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## OCCURRENCE OF GRAM POSITIVE BACTERIA AMONG SAUDI CHILDREN WITH ATOPIC DERMATITIS

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### ABSTRACT

**Objectives:** to determine the occurrences of bacterial colonization of atopic dermatitis in children of Qassim region in Saudi Arabian **Methods.** In a hospital-based study we collected 2 swabs, one from lesional skin and the other from a healthy skin area as healthy control from 80 children with atopic dermatitis. Isolation and identification were done by colony morphology, Gram stain and the automated Vitek System. The majority of children were below 5 years of age. **Results:** The mean age was 1.4 with a SD of 0.74. Bacterial colonies were grown from all patients in lesion and non-lesional skin with more species in lesional skin. Gram positive cocci, bacilli and mixed colonies were grown. *S. aureus*, *S. hemolyticus*, *S. auricularis*, *S. warnerii*, *S. simulans*, *S. scurii*, *S. capitis*, *S. xylosus*, *S. cohnii* and *S. hominis* were more in the lesion whereas *S. epidermidis* was found to be more in nonlesional skin. *Enterococci*, commonly *Ent. Faecalis*, *Ent. Faecium* and *Ent. gallinarium* were isolated mostly from lesions. Other less common isolates were *Corynebactericae* as *C. xerosis* and *C. minutissimum*.

**Conclusions:** Bacterial colonization is more frequent in atopic dermatitis than in normal skin. Staphylococcal species predominate. Streptococci, *Corynebactericae* and *Enterococci* are significant inhabitant of atopic dermatitis.

**Keyword:** Skin lesion, Colonization, Atopic Dermatitis

### INTRODUCTION

Atopic dermatitis is a chronic inflammatory pruritic skin disease affecting children and adults. The onset occurs in 45% of children during the first 6 months of life, 60% during the first year and before 5 years in about 85% of affected individuals. The estimated prevalence worldwide is ranging from 1% to 20% (1). Treatment of atopic dermatitis accounts for a significant amount of health service financial resources, clinical time and place a burden on the child, family and society (2). Atopic dermatitis was found to be predominant in

children in Qassim region, Saudi Arabia (3). The diagnostic criteria for atopic dermatitis had been set by Hanifin and Rajka in 1980 (4). Bacterial and viral infections often nonspecifically aggravate atopic dermatitis (5). "Immunomodulatory pathways in atopic dermatitis may have important implications from a therapeutic point of view because patients with atopic dermatitis may benefit from more than just anti-inflammatory treatment in the future" (6). *Staphylococcus aureus* infection was found to be the leading cause of infection of atopic dermatitis and that antibacterial treatment is

beneficial when children are clinically impetiginized (7). Most studies focused on the direct relationship between *S. aureus* and severity of atopic dermatitis (7, 8, 9 and 10) besides, the majority of the studies were conducted in adult populations but literature on whether other types of bacteria were involved is not sufficient. Moreover, worldwide studies in bacterial colonization especially gram positive, apart from *S. aureus*, are scanty and particularly no studies to determine colonizing bacteria in AD young patients were done in Saudi Arabia. The aim of this study is to determine the bacterial colonization of atopic dermatitis lesions in a group of children in compared with control to healthy areas of the skin in the same subjects.

## MATERIALS AND METHODS

### Patients and organisms

This cross-sectional descriptive controlled study was conducted in different out-patient dermatology clinics in Qassim University affiliated hospitals during the period from March 2009 to February 2010. A total of 80 subjects, all were children aged 6 months to 18 years with AD were included after written informed consent was obtained. The diagnosis of atopic dermatitis which was made by consultant dermatologists was based on the criteria of Hanifin and Rajka (4). Children who had concomitant immune system disease, severe systemic infection, systemic heart and kidney or liver diseases were excluded from the study. Moreover, children on treatment by topical steroids in the last two weeks or systemic antibiotics in the last four weeks were also excluded. Two skin swabs were taken from each subject; one from the target skin lesion and the other from the non-lesional skin which was defined as healthy skin symmetrical to the target skin lesion or at least 10 cm away from it as a healthy control. Swabs were saturated by brain

heart infusion broth (Oxoid) and transported immediately to the laboratory (11). These swabs were then cultured on blood agar base (Oxoid), Mc Conky agar, Nutrient agar, Mannitol salt agar and incubated at 37° C for 24-48 hours.

The growth colonies were identified by Gram stain, colonial morphology and biochemically by Vitek system (BioMerieux 12) automated machine with different incubation periods from 2 hours up to 24 hours according to the manufacturers' procedures (12). Records of measurements were registered each hour starting at hour zero to a maximum of 15 hours. Data were entered and analyzed into SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, U.S.A.). A P value of < 0.05 was defined as statistically significant.

## RESULTS

The age ranged between 6 months and 14 years. The majority of children were below 5 years of age constituting 59 (73.75%) of the whole sample. The mean age was 1.4 with a SD of 0.74. Males outnumber females comprising 61 (76.3%) (N=80) however, gender distribution within different age groups was insignificant (p=0.98).

