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ORIGINAL ARTICLE

Genotyping of Staphylococcal Species on the Skin of Patients with Atopic Dermatitis

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ABSTRACT

Background: *Atopic dermatitis (AD) is a prevalent illness that impacts a diverse range of individuals worldwide. The skin microbiome of AD patients is characterized by increased colonization by Staphylococci and decreased bacterial diversity, which could worsen disease symptoms.* ***Objectives:*** *To recognize staphylococcal species on skin of AD patients and to assess the association of these species with disease severity.* ***Methodology:*** *The study comprised 45 AD patients and 45 healthy controls. Full history, general and dermatological examination were performed to all the participants. Disease severity was calculated using Severity Scoring of Atopic Dermatitis (SCORAD). Microbiological examination was conducted for all skin specimens collected. Molecular identification of the detected staphylococcal species using multiplex Polymerase Chain Reaction (PCR).* ***Results:*** *There was a statistically significant higher growth of Staphylococci among cases than control group (97.8% versus 46.7%, respectively, P value<0.001). Following Genotyping, a statistically significant higher growth of Staphylococcus aureus (S. aureus) and S. hominis were detected among AD cases than control group (P value =0.0005 and 0.05, respectively). There was significant association (p value < 0.001) between severity of AD and genotypic distribution of staphylococcus species. All Patients with Severe AD were colonized with S. aureus.* ***Conclusion:*** *Staphylococcal species were relatively exclusive to AD. Increased frequency of S. aureus and S. hominis were observed in AD skin. These findings may be significant for understanding pathophysiology and severity of AD to outline new treatment options.*

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disorder marked by recurring eczematous lesions and sever itching [1]. Globally, AD affects children and adults at a rate of 15-20% and 1-3%, respectively. It is the most prevalent long-term skin condition affecting children, with a negative influence on the quality of life[2].

The pathogenesis of AD is multifactorial that includes a strong familial susceptibility, epidermal barrier breakdown, immunological dysfunction along with disturbances in skin microbiota [3]. Staphylococci are normal residents of human skin. While *Staphylococcus aureus (S. aureus)* is frequently the source of invasive and fatal infections, coagulase-negative staphylococci (CoNS) mostly cause opportunistic infections. As a result, staphylococci identification at species-level is crucial [4].

Staphylococcus aureus is a notable human microorganism which is associated with community and healthcare associated infections. However, it can establish a commensal relationship in certain individuals[5]. The dysbiosis of the cutaneous microflora, which is typified by a decrease in microbial diversity and increase in *S. aureus* colonization, is strongly related to the disease activity in AD. In healthy persons, the *S. aureus* skin colonization is about 5%, whereas it is more than 90% in patients with AD [6].

S. aureus aggravates inflammation in AD through its penetration under the epidermis, boosting protease activity and compromising the skin barrier [7,8]. Also, *S. aureus* generates a variety of enterotoxins that are superantigenic and initiate IgE mediated allergic reaction [9,10].

Likewise, CoNs are opportunistic organisms that can infect eczematous lesions; as such, their purpose in AD may differ from that in normal skin. While numerous researches have investigated *S. aureus* involvement in AD, there are relatively fewer studies that focused on role of CoNS at the species level (*S. capitis, S. caprae, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, and S. warneri*) have been published [11,12].

Numerous techniques have been used for the characterization of staphylococcus species. Standard biochemical assays are imprecise because of phenotypic variance. A quick and precise multiplex Polymerase Chain Reaction (PCR) was created to identify staphylococci up to species level, depending on the sequence of thermonuclease (*nuc*) genes [4].

This study was carried out to recognize staphylococcal species on lesional and non-lesional skin of AD patients, compared to healthy controls and to assess if there is a correlation between presence of staphylococcal species and AD severity.

METHODOLOGY

Study design

This is a case control study that included 45 AD patients and cross-matched 45 healthy control. It was conducted at the Dermatology, Andrology and STDs Department, Mansoura University Hospitals and Medical Microbiology& Immunology Department, Mansoura Faculty of Medicine from August 2022 to February 2024.

The study included cases from both genders aged 18 years or less who were diagnosed with AD in accordance to Modified Hanifin- Rajka criteria [13].

