Staphylococcus Aureus Colonization in Atopic Dermatitis Patients Attending Zagazig University Hospitals

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ABSTRACT

Background: Atopic dermatitis (AD) is a very common persistent skin disorder at which skin colonization by bacteria increases. *Staphylococcus aureus* can be found on the skin as a human commensal or as a causal agent in a variety of skin and soft tissue infections.

Objective: This study focused on detecting *staph aureus* colonization on AD lesions and if it influenced the severity of the disease.

Patients and Methods: Swabs from skin lesions of 108 atopic dermatitis patients were collected and subjected for bacterial isolation, identification, and antibiotic susceptibility testing.

Results: Among 108 AD patients, Fifty percent of patients had mild AD disease, 37% had moderate disease, and 13% had severe disease. *Staph aureus* colonization in AD patients was 61.1%. There were no significant differences between positive and negative *staph aureus* culture groups regarding age (P-value 0.57), sex (P-value 0.38), and the most prominent lesion location (P-value 0.08). There was no significant difference in the severity of AD between positive and negative *staph aureus* culture groups (P-value 0.09). Methicillin-resistant *Staph Aureus* was detected phenotypically by cefoxitin (30 µg) disc in 84.8% of isolates.

Conclusion: *Staphylococcus aureus* colonization was detected with a high percentage among atopic dermatitis patients with an extremely higher incidence in severe forms of atopic dermatitis than mild forms. However, this couldn't be proved statistically.

Keywords: Atopic dermatitis, Colonization, MRSA, *Staphylococcus aureus.*

INTRODUCTION

Atopic dermatitis (AD) is a very widespread persistent skin disease, which affects individuals having an atopic tendency together with bronchial asthma, allergic rhinitis, and food allergies. Atopic dermatitis patients complain usually about itchy skin, especially at night which is the predominant symptom, dry skin, eczema lesions in flexural areas, and recurrent skin infections **(1).**

The pathophysiology of atopic dermatitis (AD) is complicated, including elements of barrier malfunction, changes in cell-mediated immune responses, and environmental variables. During AD flares, skin colonization by bacteria increases with increased density on acutely inflamed AD lesions more than 1000-fold higher than on non-lesional AD skin. The improvement of atopic dermatitis clinically is related to the reduction in bacterial colonization using antibiotics and antiseptics **(2).**

Staphylococcus aureus is gram-positive bacteria that can be found on the skin as a human commensal or as a causal agent in a variety of skin and soft tissue infections. *Staph aureus* can be found in the nasal cavity, skin, perineum, and pharynx in healthy people, with the anterior nares being the most prevalent location of carriage **(3)**.

The carriage rate of *staph aureus* on the skin varies from five to thirty percent, relying on the body site or if the person is a persistent nasal carrier **(4)**.

This study was designed to investigate whether *staph aureus* could be a skin colonizer that influences severity in atopic dermatitis patients attending Zagazig University Outpatient clinics.

PATIENTS AND METHODS

This cross-sectional study was conducted in the Microbiology and Immunology Department, and Dermatology, Venereology, and Andrology Department, Faculty of Medicine, Zagazig University, Egypt. One hundred and eight patients complaining of atopic dermatitis lesions were recruited from the Dermatology outpatient clinic in Zagazig University Hospitals without any limitations for age, or sex during the period from December 2019 to June 2021. Patients with a medical history of topical antibiotic application, or suffering from another skin disease than atopic dermatitis were excluded.

Personal history was acquired from all included patients. The severity of AD was assessed with the SCORAD index to mild, moderate, and severe according to the extent of the lesion, swelling, redness, crusting, skin thickening, scratch marks, dryness, itching, and sleeplessness.

Samples were collected from open or inflamed skin lesions using sterile cotton swabs. Bacterial isolation was done on nutrient, blood, mannitol salt agar, also on MacConkey's and sabouraud dextrose agar to exclude infection by other organisms. *Staph aureus* identification was confirmed by colonial morphology, gram-stained films, and conventional biochemical reactions.

The disc diffusion method was used to test the antimicrobial susceptibility using /the following antibiotic discs: penicillin (10 units), cotrimoxazole (1.25/23.75 µg), clindamycin (2 µg), azithromycin (15 µg), doxycycline (30 µg), linezolid (30 µg),

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levofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), fusidic acid (10 µg). Screening for methicillin resistance was done by cefoxitin disc (30 µg). The diameters of the inhibition zones were interpreted according to zone diameter interpretative standards CLSI 2020 [5]. Minimal Inhibitory Concentration (MIC) for vancomycin was detected by tube dilution method according to CLSI 2020, (≤ 2µg/ml) for susceptible, (4-8 µg/ml) for intermediate, and (≥ 16µg/ml) for resistance [5].

Ethical Consideration:

The study was approved by the Local Ethical Committee of Zagazig University. Written consent was obtained from every patient prior to the procedures. This study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:

Data were analysed using statistical packages (EPI-info Version 6.04 and SPSS Version 20 inc. Chicago, USA). Mann-Whitney test was used to compare quantitative non-parametric data. The Chi-square test (χ^2) was used to compare proportions. A P-value <0.05 was considered to be statistically significant at a 95% confidence interval.