Bacterial colonies were grown from all patients of atopic dermatitis in both lesion and non-lesional skin. A total of 240 of different bacterial colonies were grown from 80 subjects with AD in contrast to 193 colonies from non-lesional or healthy areas of skin of the same subjects. The lesion/non-lesion ratio of grown bacterial colonies was 3/2.4. Bacterial species were found to be more in the lesions than in the non-lesional skin where 31 species were recovered from lesions whereas non-lesional areas yielded a count of 25 species. Out of 80 patients, gram positive cocci were found in 78 (97.5%) of the lesions and 77 (96.25%) of the non-lesional healthy skin (p=0.001). Gram positive bacilli and mixed colonies were also

isolated more from lesion than non-lesional areas. Gram positive cocci were isolated from both lesion and non-lesion in 77 (96.25%) patients and in one patient (1.25%) in the lesion alone, whereas 2(2.5%) patients did not grow them in either lesion or non-lesional areas of skin.

*Staphylococci spp.* was found in the lesions more than in the controls. Those were *S. aureus* ( $p=0.007$ ), *S. hemolyticus* ( $p=0.004$ ), *S. auricularis* ( $p=0.003$ ), *S. warnerii* ( $p=0.000$ ) and *S. hominis* ( $p=0.009$ ). The following strains were also grown more in the lesion than healthy nonlesional skin: *S. simulans*, *S. scurii*, *S. capitis*, *S. xylosus* and *S. cohnii* but this finding is not statistically significant (table). The exception was for *S. epidermidis* which was found to be more in the nonlesional skin, however this was also not significant ( $p=0.07$ ). Streptococci colonized 10% of both lesional and non lesional skin. Isolated *Streptococci* were: *S. bovis* in 3(3.8%) lesions and in 1(1.2%) nonlesion ( $p=0.03$ ); *S. agalactiae* was more in nonlesional 3(3.8%) than in lesion 2(2.5%) lesions ( $p=0.001$ ) likewise *S. viridians* 2(2.5%) in lesion and 1(1.2%) in nonlesion ( $p=0.02$ ) whereas, *S. acidominimus* and *S. salivarius* were isolated only from nonlesions in 1(1.2%) and 2 (2.5%) respectively. Equal isolation for *S. pneumoniae* was found in both lesion and nonlesion areas but the finding is statistically insignificant ( $p=1.0$ ). ( Table1and figure1).

*Enterococcus gallinarum* were isolated only in the lesion. Whereas *Ent. Faecalis* and *Ent. Faecium* were isolated from both (table). Other gram positive bacilli isolates were *Corynebacterium xerosis* and *Corynebacterium minutissimum* which were recovered from 10 (12.5%) and 3 (3.8%) lesions and 7(8.8%) and 1(1.2%) nonlesional skin respectively ( $p=0.002$ ). ( Fig.1 and tab.1).

#### Discussion

Bacterial colonies in this study were grown from all lesion and healthy skins of children with atopic dermatitis. This was reported, in lesser values, in a similar study which was done by Alsaimaru et al 2006 the were found that 94.4% and 86.36% grown of the positive culture in lesion and healthy skin area from AD respectively (9). However their study was conducted in different age groups whereas ours was primarily in children population. Moreover Gong et al (8) was reported similar information compared to our results by finding colonization of bacteria from all lesions of patient with AD but their results was not controlled by healthy areas of skin as well as their patients was aged group of 2 to 65 years. A lower positive culture rate was shown by Farajzadeh et al (13) where they detected a positive culture in 74% of the lesion in children with AD. The reason for the high colonization rate in all patients in this study could be attributed to the chronic lesion of atopic dermatitis despite sampling was taken from exposed skin to the environment i.e. upper limbs and face, which has a rate of occurrence of the normal inhabitant of the skin (14). Moreover no patient was receiving any antibiotic before and during sampling.

Staphylococcal colonization of AD was reported as common in many studies. Hill SE et al isolated *S. aureus* from 68 patients out of hundred who are all children (15). Gong J. Q et al (8) reported more or less similar results in adult population. Moreover earlier studies by Leyden JJ et al and Ring J 1 yielded that *S. aureus* is the most common skin infecting agent in AD which was found in more than 90% of the patients compared to 5% normal individual in both lesional and non lesional AD skin (16). Guzik TJ et al found, more or less, similar results to ours concerning *S. aureus* but they also studied colonization during exacerbation with the finding of significant correlation between the density of colonization and the severity of

dermatitis (17). *S. aureus* was isolated in 30(37.5%) lesional areas in contrast to 12(15%) non-lesional in this study however, *S. aureus* is not considered as normal skin resident (18). This finding is in line with the above mentioned reports but colonization of healthy skin is even higher in our study. Variation of the occurrence of *S. aureus* in uninvolved skin has been reported in several studies (19). High colonization of the healthy skin could be due to contamination from lesional skin.