Children with other inflammatory skin conditions (e.g. Scabies, contact dermatitis or seborrheic dermatitis), systemic diseases, hormonal abnormalities and topical or systemic antibiotics, tacrolimus or corticosteroids use in the preceding 2 weeks, were excluded.

Dermatological examination

A full history, general and dermatological examinations were performed to diagnose other atopic conditions as bronchial asthma. Clinical evaluation of the degree of AD was done in accordance to Severity Scoring of Atopic Dermatitis (SCORAD). This clinical test is used to appraise the extent and severity of eczema. It integrates subjective indicators (diurnal pruritus and sleeplessness) with objective indicators (lesion extent and intensity). SCORAD index formula is A/5 + 7B/2 + C (A=Affected area, B=Intensity of AD signs, C=subjective symptoms). Patients were classified according to SCORAD into; mild (<25), moderate (25-50) and severe AD (>50) [14].

Microbiological examination

Sample collection & processing

Skin samples were taken with sterile cotton swabs moistened with sterile saline. The sampling was applied to a 25 cm^2 region of skin. For patients, samples were taken according the site of eczema, typically from the volar forearm and the antecubital crease. Specimens from controls were taken from the antecubital crease. The swabs were put in transport media [15,16].

All samples were processed within 2 hours in the Medical Microbiology and Immunology Department at Mansoura Faculty of Medicine. Samples were cultured in Nutrient agar, Sheep Blood agar, Mannitol salt agar and then incubated aerobically at 37˚ c for twenty-four hours.

Phenotypic identification of staphylococci

Skin colonization by staphylococci were identified by using microscopic detection of Gram positive cocci, colony morphological features and standard biochemical tests (catalase, coagulase and DNase tests) [15]. The identified strains were stored at −20°C in Nutrient broth with 30% sterile glycerol until molecular analysis [16].

Molecular characterization of the staphylococcal genotypes

Genomic DNA was isolated from overnight fresh cultures of staphylococci grown on blood agar. DNA was extracted using Boiling method. The extracted DNA was stored frozen at −20 °C until further amplification procedures [16].

Identification of staphylococcal isolates up to species level was done by multiplex PCR. Primers (Analysis for life Company, Egypt) specific for each species were used (Table 1)**.** The reaction mixture contained 2 µl template DNA, 0.2 µM of each primer, 12.5 µl Master mix (Willowfort,W1020300X) and 6.9 µl distilled water in a final volume of 25 µl. A PCR thermal cycler was used for amplification, with an initial denaturation step (95°C, 5 min); 30 cycles of denaturation (95°C, 30 s), annealing (58°C, 30 s), and extension (72°C, 70 s); and a final elongation step at 72°C for 2 min. PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide [4].

Table 1: Oligonucleotide primers for identification of staphylococcal species [4].

Staphylococcus Species	Primer	Sequence (5′–3′)	Size of PCR product (bp)
S. hominis	hom-F	TACAGGGCCATTTAAAGACG	177
	hom-R	GTTTCTGGTGTATCAACACC	
S. epidermidis	epi-F	TTGTAAACCATTCTGGACCG	251
	epi-R	ATGCGTGAGATACTTCTTCG	
S. aureus	aur-F	TCGCTTGCTATGATTGTGG	359
	aur-R	GCCAATGTTCTACCATAGC	
S. haemolyticus	hae-F	TAGTGGTAGGCGTATTAGCC	434
	hae-R	ACGATATTTGCCATTCGGTG	
S. capitis	cap-F	ACTACGCCTATGATTATTGC	525
	cap-R	GAYGCTTCTTTACCATAGGG	
S. lugdunensis	lug-F	TCCAATGATGGTAACGAGGC	695
	lug-R	TTTTGCGCCTCGTTTTGTGC	
S. saprophyticus	sap-F	TTTTGGATGCGATAGATTGG	843
	sap-R	TCTTCAGACTTTTCAAAGGC	
S. warneri	war-F	CGTTTGTAGCAAAACAGGGC	999
	war-R	GCAACGAGTAACCTTGCCAC	
S. caprae	rae-F	TTGTTCTWGCACTYATTGCG	1,227
	rae-R	TTTTATAGAACAGGGTCGAC	

Ethical approval

The whole study design was accepted by the Institutional review board (IRB), Mansoura Faculty of Medicine (Code R.21.09.1643). A written consent was obtained from participants. Confidentiality were respected in all levels of the study. Patients feel free to withdraw from the study at any time. The study is conducted in accordance with Helsinki Standards for Medical Research.

