

# Bacteriological profile in patients of atopic dermatitis: Comparison between normal and lesional skin

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## Abstract

**Objective** To determine the bacteriological profile in normal and lesional skin in patients of atopic dermatitis.

**Methods** In this cross sectional study, patients of either sex, fulfilling the modified Hanifan & Rajka criteria for atopic dermatitis were enrolled. Patients on topical and / or systemic antibiotics or having concurrent scabies were excluded. After washing with normal saline, swabs were taken from selected sites and were sent to pathology laboratory preferably within an hour, where cultures were performed aerobically for growth identification.

**Results** A total of 115 patients were enrolled, mean age was  $50.02 \pm 11.4$  years [range 02-65years]. In lesional skin, 109 (95%) patients were culture positive while in non lesional skin 89 (77%) were culture positive (p value 0.0002). In lesional skin, swabs were positive for Staph aureus in 47% followed by Streptococci (31%), Staph & Streptococci (22%), Staph epidermidis (8.5%), Staph & enterococcus (6.5%), Pseudomonas (3%), and E coli and proteus (1% each). No growth was seen in 5%. In non lesional skin, swabs were positive for Staph epidermidis in 34% followed by Streptococci in 26%, Staph aureus in 17%, while 23% had no growth.

**Conclusion** Staph aureus and Streptococci, or both Staph.+Streptococci are commonly found microorganisms from lesional skin of patients with atopic dermatitis while Staph epidermidis and Staph. + Streptococci are commonly found organisms on the normal skin.

## Key words

Bacteriological profile, atopic dermatitis.

## Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease of the skin, characterized by xerosis and pruritus with increased transepidermal water loss.<sup>1</sup> Fifteen to 20% of the childhood population has AD.<sup>2</sup> It usually begins

in infancy or childhood and follows a remitting/ flaring course that may continue throughout life.<sup>3</sup> AD is a multifactorial disease in which both hereditary and environmental factors play a role.<sup>1</sup>

AD is associated with disruption of the normal skin barrier and increased colonization by pathogenic organisms, both bacterial and fungal.<sup>3</sup> The defective epidermal barrier in patients with AD results from abnormalities in both lipids (ceramide and sphingosine

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deficiencies) and proteins (increased serum protease levels and decreased filaggrin expression).<sup>2</sup>

Disturbances of the skin bacteriological composition with reduced microbial diversity and overabundance of *Staphylococcus* species are associated with AD inflammation.<sup>4</sup> It is recognized as an important triggering factor for the maintenance of skin inflammation and acute exacerbations of the disease. The common long-term treatment concept for atopic dermatitis is based on daily application of emollients with or without antibacterial ingredients accompanied with symptomatic anti-inflammatory therapy consisting of topical glucocorticoids or topical calcineurin inhibitors (TCI) on an as need basis.<sup>5</sup> Clinical practice and studies<sup>2</sup> have shown improvement in disease with addition of antibiotics to conventional treatment modalities in AD patients.

Various studies<sup>6-8</sup> have been conducted on the bacteriological profile in AD patients but none in Pakistan. Therefore, this study was conducted to determine the bacteriological profile in normal and lesional skin in patients of AD. It will be helpful in understanding the bacteriological role in AD in our patients.

## **Patients and Methods**

This cross sectional study was carried out at the Department of Dermatology, Fauji Foundation Hospital, Rawalpindi, Pakistan. Using World Health Organization (WHO) sample size calculator, with anticipated population 12%, absolute precision 6% and with confidence interval >95%, sample size (n=115) was calculated. Non probability consecutive sampling was done. Patients between 2 years to 65 years of age and either sex fulfilling the Hanifin and Rajka criteria for atopic dermatitis were enrolled. Atopics already getting any

topical or/ and systemic antibiotics or having scabies were excluded from the study. Permission for this study was obtained from Hospital Ethical Committee. Patients were recruited through Dermatology OPD. Purpose of the study was explained and informed consent was taken. Data was collected according to pre designed proforma. Detailed history and examination of patients was carried and sites for taking swabs were selected in each patient, both lesional and non-lesional. After washing with normal saline, swabs were taken and sent to Pathology laboratory preferably within an hour. The swabs were cultured aerobically on blood agar, Mac-Conkeys agar and chocolate agar at 35-37°C for 48 hours. Growth identification was done by Gram staining. Further testing was done to differentiate catalase and coagulase positive and negative bacteria. API 20E/NE (Analytical Profile Index) Kits were used to identify gram negative organisms. Patients found to be culture positive were treated with appropriate antibiotics. The tests and requisite treatment was free of cost.

