Original Article

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# Pattern of Bacterial Colonization of Atopic Dermatitis in Saudi Children

Paediatrics Section

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## ABSTRACT

**Background:** Atopic dermatitis is an inflammatory skin disorder. Although it is not a life threatening condition, it may become infected with microorganisms, especially in children.

**Objectives:** The aim of this study was to determine bacterial colonisation in children with atopic dermatitis.

**Methods:** A total of 80 children were randomly included in this study. Two swabs were taken from each child, one from the eczematous skin lesion and the other from apparently healthy skin, as a control. Bacteria were isolated and identified on the basis of the colonial morphology, gram staining and the Vitek System.

**Results:** The mean age of children in this study was 1.4 years, with no gender difference (p=0.98) (n=80). A total of 240 bacterial colonies were grown from atopic dermatitis lesions in contrast to 193 colonies from non–lesional skin. Gram–positive cocci were found in 78 (97.5%) lesions and in 77 (96.2%) non–lesional skin. *Staphylococci* species were significantly detected in the lesions than in the non–lesional skin. *Ent. Faecalis, Ent. Faecium, Ent. gallinarium and C. minutissium* were significantly isolated from lesions as compared to non–lesional skin, whereas *C. xerosis* was insignificantly found to be more in the lesions (p=0.21). Gram–negative bacteria were isolated from 7(8.8%) lesions, but none were isolated from non–lesional skin. Recovered species were *Pantoea agglomerans, Enterobacter cloacae, Chryseobacterium indologenes and Acinetobacter Iwoffii.*

**Conclusion:** Atopic dermatitis in children is complicated with streptococcal and gram–negative bacterial colonisations and the latter was correlated with the severity of the lesions. *Enterococci* and *Corynebacterium* species were significant residents. *S. aureus* remained the chief inhabitant. No causal relationship could be established between the skin microbiota and atopic dermatitis.

**Key words:** Dermatitis, Atopic, Bacteria, Child, Saudi Arabia

## INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory pruritic skin disease which affects children and adults, with an estimated 1-20% worldwide prevalence [1]. *Staphylococcus aureus* is the leading cause of the infection in AD patients and an antibacterial treatment is beneficial in children [2]. Previous studies were conducted on adult populations and few studies were carried out to determine the colonising bacteria in children worldwide [3]. Furthermore, and to the best of our knowledge, no studies were published on the colonizing bacteria in children with AD in Saudi Arabia. The aim of this study was to determine the bacterial colonisation in children with atopic dermatitis.

## METHODS

We conducted this cross-sectional, descriptive study after it got approval from the Research and Ethical Committee of the College of Medicine and the Deanship of Scientific Research, Qassim University. It was carried in the Outpatients Clinics; Qassim University affiliated hospitals, during the period from March 2, 2010 to February 29, 2011. We randomly selected 80 children with AD, who were aged less than 14 years. Their parents signed written consents after being informed about the study. The inclusion criteria were children who fulfilled the criteria of Hanifin and Rajka [4]. By using the SCORAD index, the severity of dermatitis was determined and it was classified into mild (<15), moderate (between 15 and 40) and severe (≥40) [5]. Children who had immune system disease, systemic infections, systemic heart and kidney or liver diseases were excluded from the study. Children who were on steroids or any immunosuppressive therapy in the past two weeks or on antibiotics in the last four weeks were excluded, as well. We collected two skin swabs from each subject; one from the AD lesion at the following sites: the face and the extensor and flexor surfaces of the knees and the elbow. The second was from the non-lesional skin, which was apparently healthy skin which was symmetrical to the target lesion or 10 cm away from it, which we used as a control. Sterile cotton-tipped swabs were saturated with brain heart infusion broth (Oxoid). They were then collected after rolling twice over the skin surface and were immediately transported to the laboratory. They were cultured on blood agar base (Oxoid) and Mc Conkey's, nutrient and mannitol salt agars and the plates were incubated at 37° C for 24-48 hours. The colonies were identified on the basis of gram staining, their morphologies and the Vitek system (BioMerieux 12), an automated machine with incubation periods from 2 to 24 hours [6]. Measurements were registered hourly for 15 hours. Data were entered and analysed into SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, U.S.A. 2007). Pearson's Chi–Square test was used to assess the differences between bacterial isolates between the lesional and non–lesional skin and a bivariate correlation was done to assess the differences between lesion severity and bacterial colonisation. A multiple regression was conducted for predictors of atopic dermatitis severity. A p value of $< 0.05$ was considered as statistically significant.

## RESULTS

The total sample was 80 children with AD and a majority of them, 59(73.75%), were below 5 years of age. The age of the children ranged from 9 months-14 years, with a mean of 1.4 years ± 0.74 years. Male to female ratio was 3/1 however; the gender distribution was insignificant (p=0.98). A total of 240 bacterial colonies were grown from AD lesions of the 80 subjects in contrast to 193 from non-lesional skin. Thirty–one species were recovered from lesions, whereas non-lesional areas yielded 25. Gram–positive cocci were

found in 97.5 % (78/80) of the lesions and in 96.25% (77/80) of the non–lesional skin (p=0.001). Gram–positive bacilli and mixed colonies (gram positive cocci and bacilli) were seen more in lesions than in non–lesional areas. Gram–positive cocci were recovered from both lesion and non–lesion in 77 (96.25%) patients, and in one patient (1.25%), from the lesion alone. Two (2.5%) patients showed no growth in either lesion or non–lesional skin.