Statistical analysis

The collected data were analyzed with SPSS version 26. Qualitative data were shown as number and percent. The Chi-Square test made the comparison between groups. Parametric data were shown as median ± SD. To compare three groups with normally distributed quantitative variables, one-way ANOVA test was used and Kruskal Wallis test was used if the data were abnormally distributed. For all tests, P value ≤ 0.05 was considered significant.

RESULTS

The study involved 45 cases with AD and 45 healthy control. Regarding sociodemographic characters of the studied groups, there was no statistical significant difference as regard their age, gender and residence. Median age of the studied cases and control group was 3 years ranging from 2 months to 15 years. Interestingly, cases with AD were more distributed in urban (71.1%) than rural areas (28.9%).

In the AD cases group, 88.9% of the studied lesions involve flexor surfaces, 84.4% present in head & neck and 68.9% in limbs. Also, 62.2% of the cases had acute disease onset & 37.8% had subacute onset (Table 2). Moreover, 44.4% of the studied cases had personal history of asthma and 8.9% had allergic rhinitis. Regarding the family history, 48.9% of the cases had family history of AD and 15.6% had asthma (Table 3).

Table 2: Distribution of the AD cases according to type, site & stage of lesion

Lesion	AD cases (n=45)	%
1.Type of involvement		
Flexor surface	40	88.9
Extensor surface	8	17.8
2.Site of involvement		
Head & neck	38	84.4
Trunks	12	26.7
Limbs	31	68.9
Genitalia	9	20.0
3. Stage of the lesion		
Acute	28	62.2
Subacute	17	37.8

Table 3: Distribution of the AD cases according to personal & family history.

	AD cases (n=45)	%
1.Personal history of allergy		
Asthma	20	44.4
Allergic rhinitis	4	8.9
2.Family history of allergy		
Atopic dermatitis	22	48.9
Asthma	7	15.6
Allergic rhinitis	3	6.7

Regarding the clinical characteristics of the patients with AD, the most common aggravating factor for the lesion was food (97.8%) followed by climate (46.7%), infection (44.4%) and exercise (31.1%). The most prevalent associated symptom was dry skin (93.3%). The median disease duration was 6 months ranging from 0.06 to 72 months. For diagnosis, all studied cases had itchy skin, 91.1% had dermatitis, 93.3% had dry skin, 68.9% had visible flexural eczema, 44.4% had asthma and 48.9% had family history of AD. The mean SCORAD index was 42.1, mean percent of lesion extent was 28.51, most of cases were moderate cases (77.8%), 15.6% were severe and 6.7% were mild cases, as demonstrated in table 4 and figure 1 (a, b & c).

Table 4: Clinical criteria of AD in the cases group

Clinical criteria	AD cases (n=45)	%
1-Aggravating factor		
Food	44	97.8
Climate	21	46.7
Infection	20	44.4
Exercise	14	31.1
Psychosomatic	0	0.0
Trauma	0	0.0
2-Associated symptoms:		
Dry skin	42	93.3
Facial pallor	5	11.1
Cheilitis	12	26.7
3-Disease duration		
Disease duration (months)	6(0.06-72)	
Duration of treatment (days)	7(1-90)	
Last attack (days)	7(1-90)	
4-Diagnostic Criteria:		
Itchy skin	45	100.0
Visible flexural eczema	31	68.9
Dry skin	42	93.3
*P/H of dermatitis	41	91.1
*P/H of asthma	20	44.4
**F/H of atopic dermatitis	22	48.9
**F/H of asthma	7	15.6
**F/H of allergic rhinitis	3	6.7
5-SCORAD		
Index mean± SD (Min-Max)	42.10±9.74 (23.9-66.5)	
Extent (%) mean± SD (Min-Max)	28.51±18.86 (4-76.5)	
Severity of AD Mild Moderate Severe	 3 35 7	 6.7 77.8 15.6