The most frequently isolated *Staphylococci* species other than *S. aureus* in lesional AD skin in our study were *S. epidermidis* 42.5%, *S. hemolyticus* 37.5%, *S. auricularis* 23.8%, *S. warneri* 21.2%, *S. hominis* 18.8%, *S. scuri* 11.2%, *S. capitis* 7.5%, *S. saprophyticus* 6.2%, *S. xylosus* 6.2% and others. The isolation of these species was lower in non-lesional skin, with exception for certain species which were *S. epidermidis* 58.8%; *S. auricularis* 23.8%, *S. hominis* 18.8%, *S. capitis* 7.5%, *S. saprophyticus* and *S. xylosus*; the later 2 species were isolated each in the rate of 6.2% of the lesions. About 12 species of coagulase negative staphylococci are commonly found on human skin and the most abundant were *S. epidermidis*, *S. haemolyticus* and *S. hominis* (20). We isolated 13 species in this study. In contrast to Hoeger P. H. et al (21) colonization was not equal in lesional and non-lesional areas but denser on the lesional skin of our patients in most of the isolated species. These species are known as part of the normal flora which usually inhabits the perineum (22); their isolation in the exposed areas of the body may be due to matters of hygiene but whether they are related to atopic dermatitis or not may need further elucidation. Gong JQ et al results (8) compared to ours was different in that *S. epidermidis* was more in the lesional skin. Hoeger H.P. et al (21) results, however, were similar to ours regarding *S. epidermidis* which

they reported to be higher in the non-lesional skin.

*Streptococci* are rarely seen on normal skin especially  $\beta$ -hemolytic *streptococci* (20). This is attributed in part to the lethal effect of lipid on them (14). The finding of almost 10% colonization in both healthy and AD skin could be attributed to atopic dermatitis. While we did not specifically address the cause/effect factor, our results do not support the rare existence of streptococci in healthy or non-complicated AD skin, nor did a previous report. However David T.J. and Cambridge G.C. (23) reported recovery of *beta hemolytic streptococci* in combination with *S. aureus* in 62% of episodes of infection in children with AD but not as colonizing agents.

Gram positive bacilli occurrence was not significantly different between lesional and non-lesional areas of skin in this study. The commonly isolated gram positive bacilli in this study were *C. xerosis* and *C. minutissimum* among others (tab.1). These strains are normally colonizing areas of skin rich in lipids or sebum such as the axilla (24) and in our study the isolates were from exposed areas which are poor in sebum. Some *Corynebacterium* species can cause serious infection and may even cause endocarditis (25).

In humans, *Enterococci* can be isolated from almost 100% of faecal samples, with *Ent. faecalis* being reported more commonly and in higher numbers than *Ent. faecium* in most studies (26). *Enterococci* species were isolated from both lesional and non-lesional skin in this study. However, no previous report of them being as part of the normal microflora of the human skin, they can be recovered from the vagina and oral cavity and the carriage rate may be increased in hospitalized patients (27). *Enterococci*, especially *Ent. Faecium* can cause epidemic of vancomycin resistant in clinical settings (28) and multiple sites infection resistant to multiple antibiotics (29). The

presence of *enterococci* species in atopic dermatitis needs further verification.

### CONCLUSION

Bacterial colonization rate is higher in diseased as well as healthy skin of children with atopic dermatitis but the colony count and colonization with pathologic strains are more in atopic dermatitis skin. *Staphylococci*, especially *S. aureus* and *S. epidermidis*, predominate. There is also high colonization rate with *streptococci* in atopic dermatitis and equal colonization density of gram positive bacilli. *Enterococci* and *Corynebacterium* species were also isolated in considerable proportion.

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**Table1. Different bacterial species colonizing in lesional and non-lesional skin of children with atopic dermatitis (N=80)**

Bacterial species	% colonization of lesional skin	% colonization of non-lesional skin	P- value
<b>Staphylococci</b>			
<i>S. epidermidis</i>	42.5	58.8	0.07
<i>S. aureus</i>	37.5	15	0.007
<i>S. hemolyticus</i>	37.5	21.2	0.004
<i>S. auricularis</i>	23.8	17.5	0.003
<i>S. warneri</i>	21.2	13.8	0
<i>S. hominis</i>	18.8	20	0.009
<i>S. simulans</i>	15	0	0.281
<i>S. scurii</i>	11.2	6.2	0.095
<i>S. capitis</i>	7.5	12.5	0.161
<i>S. saprophyticus</i>	6.2	7.5	0.33
<i>S. xylois</i>	6.2	10	0.76
<i>S. cohnii</i>	3.8	6.2	0.178
<i>S. lentus</i>	2.5	0	-
<b>Streptococci</b>			
<i>S. bovis</i>	3.8	1.2	0.03
<i>S. agalactae</i>	2.5	6.2	0.001
<i>S. viridians</i>	2.5	1.2	0.02
<i>S. pneumonia</i>	1.2	1.2	1
<i>S. salivarius</i>	0	2.5	-
<i>S. acidominimus</i>	0	1.2	-
<b>Enterococci</b>			
<i>Ent. faecalis</i>	16.2	17.5	0.001
<i>Ent. faecium</i>	6.2	7.5	0.002
<i>Ent. gallinarum</i>	2.5	0	-
<b>Corynebacteriae</b>			
<i>C. xerosis</i>	12.5	8.8	0.21
<i>C. minutissimum</i>	3.8	1.2	0

