients ($n = 73$; 73%) had AD only, but associated diseases were seen in the other 27

patients (with some having < 1 condition): 15 (15%) had allergic rhinitis, 18 (18%) had asthma and 12 (12%) had food allergies. Over half of the patients ($n = 60$; 60%) also had a family history either of AD or of other allergic diseases such as allergic rhinitis, asthma or food allergies. The control group comprised 120 age-matched people (60 male, 60 female patients; mean $\pm$ SD age 14.9 ± 12.3 years, range 0.1–47) who did not have any history of asthma, AD or allergic rhinitis.

Culture and identification of *Staphylococcus aureus*

Skin swabs were collected from eczematous lesions and nonlesional areas from patients with AD (swabs from two lesions and two nonlesional areas) and also from healthy controls (from left and right antecubital fossae). The swabs were first dipped in sterile saline and an area of skin 1 cm^2 in size was rubbed for 30 s. Swabs from the anterior nares were obtained from both groups to determine the nasal carriage of SA.

Swabs were inoculated onto mannitol salt agar (Hi-Media Laboratories, Mumbai, India) and incubated aerobically for 24–48 h at 37°C . Characteristic yellowish, mannitol-fermenting colonies were identified as SA by Gram staining and catalase test. Coagulase slide and tube tests were performed to confirm the identity of the SA isolates. SA colony counts were also obtained for skin samples.

Antibacterial susceptibility testing and beta-lactamase production

A colony suspension of each SA isolate was prepared to a 0.5 McFarland standard, and antimicrobial susceptibility (except for methicillin) was tested by the Stokes disc diffusion method on Mueller–Hinton agar (Hi-Media Laboratories) with antimicrobial discs (Hi-Media Laboratories) and using *S. aureus* NCTC 6571 as the control strain. The plates were incubated for 24 h at 37°C prior to interpretation of antibacterial susceptibility test results. Methicillin resistance was tested using the Clinical Laboratory Standards Institute disc diffusion method, with Mueller–Hinton agar plates and cefoxitin $30 \mu\text{g}$ discs (Hi-Media Laboratories) incubated for 24 h at 35°C . Isolates with an inhibition zone diameter of $\leq 21 \text{ mm}$ were identified as methicillin-resistant (MR)SA.¹⁸ We also tested for erythromycin-induced clindamycin resistance at the same time. The erythromycin disc and a clindamycin disc were placed 15 mm distant from each other, and zone diameters were recorded at 16–18 h for all discs. Results were

interpreted according to the CLSI guidelines.¹⁸ Detection of β -lactamase enzyme production in isolates was performed with nitrocefin discs (Mast Diagnostics, Bootle, Merseyside, UK) according to the manufacturer's instructions.

Results

SA colonization patterns

SA colonization was seen in 59 patients (59%) and 16 controls (13%). Skin colonization was seen in 57 patients (57%) compared with 10 controls (8%), and nasal colonization of SA was seen in 45 patients (45%) and 9 controls (8%). In lesional skin, SA colonization was present in 28 patients (46%) with mild AD, 22 (73%) with moderate AD, and all 9 patients (100%) with severe AD. SA colonization was also seen in nonlesional skin in 10 patients (16%) with mild AD, in 13 patients (43%) with moderate AD and in all 9 patients (100%) with severe AD. Almost all patients with skin colonization (52/57; 91%) had SA densities of > 300 CFU/cm² in lesional skin. Six patients (10%) with mild AD, eight (27%) with moderate AD and seven (78%) with severe AD had SA densities of > 300 CFU/cm² in nonlesional skin. SA densities were significantly lower in healthy controls than in patients with AD (mean $\pm$ SD 8.3 ± 6.8 CFU/cm²; $P < 0.05$).