*P/H: personal history **F/H: family history

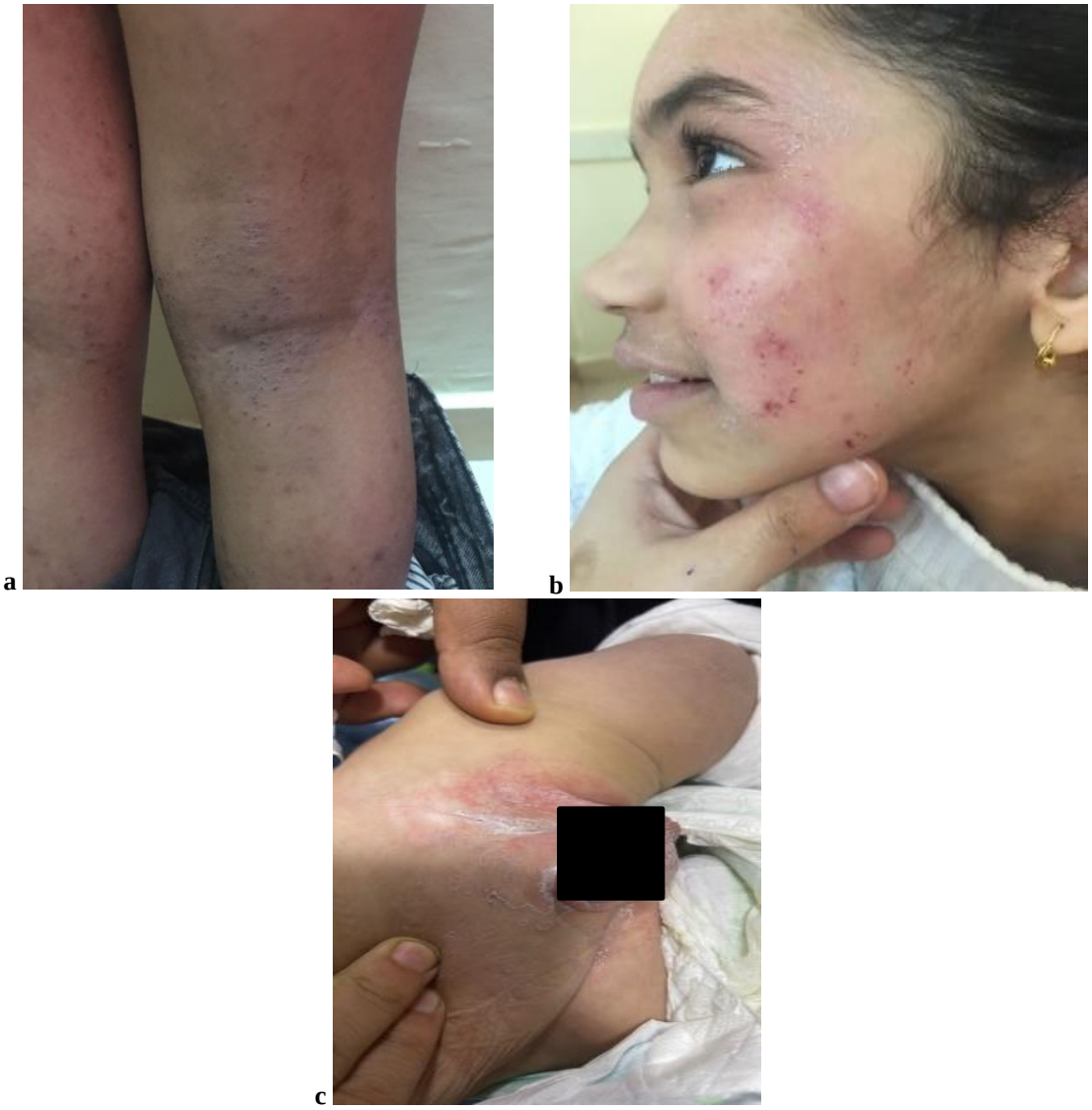

Fig. 1: AD lesions in some studied cases. (a) A child 7 years old with AD in popliteal fossa. (b) Female child with AD in the face. (c) An infant 5 months old with AD in genitalia.

As shown in table 5, there was a statistically significant higher growth of staphylococcal species among AD cases than control group, as detected by phenotypic methods (97.8% versus 46.7%, respectively). A statistically significant higher no growth frequency was detected among control than cases (46.7%versus 2.2%).