Data was entered and analyzed by software SPSS version 10. Descriptive statistics were calculated for both qualitative and quantitative variables. For quantitative variables like age mean±SD were calculated. For qualitative variables like gender and bacterial cultures positive, frequency, type of organism or percentage were calculated. Effect modifiers like age, gender, skin type were controlled by stratification. Post stratification chi square test is significant. A p value of < 0.05 was considered significant.

## **Results**

A total of 115 patients were enrolled in the study. The mean age of the patients was 50.02±11.4 years [range 02–65years]. There were 31(27%) patients less than 30 years of age

**Table 1** Comparison of different organisms isolated from lesional and normal skin (n=115).

Isolated Organisms	Lesional Skin	Normal Skin	P - Value
	( yes   no for that specific organism)		
Staph aureus	37   78	17   98	0.0029
Staph epidermidis	09   106	29   86	0.0006
Streptococci	27   88	24   91	0.7511
Staph. + Streptococci	21   94	11   104	0.0852
Staph +Enterococcus	07   108	03   112	0.3328
Pseudomonas	04   111	01   114	0.3695
Escherichia coli	02   113	01   114	1.0000
Proteus	02   113	03   112	1.0000
No growth	06   109	26   89	0.0002

and 84 (73%) patients were more than 30 years of age.

In this study, 43 (37%) patients were male while 72 (63%) patients were female. A total of 230 skin swabs were cultured, 115 each from lesional skin and normal skin. One hundred and ninety eight (86%) were found to be culture positive, 109 from lesional skin and 89 from normal skin and 32 (14%) were culture negative, 6 from lesional skin and 26 from non lesional skin ( p-value 0.0002 **Table 1**).

When data was stratified with respect to gender there was no significant difference between males and females (p-value 0.5556 ).

Comparing different organisms isolated from lesional and non lesional skin, p-value was significant for Staph aureus, Staph epidermidis, Staph+Streptococci, Staph.+Enterococci, and Pseudomonas (**Table 1**).

When organisms from lesional and non lesional skin were compared it was seen that in male patients, p-value was significant for Staph epidermidis, Streptococci, Staph.+Streptococci and Escherichia coli while in females it was for Staph aureus, Staph epidermidis and Pseudomonas.

Similarly young patients of age less than 30 years, had significantly more positive cultures for Staph aureus, and Staph.+Enterococci. In

older patients of more than 30 years, comparing lesional and normal skin p-value was significant for Staph aureus, Streptococci, Staph.+Streptococci and Escherichia coli and Proteus.

## Discussion

After successful topical AD treatment, there is an increased biodiversity of cutaneous microbiome.<sup>9</sup>

The hygiene hypothesis of atopic diseases links microbes with atopic dermatitis (AD) both as drivers and modulators of skin pathology.<sup>10</sup> A hypothesis known as outside-inside model of AD considers a genetic skin barrier defect compounded by a skin microbiota dysbiosis as primary pathogenic event.<sup>11</sup> Cultivation microbiology has demonstrated strong evidence of skin colonization with superantigen-encoding *Staphylococcus aureus* in AD patients; microbiota and *S. aureus* abundance fluctuates and parallels clinical symptoms.<sup>6,12</sup>

Flares of AD are associated with an increased colonization of *Staphylococcus aureus* on lesional skin without clinical infection and a substantial loss of biodiversity in skin microbiome. Disturbances in skin microbiome represent an independent risk factor for the development of AD. In ~90% of patients suffering from AD, the skin becomes colonized by *S. aureus* 50% of which are toxin producing.

These toxins can contribute to inflammation and skin barrier dysfunction via activating the host inflammatory response.<sup>9</sup> Membrane vesicles released from these bacteria can penetrate the epidermis and cause a massive infiltration of inflammatory cells. Clinical trials showed that various treatments reducing *S. aureus* skin load also reduced AD symptoms, suggesting *S. aureus* as a potential critical driver of AD and a target for antimicrobial interventions other than antibiotics.

AD patients having scabies or who were taking any topical or systemic antibiotics were excluded from the study, as it may modify the microflora of the skin. Severity of AD was not considered in our study.