Colonization rates with SA significantly increased with increasing age (Spearman $R = 0.9$, $P < 0.05$). SA colonization was seen in 47% of patients (19/40) aged < 1 year, rising to 75% in patients aged > 15 years (Table 1). Increased colonization rates were also seen with increasing duration of AD (Spearman $R = 0.87$, $P < 0.05$); for instance, 7 of 29 patients (24%) with disease duration of < 6 weeks had SA colonization, compared with 19 of 27 (70%) with disease duration of 3–6 months (Table 2). SA colonization was also higher in patients with other atopic diseases (19/27; 70%) such as asthma, allergic rhinitis and food allergies, compared

with patients who had AD only (38/73; 52%). However, AD was more severe in patients with other allergic diseases (56% had severe or moderate AD) and all of them were > 1 year old. SA skin colonization was also higher (39/60; 65%) in patients with a family history of atopy than in those patients without such family history (18/40; 45%), but this was not significant. Family history was also associated with severity; 27 of 60 patients (45%) with AD who had a family history of atopy had moderate or severe AD, and 19 (32%) of them were aged < 1 year. No association was seen with SA colonization and sleep disturbance, body surface area affected, acute or chronic lesions, and lichenified or nonlichenified lesions (Table 2).

Antibiotic sensitivity patterns of *Staphylococcus aureus* isolates

Of the 59 SA isolates from patients, 8 (13.55%) were MRSA. According to Centre for Disease Control criteria, these were consistent with community-acquired (CA)-MRSA infection, as this was an outpatient setting and the patients gave no history of MRSA infection or colonization, and had not been hospitalized in the previous year, had been admitted to a community healthcare facility and had not undergone any invasive procedures. Furthermore, five of the eight MRSA strains also carried the gene for Panton–Valentine leucocidin (PVL), which is usually expressed only by CA-MRSA

Table 2 SA colonization rates in patients.

Magnitude score for magnitude*	SA colonization rates, n/total n (%)		
	Vs. disease duration	Vs. sleep disturbance	Vs. BSA affected
1	7/29 (24)	30/59 (51)	8/14 (57)
2	19/29 (66)	2/4 (50)	11/21 (52)
3	19/27 (70)	6/12 (50)	8/23 (35)
4 and 5	12/15 (80)	19/25 (76)	30/42 (71.4)

*Emerson *et al.*¹⁷ BSA, body surface area.

Table 1 Skin and nasal colonization of SA in different age groups of patients with AD.

Age category, years	SA colonization in patients, n (%)			SA colonization in different types of AD lesions, n/total n (%)			
	Nasal	Nonlesional skin	Lesional skin	Acute	Chronic	Lichenified	Non-lichenified
< 1 ($n = 40$)	14 (35)	13 (32)	18 (45)	16/38 (42)	2/2 (100)	3/4 (75)	15/36 (42)
1–5 ($n = 23$)	11 (48)	10 (43)	13 (57)	6/14 (43)	7/9 (78)	10/11 (91)	3/12 (25)
5–10 ($n = 23$)	13 (57)	6 (26)	16 (70)	8/10 (80)	8/13 (62)	9/13 (69)	7/10 (70)
10–15 ($n = 6$)	3 (50)	2 (33)	4 (67)	2/3 (67)	2/3 (67)	2/3 (67)	2/3 (67)
> 15 ($n = 8$)	4 (50)	1 (13)	6 (75)	4/4 (100)	2/4 (50)	2/3 (67)	4/5 (80)

AD, atopic dermatitis; SA, *Staphylococcus aureus*.

strains, further supporting that the MRSA isolated were acquired in the community.

Of the 59 isolates, only 6 (10%) had susceptibility to penicillin and 22 (37%) to erythromycin, while 51 (86.4%) were susceptible to cloxacillin. Although 58 isolates (98%) were sensitive to clindamycin, 28 (47%) had erythromycin-induced resistance. All eight MRSA strains were susceptible to ciprofloxacin, cotrimoxazole, gentamicin, rifampicin and vancomycin, and one was susceptible to clindamycin. None of the MRSA strains was susceptible to erythromycin. Antibiotic resistance was not seen in the 16 SA isolates from healthy controls. Of the 75 isolates from both groups, 67 (89%) isolates were β -lactamase producers.