Furthermore, by multiplex PCR, a statistical significant higher growth frequency of *S. aureus* & *S. hominis* were detected among AD cases than control group (P value =0.0005 &0.05, respectively). Control group showed growth of all staphylococcal species except *S. caprae*, but, only 5 staphylococci species were detected in AD cases (17 *S. aureus*, 10 *S. epidermidis*, 8 *S. hominis*, 7 *S. haemolyticus*, 2 *S. capitis*), as demonstrated in table 5 and figure 2 (a & b).

Table 5: Comparison of distribution of staphylococcal species between AD cases (lesional skin) & control group by both phenotypic & genotypic (multiplex PCR) methods.

Bacterial growth	AD Cases		Control		P value
	n=45	%	n=45	%	
1-Phenotypic identification					
No growth	1	2.2	21	46.7	<0.001*
Staph species	44	97.8	21	46.7	<0.001*
*Others***	0	0.0	3	6.7	0.08
2-Genotypic identification					
No growth	1	2.2	21	46.7	<0.001*
S. haemolyticus	7	15.6	6	13.4	0.10
S. capitis	2	4.4	2	4.4	1.0
S. warneri	0	0.0	1	2.2	0.30
S. saprophyticus	0	0.0	2	4.4	0.14
S. lugdunensis	0	0.0	1	2.2	0.30
S. hominis	8	17.8	2	4.4	0.05*
S. epidermidis	10	22.2	4	8.9	0.09
S. aureus	17	37.8	3	6.7	0.0005*
S. caprae	0	0.0	0	0.0	0
*Others***	0	0.0	3	6.7	0.08

*significant p value ≤ 0.05% **Others* (Anthracoid & Gram negative rods)

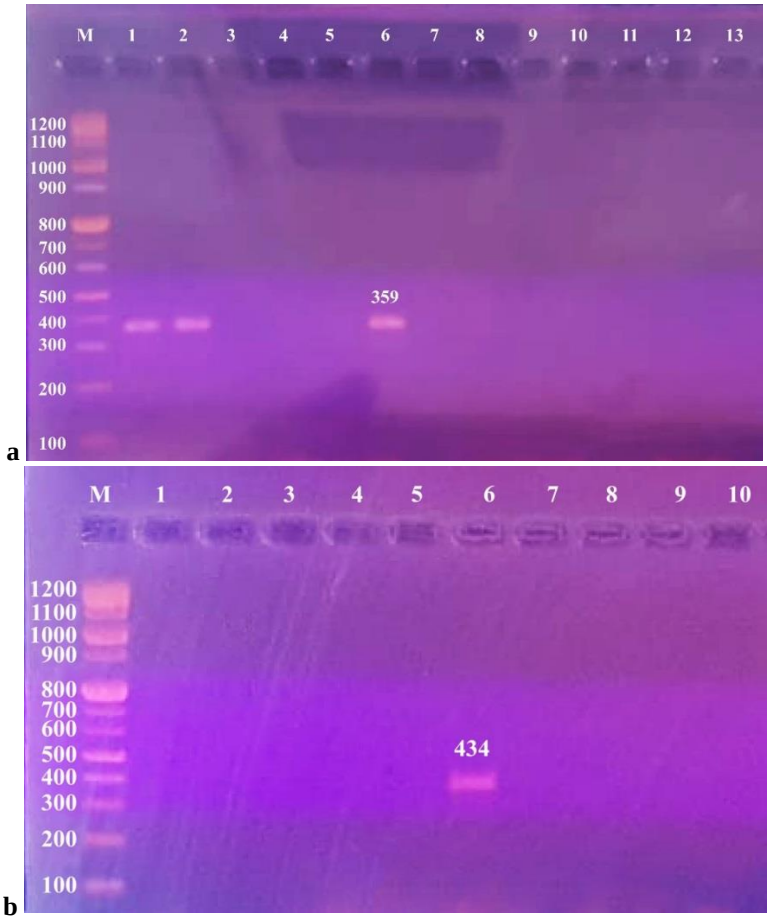

Figure 2: Gel electrophoresis showing genotyping of staphylococcal species by multiplex PCR amplification among AD cases. (a) Lanes1,2 and 6 showed band of *S. aureus* at 359 bp. (b) Lane 6 showed band of *S. haemolyticus* at 434 bp. The M lane is 100 bp DNA marker.