In a study comprising of fifty atopics by Thakur,<sup>3</sup> cultures were positive in 96% of lesional and 58% from the non-lesional swabs. In the swabs taken from lesional skin, *Staphylococcus aureus* was isolated from 42% of the samples, followed by *Staph epidermidis* from 12% of the swabs. Among the swabs taken from non-lesional skin, 42% cases did not show growth of any microbe. *Staph epidermidis* was isolated from 30% cases.

In a study by Reddy JR *et al.*<sup>6</sup> on 280 atopic patients, cultures were positive in 93% of lesional and 44% from the non-lesional swabs. In the swabs taken from lesional skin, *Staphylococcus aureus* was isolated from 49% of the samples, followed by *Staph epidermidis* from 22%. 7% of the samples did not show growth of any microbe. Among the swabs taken from non-lesional skin, 49% cases did not show growth.

Research in the field of AD is mostly done on children; however, AD is not just a disease of the children, it often persists in adulthood and even in elderly. We studied a wide range of age

group. There were total 115 patients included in our study. The mean age of the patients in the study was  $50.02 \pm 11.4$  years [range 02 –65years]. In lesional skin, 109 (95%) patients were culture positive while in non lesional skin 89 (77%) were culture positive. Stratification on the basis of culture positivity in lesional and normal skin, yielded p-value of 0.0002.

In lesional skin *Staph aureus*, positive in 37 patients was the most common organism found, followed by *Streptococci*, and *Staph.+ Streptococci*. Comparing lesional and non lesional skin p value for *Staph aureus* was found to be very significant 0.0037. Increased colonization of *staphylococcus aureus* in the lesional skin of AD patients has been found in many studies.<sup>3,6</sup>

In healthy skin among the Gram-positive *Staphylococcus* species, *Staphylococcus epidermidis* is the dominant type with the ability to inhibit the growth of *Staphylococcus aureus*. In our study also it was seen that in normal skin 89(77%) patients were culture positive and *Staphylococcus epidermidis* was the most common organism found.

In our study, no significant difference (p-value 0.55) was found in culture positivity of male and female patients.

A study done in Sweden included 21 adult patients of AD. Of which 71% (15/21) of the patients were found to be colonized with *S. aureus* on lesional skin at one or more visits while 90% (19/21) of patients were colonized with *S. aureus* on non-lesional skin at least once.<sup>7</sup>

As a part of the National Institute of Allergy and Infectious Diseases (NIAID) Atopic Dermatitis Research Network (ADRN)<sup>8</sup> in a study<sup>8</sup> of 100 patients, cultures were positive in

99% of lesional and 73% from the non-lesional swabs. In the swabs taken from lesional skin, *Staphylococcus aureus* was isolated from 92% of the patients. Race-specific *S. aureus* selection may account for differences in virulence factor profiles.

However in our study, in lesional skin, 95% patients were culture positive while in normal skin 77% were culture positive (p value 0.0002). Percentage of *Staphylococcus aureus* from lesional skin is not as high as in above mentioned studies.<sup>7,8</sup>

An explanation of this difference of results could be climate of our region. The study was conducted in summer months and UV exposure and warm climate can affect the severity and the micro biome of skin in AD patients.<sup>13</sup> The density of microorganisms and strain identification was beyond the scope of our study.

Despite extensive research to understand the complex interplay between *S. aureus* and atopic skin the question still remains: Is *S. aureus* a primary trigger of dermatitis or is colonization a secondary event due to a favourable environment for *S. aureus* in the altered epidermal barrier of AD? Whatever the reason intermittent long-term treatment with topical preparations such as clioquinol, potassium permanganate or dilute bleach baths<sup>14,15</sup> could prevent the dominance of *S. aureus* and promote microbial diversity on AD skin.<sup>14</sup> This could potentially reduce overall disease severity, number of flare-ups and antibiotic consumption for patients with AD who are persistent carriers of *S. aureus* on lesional skin.

Studies investigating the degree of bacterial colonization and AD severity and the effects of anti-staphylococcal measures in patients with AD persistently colonized with *S. aureus* are

recommended.

**Study limitations:** The present study had female to male ratio 1.7:1. It does not indicate gender difference in AD in general population. The higher female patient ratio is due to the fact that Fauji Foundation Hospital, Rawalpindi is a trust hospital for veterans and their families. Male children of these employees are entitled up to the age of 18 years, while daughters are entitled till their marriage.

## Conclusion

*Staph aureus*, *Streptococci*, or both *Staph.+ Streptococci* are commonly found microorganisms from lesional skin while *Staph epidermidis* and *Pseudomonas* are commonly found in normal skin in patients of AD.

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