Discussion

Although the SA colonization rates of patients from European countries, the USA,¹⁹ and some Asian countries²⁰ have been reported as 80–100%,^{6,21} the colonization rates in our study (57%) are comparatively lower. Because 61% of our patient population consisted of patients with mild AD, this may have contributed to the lower colonization rates. However, SA colonization rates in healthy Sri Lankans (13%) are in the range reported in healthy Europeans (6–30%).²² SA colonization rates have been shown to be associated with increasing severity^{6,21,23} and age, findings that were confirmed in this study.

Epidermal barrier defects and immune dysfunction are thought to be important factors in the pathogenesis of AD.³ Most (91%) of our patients had heavy densities of SA colonization (> 300 CFU/cm²) in lesional skin, implying that the prevailing inflammatory conditions support SA colonization. The severity of AD was also associated with heavy SA colonization in nonlesional skin. The percentage of patients with heavy densities (> 300 CFU/cm²) of SA in nonlesional skin increased with AD severity, from mild (10%) to moderate (27%) to severe (78%), and the density was significantly lower in healthy controls (8.3 ± 6.8 CFU/cm²).

SA colonization was more prevalent in patients with other atopic diseases in addition to AD (70% had SA), compared with patients who only had AD (52%), probably because patients with coexisting allergic diseases also had more severe AD. Patients with AD who had a family history of atopic disease were also more likely to have SA (65% had SA) compared with those without such history (45%).

The percentage of colonization and the antibiotic sensitivity test patterns show that eczematous lesions of patients with AD are a source of virulent SA strains. The

presence of methicillin resistance along with the expression of the PVL gene by five of the eight MRSA isolates implies that AD skin is a favourable reservoir for CA-MRSA. CA-MRSA usually produces PVL,²⁴ which is a cytotoxin that forms nonspecific pores in leucocyte plasma membranes, resulting in leucocyte lysis.²⁵ It is also associated with necrotic skin lesions and with severe necrotizing pneumonia.^{26,27} Therefore, it is possible that MRSA may further contribute to the pathogenesis of AD. However, as the prevalence of CA-MRSA in Sri Lanka is not known, we cannot say whether the frequency in our study is greater than that in the general population. However, a study carried out in a tertiary care hospital on patients who were awaiting surgery in Sri Lanka found that 7.4% were colonized with MRSA, therefore the current data would be compatible with a relatively high rate of CA-MRSA in patients with AD in Sri Lanka.

The presence of β -lactamase-producing SA strains in both patient and control groups is indicative of a high prevalence of β -lactam-resistant SA strains in the Sri Lankan community. Importantly, as most strains (61%) had resistance to erythromycin, this antibiotic is likely to be ineffective as a first-line treatment for infected AD lesions. In addition, 47% of SA strains from patients had erythromycin-induced resistance to clindamycin. Although these two antibiotics belong to separate classes, they both act by inhibiting protein synthesis through binding to the 50S ribosomal subunits of bacterial cells. Resistance to erythromycin in SA may occur through either the *erm* or *msrA* genes. SA strains with the *erm* gene induce resistance to clindamycin also in the presence of erythromycin. As antibiotics are often used to treat exacerbations of AD, it would thus be useful to perform microbial susceptibility testing with multiple samples before antibiotic treatment is begun.

Conclusion

In summary, we found SA colonization rates to be significantly associated with increasing patient age and AD severity in Sri Lankan patients, and colonization was also higher in patients who had eczematous lesions for a longer duration. Higher colonization densities were also seen in both lesional and nonlesional skin of patients with more severe AD. Colonization rates were also higher in patients with other coexisting allergic diseases and those with a family history of atopy. Patients with AD were also more likely to be colonized with SA strains resistant to conventional antibiotics, therefore routine microbiological characterization of clinically infected AD should be undertaken.

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What's already known about this topic?

- Most patients with AD have SA present on skin swabs.
- Exacerbations of AD can be associated with SA infection.

What does this study add?

- This study provides supportive evidence that in Sri Lanka, SA colonization rates are significantly associated with increasing age and severity of AD, and particularly with duration of lesions.
- Patients with AD are more likely to have SA that is resistant to conventional antibiotics.
- Emergence of community-acquired MRSA appears to be an important problem in Sri Lanka.

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