Table (6) illustrates statistical significant difference between lesional and non lesional skin of the studied AD cases, with higher frequency of no growth among non lesional skin than lesional skin (22.2% versus 2.2%, respectively). A higher frequency of staphylococcal species growth was detected among lesional skin than non lesional skin with statistically significant difference between them (p value= 0.009). a statistically significant higher frequency (p value= 0.003) of *S. hominis* growth among lesional than non lesional skin, as detected by multiplex PCR.

Table 6: Comparison of distribution of staphylococcal species between lesional & non lesional skin for AD cases by both phenotypic & genotypic methods.

Bacterial growth	AD Cases				χ^2/FET	P value
	Lesional skin		Non lesional skin			
	n=45	%	n=45	%		
1-Phenotypic identification:						
No growth (NG)	1	2.2	10	22.2	8.39	0.003*
Staph. species	44	97.8	33	73.3	10.88	0.009*
*Others***	0	0.0	2	4.4	2.05	0.15
2-Genotypic identification:						
No growth	1	2.2	10	22.2	8.39	0.003*
S. haemolyticus	7	15.6	10	22.2	0.836	0.360
S. capitis	2	4.4	2	4.4	0.002	0.963
S. hominis	8	17.8	0	0.0	8.41	0.003*
S. epidermidis	10	22.2	6	13.4	1.75	0.19
S. aureus	17	37.8	15	33.4	0.079	0.777
S. warneri	0	0.0	0	0.0	0	0
S. saprophyticus	0	0.0	0	0.0	0	0
S. lugdunensis	0	0.0	0	0.0	0	0
S. caprae	0	0.0	0	0.0	0	0
*Others***	0	0.0	2	4.4	2.05	0.15

*significant p value ≤ 0.05% **Others* (Anthracoid & Gram negative rods)

Table (7) illustrates statistically significant association (p value < 0.001) between severity of AD and genotypic distribution of staphylococcal species in such cases. Severe AD was found only among cases with *S. aureus* colonization, moderate disease was detected among 85.7% of cases with *S. hemolyticus*, 100% of *S. hominis*, 100% of *S. epidermidis* and 58.8% of *S. aureus*.

Also, there was a statistically significant (p value = 0.005) higher mean SCORAD index among cases with *S. aureus* (48.06±10.71), followed with *S. epidermidis* and *S. hominis*. However, there was statistically insignificant relation between extent of lesion or disease duration and genotypic findings among cases.

The table also demonstrated a statistically significant higher median time elapsed since last attack among AD cases for cases with *S. capitis*, followed by *S. hominis*, *S. epidermidis*, *S. aureus* and *S. hemolyticus* (20, 14.5, 14, 7 and 3, respectively).

Table 7: Relation between Genotyping of Staphylococcal species in AD cases (lesional skin) and their clinical criteria:

Clinical criteria	Genotyping of staphylococci among AD cases					P value
	***S. haemolyticus* (n=7)**	***S. capitis* (n=2)**	***S. hominis* (n=8)**	***S. epidermidis* (n=10)**	***S. aureus* (n=17)**	
1-SCORAD	n(%)	n(%)	n(%)	n(%)	n(%)	
Severity Mild (3) Moderate (34) Severe (7)	 1(14.3) 6 (85.7) 0	 2(100) 0 0	 0 8 (100) 0	 0 10 (100) 0	 0 10 (58.8) 7 (41.2)	 <0.001*
Index	33.19±5.42	33.15±8.69	39.61±7.4	41.42±5.67	48.06±10.71	0.005*
Extent	22.5 (9-58.5)	54 (31.5-76.5)	23.5 (6.5-63.0)	28 (6.5-67.5)	22.5 (4-63.0)	0.367
2-Disease duration (months)	5(1-48)	42(24-60)	9(0.06-24)	6(2-72)	5(0.1-60)	0.223
`3-Last attack (days)	3(1-3)	20(10-30)	14.5(2-21)	14(2-90)	7(1-30)	0.002*

*significant p value ≤ 0.05%

DISCUSSION

Skin is a complex and dynamic ecosystem inhabited by a large varieties of microbes; like *Staphylococci*, *Micrococci*, *Propionibacterium* and *Corynebacterium* species. Many AD patients get recurring skin infections and are vulnerable to staphylococcal species, especially *S. aureus*, colonization [17].