*Staphylococcal* species were significantly detected in lesional than in the non–lesional skin. [Table/Fig-1] shows the most recovered species. The difference between lesional and non-lesional skin was statistically insignificant regarding *S. simulans*, *S. scurii*, *S. capitis*, *S. xylosus* and *S. cohnii*. As shown in [Table/Fig-1], *S. epidermidis* was found to be insignificantly (p=0.07) present in the non-lesional skin.

| Bacterial species | Percent colonisation of skin | | p value |
|---|---|---|---|
| | lesion | Non-lesion | |
| ***Staphylococci*** | | | |
| *S. epidermidis* | 42.5 | 58.8 | 0.07 |
| *S. aureus* | 37.5 | 15 | 0.007 |
| *S. hemolyticus* | 37.5 | 21.2 | 0.004 |
| *S. auricularis* | 23.8 | 17.5 | 0.003 |
| *S. warneri* | 21.2 | 13.8 | 0 |
| *S. hominis* | 18.8 | 20 | 0.009 |
| *S. simulans* | 15 | 0 | 0.281 |
| *S. scurii* | 11.2 | 6.2 | 0.095 |
| *S. capitis* | 7.5 | 12.5 | 0.161 |
| *S. saprophyticus* | 6.2 | 7.5 | 0.33 |
| *S. xylosis* | 6.2 | 10 | 0.76 |
| *S. cohnii* | 3.8 | 6.2 | 0.178 |
| *S. lentus* | 2.5 | 0 | - |
| **Streptococci** | | | |
| *S. bovis* | 3.8 | 1.2 | 0.03 |
| *S. agalactae* | 2.5 | 6.2 | 0.001 |
| *S. viridians* | 2.5 | 1.2 | 0.02 |
| *S. pneumonia* | 1.2 | 1.2 | 1 |
| *S. salivarius* | 0 | 2.5 | - |
| *S. acidominimus* | 0 | 1.2 | - |
| **Enterococci** | | | |
| *Ent.faecalis* | 16.2 | 17.5 | 0.001 |
| *Ent. faecium* | 6.2 | 7.5 | 0.002 |
| *Ent.gallinarium* | 2.5 | 0 | - |
| **Corynbacteriae** | | | |
| *C. xerosis* | 12.5 | 8.8 | 0.21 |
| *C. minutissimum* | 3.8 | 1.2 | 0 |

**[Table/Fig-1]:** Percent distribution of bacterial species colonizing lesional and nonlesional skin of children with atopic dermatitis in the study (N=80).

Streptococci colonised 10% of both lesional and non-lesional skin. These was *S. bovis*, which was grown from 3(3.8%) lesions, and 1(1.2%) non–lesion; *S. agalactiae* was seen more in non-lesional skin 3 (3.8%) than in lesions; 2 (2.5%); (p=0.001). *S. viridians*, *S. acidominimus* and *S. salivarius* were isolated only from non-lesions. *S. pnemoniae* was equally found in both lesional and non-lesional skin, but this finding was statistically insignificant (p>0.05).

*Ent. gallinarium* was isolated only from lesions, whereas *Ent. Faecalis* and *Ent. Faecium* were isolated from both lesional and non-lesional skin. Other gram-positive bacilli isolates were *Corynebacterium xerosis* and *Corynbacterium minutissimum*, which were recovered from 10 (12.5%) and 3 (3.8%) lesions and 7(8.8%) and 1(1.2%) non-lesional skin respectively.

Four gram-negative bacterial species were grown from 7 (8.8%) AD lesions, but none were recovered from non-lesional skin. *Pantoea agglomerans* was grown from 3 (3.8%) lesions and *Enterobacter cloacae* was grown from 2 (2.4%) lesions. *Acinetobacter iwoffii* and *Chryseobacterium indologenes* each was grown from 1 (1.2%) lesion.

Based on the SCORAD index for severity of dermatitis [5], gram-negative bacteria were isolated from lesions, which were either moderate or severe, but none were isolated from mild lesions (p < 0.0001). Severity of atopic dermatitis was negatively correlated with colonisation of gram–negative bacilli (r = –0.5, p=0.001). However, there was no correlation between gram–positive bacterial colonisation and the severity of AD lesions (p >0.05). A logistic regression was conducted to determine as to what impact the 3 variables; gram–negative bacilli, gram-positive cocci and gram–positive bacilli lesion–colonisation, as predictors, had on the severity of dermatitis (as mild or moderate) as an outcome. None of the colonisations were found to be a predictor of severity of AD lesions (OR was0.04, 3.9, 0.26; 95% CI 0.08-2.09, 0.23-67.9, 0.08-2.09 and p values were >0.05) respectively.