This study was conducted on 45 AD patients with matched healthy control. It was found that prevalence of AD in urban is higher than in rural areas (71.1% versus 28.9%, respectively). Environmental factors such as dietary habits, hygiene variations, vaccinations, exposure to microbes and allergens could explain the effect of urbanization on AD [18].

This study demonstrated that 88.9% of the lesions involve flexor surface, 84.4% in head & neck and 68.9% in limbs. In accordance to our findings, another studies had shown that hands, fingers, legs, dorsal foot, popliteal and antecubital fossae were the most common sites for AD lesions [19, 20].

The present study revealed that 44.4% of the AD patients had asthma and 48.9% of the cases had family history of AD. Similarly, two recent studies demonstrated a significant association between AD and asthma, which support their genetic relatedness [20,21].

As regard the aggravating factors, our study found that the most prevalent factor was food (97.8%), followed by climate, infection and exercise. Similarly, Kayode et.al. [22] had observed that children who played on school grass and consumed fruits, potatoes, and cereal had statistically significant higher risks associated with AD.

Regarding phenotypic character, this study concluded that there was a statistically significant higher growth of staphylococci among cases when compared to control group (97.8% versus 46.7%, respectively). Parallel to our findings, AD cases showed extremely higher (10–100 times) prevalence of staphylococci than in normal individuals [23].

It's interesting to note that the multiplex PCR could be a quick and precise method for genotyping of diverse staphylococcal species isolated from AD patients' skin. Current study demonstrated decreased diversity of staphylococcal species (5 out of 9 species) in AD skin with a statistically significant higher growth frequency of *S. aureus* (37.8%) and *S. hominis* (17.8%) than control group. This was consistent with Soares et. al. [12] study (35.6% *S. aureus*, 30.4% *S. epidermidis* and 27.8% *S. hominis*). *Moreover, previous study in Egypt detected high prevalence of S. aureus colonizing skin (25%) and nose (30%) of AD cases* [24]. Nevertheless, another study showed that 100% of AD cases had *S. aureus* on their lesion [25].

The above variations could be explained by simultaneous fluctuations between skin flora [26]. Concurrent prevalence of both *S. aureus* and *S. epidermidis* in Portuguese AD patients was documented in a prior study. These two species could share a commensal association to resist antimicrobial peptides in inflamed AD skin [27].

Furthermore, researches had concluded that AD patients colonized by *S. aureus*, particularly resistant strains, commonly became hospitalized and used steroid therapy [24, 28]. Early-life *S. aureus* colonized skin had been associated with early AD in infancy by stimulating Th2/Th17-type inflammations [29].

The present study found that there was a statistically significant higher growth of all staphylococcal species and specifically S. hominis *in lesion than non-lesion skin among AD cases. Similarly, many studies had discovered a much higher bacterial load on lesion compared to non-lesion skin* [30-32]. When *S. hominis* was applied topically to AD, the absolute abundance of *S.*

aureus was significantly reduced. This is due to production of bacteriocins by *S. hominis* that work against *S. aureus* [33].

The current study lastly concluded that there was a significant association between severity of AD and genotyping of staphylococci. Severe AD was found among cases colonized by *S. aureus*. There was a statistically significant higher mean SCORAD index among cases with *S. aureus*, followed with *S. epidermidis*. In the same line, colonization of the lesion, normal skin and the nose of AD cases by *S. aureus* is correlated to greater serum IgE, recurrent flares and more severe AD [34-36]. Moreover, the density of *S. aureus* on affected AD skin had been linked to increased SCORAD index [37]. This is due to production of pro-inflammatory cytokines and toxins by *S. aureus* that impact keratinocytes and other cells in AD skin. The elimination of *S. aureus* from infected skin by the topical antibiotics is, therefore, one strategy used to treat AD [38].

CONCLUSION

Colonization with staphylococci were relatively exclusive to patients with AD. Decreased diversity of the skin microflora and increased frequency of *S. aureus* and *S. hominis* were observed in AD skin. These findings may be significant for understanding pathophysiology and severity of AD and to outline new management and treatment guidelines using antimicrobial therapy.

Authors' contributions:

N.M.M. performed the practical work and analyzed the data in microbiology lab. All authors have contributed in the design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Availability of data: All data are included in the manuscript and any other data are available upon reasonable request.

Conflict of interest: This study has not been published before and is not under consideration in any other reviewed media. All authors report no conflict of interest relevant to this work.

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