## DISCUSSION

In the present study, bacterial colonies were isolated from 97.5 % (78/80) AD lesions in children and 96% (77/80) no-lesional skin. Gong et al., [3] reported even lower values. This difference might be due to different age groups. A lower positive culture rate was demonstrated by Farajzadeh et al., [7] in 74% of lesions in children with AD. However, their results were not controlled by healthy skin in the same subjects as in ours. The reason for the high percentage of colonisation in our study could be attributed to the AD lesions in those children.

In this study, *S. aureus* colonisation in AD lesions was high compared to that in non-lesional skin (38% compared to 15%) and the difference was highly significant ($\chi^2$ =8.47, p=0.007). This result was in line with what was reported by Gong et al., [3] However Guzik et al., [8] accounted *S. aureus* in all their 34 patients and in nine (26%) uninvolved skin. High colonisation of the healthy skin could, possibly be due to contamination from AD skin.

We isolated 13 species of coagulase-negative in this study. In our patients, colonisation was found to be more in the lesional skin. A similar result was obtained by Hoeger et al., [9]. Their isolation from the exposed areas of the body may be due to matters of hygiene, but whether they were related to AD or not, may need further elucidation to find out.

*Streptococci* are rarely seen on normal skin, especially β-*haemolytic Streptococci* [10]. The finding of almost a 10% *Streptococcal* colonisation in both healthy and AD skin even in the absence of overt infection, in this study, could be due to contamination from the AD lesions. While we did not specifically address the cause/effect factor, our results did not support the rare existence of *Streptococci* in healthy or uncomplicated AD skin, nor did a previous report do it.

Two gram positive bacilli, namely, *C. xerosis* and *C. minutissimum* were isolated in both lesional and non-lesional skin, in this study, with no significant difference. These strains normally colonize areas of the skin which are rich in lipids or sebum, such as the axillae [11]. *Enterococci* were isolated from both lesional and non-lesional skin in this study. Yet, they are not known as a part of the normal microflora of the human skin. However, Hardis and Wiley [12], reported their recovery from the oral cavity with an increased carriage rate in hospitalized patients. The *Enterococcal* colonisation of atopic dermatitis in this study needs further substantiation.

Gram–negative organisms are uncommon as a part of the normal skin flora, other than transient residents of the skin [13]. This was proved true for the non–lesional skin in this study, but the finding of an 8.7% colonisation of atopic dermatitis in our sample has not been, to the best of our knowledge, reported before. However, patients were investigated only once during this study; thus, no overall conclusion can be reached as to the rate of persistence in contrast to transient skin carriage. We recommend that multiple samples be collected, to prove or refute the persistence of colonisation of gram negative bacilli. The commonest genus which is known to inhabit skin is

*Acinetobacter*, where it was found in 40–50% of normal individuals. Seifert et al., [14] documented *Acinetobacter* chiefly in the axillae, the perineum and in the antecubital fossa and not in the dry, exposed areas of the skin, as in our sample. *A. lwoffii* was the only species of the genus, *Acinetobacter*, which was recovered from AD lesions in this study. No similar report was found to describe the colonisation by *A. lwoffii* in children with atopic dermatitis; however, Scheinfeld, [15] reported a case of a child with severe AD and a pathogenic infection with *Acinetobacter* species, especially *A. lwoffii*. AD may be thought of as a cause of such a colonisation, but its role in the aetiology needs confirmatory studies.

The gram negative organism which was most frequently recovered in this study was *Pantoea agglomerans,* where it was grown from 3.8% AD lesions. It is a member of Enterobacteriacae, that inhabits plants, soil and water. Such species include bacteria which are commensals and pathogens of animals and humans, as was reported by Gavini *et al.*, [16]. It is a known cause of septic arthritis following a thorn injury [17]. Its isolation from AD lesions in this study has not been, to the best of our knowledge, reported before.

*Enterobacter cloacae* is known as an important nosocomial pathogen which causes up to 5% of the hospital-acquired septicaemia [18]. However, it has not been reported as a colonizing agent, either in atopic dermatitis or as a part of normal skin flora.

The SENTRY Antimicrobial Surveillance Program, [19] isolated *C. meningosepticum* and *C. indologenes* which are commonly among the elderly; we isolated these two species from our patients who had no evidence of an infection.

Gram-positive bacteria were found to have no effect on the severity of dermatitis, in this study. However, the effect of gram positive cocci, especially *S. aureus,* on the disease severity was reported by Breuer et al., [20] to be more conspicuous in patients with an initial SCORAD of 50, on starting treatment. However, the highest SCORAD record in the subjects of this study was 46.

Owing to their isolation from moderate and severe lesions and none from mild lesions and the negative correlation with the severity of AD, the colonisation of gram-negative bacilli may be a factor in the exacerbation of these lesions. However, a causative relationship could not be established in this study.

## CONCLUSION

In this study, it appeared that atopic dermatitis in children was complicated with *Streptococcal* and Gram–negative bacterial colonisation and the latter was correlated with the severity of the lesions. *Enterococci* and *Corynebacterium* species were significant residents. *S. aureus* remained the chief inhabitant. No causal relationship could be established between the skin microbiota and atopic dermatitis.

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