RESULTS

Among 108 patients included in our study, the age of the studied patients ranged from 4 to 33 years (10.2 ± 6.54), male to female ratio was 52/56. Fifty percent of patients had mild AD disease, 37% had moderate disease, and 13% had severe disease. The most prominent lesion was in the leg and thigh (37%) followed by the arm and forearm (31.5%) then the abdomen (19.4%) and the least common area of prominent lesion was the neck (12%). The prevalence of *staph aureus* colonization was 61.1%, 66 patients were colonized by *staph aureus* in their skin lesions **(Figure 1)**.

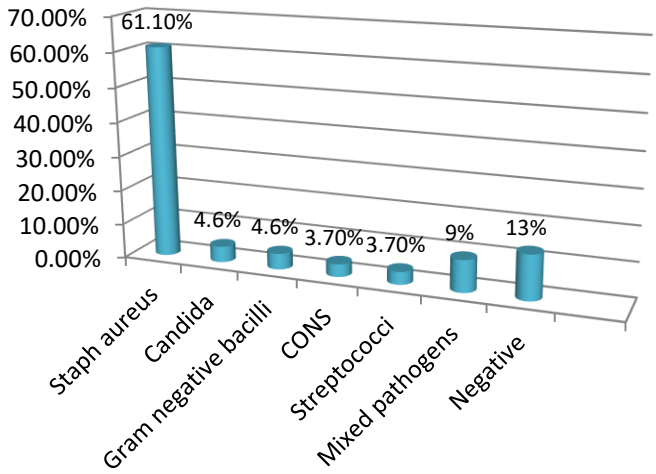

Figure (1): Distribution of isolated pathogens among studied patients with AD.

When we studied the relation between *staph aureus* colonization and age, sex, and the most prominent lesion location, we found no significant differences between positive and negative *staph aureus* culture groups. Regarding the severity of the disease, *staph aureus* colonization was detected with a higher percentage among patients with severe atopic dermatitis than those with mild lesions 85.7% versus 53.7%; however, no statistically significant difference was found **(Table 1)**.

Table (1): Relation between staph aureus colonization and general characteristics of the studied patients

	Positive *staph aureus* N=66		**Negative *staph aureus* N=42**		**X^2 MW test#**	**P Value**
	N	**%**	**N**	**%**		
Age						
Range	4 - 32		4 – 33			
Median	8		8		0.59#	0.57
mean± SD	9.89 ± 5.6		10.76 ± 7.9			
Sex						
Male	34	51.5	18	42.9	0.77	0.38
Female	32	48.5	24	57.1		
SCORAD						
Mild (n=54)	29	53.7	25	46.3	4.85	0.09
Moderate (n=40)	25	62.5	15	37.5		
Severe (n=14)	12	85.7	2	14.3		
The most prominent lesion location						
Leg and Thigh	28	42.4	12	28.6		
Arm and Forearm	15	22.7	19	45.2	6.83	0.08
Neck	10	15.2	3	7.1		
Abdomen	13	19.7	8	19.0		

Antibiotic susceptibility testing to our isolates revealed 100% resistance to penicillin, 98.5% were resistant to fusidic acid, and 60.6% were resistant to levofloxacin while all our isolates were susceptible to linezolid, and 93.9% were susceptible to azithromycin. Minimal Inhibitory Concentration (MIC) for vancomycin revealed that 60 *staph aureus* isolates were susceptible to vancomycin (90.9%). When we studied methicillin-resistance phenotypically using cefoxitin disc 10 *staph aureus* isolates were susceptible and 56 were resistant, 84.8% were phenotypically identified as MRSA **(Table 2)**.

Table (2): Antibiotic susceptibility among staph aureus isolates

	***Staph aureus* positive cases N=66**	
	N	**%**
Penicillin: Resistant	66	100
Cefoxitin:		
Sensitive	10	15.2
Resistant	56	84.8
Gentamicin:		
Sensitive	34	51.5
Resistant	32	48.5
Chloramphenicol:		
Sensitive	58	87.9
Resistant	2	3.0
Intermediate	6	9.1
Levofloxacin:		
Sensitive	21	31.8
Resistant	40	60.6
Intermediate	5	7.6
Azithromycin:		
Sensitive	62	93.9
Resistant	3	4.5
Intermediate	1	1.5
Sulphamethoxazole-trimethoprim:	57	86.4
Sensitive	9	13.6
Intermediate		
Doxycycline:		
Sensitive	29	43.9
Resistant	31	47.0
Intermediate	6	9.1
Clindamycin:		
Sensitive	45	68.2
Resistant	6	9.1
Intermediate	15	22.7
Linezolid: Sensitive	66	100
Fusidic acid:		
Sensitive	1	1.5
Resistant	65	98.5
Vancomycin:		
Sensitive	60	90.9
Resistant	6	9.1

DISCUSSION

Atopic dermatitis is a widespread chronic skin condition that impacts both patients' and their families' quality of life. It was reported that atopic dermatitis patients colonized with bacteria on their skin at a higher percentage than healthy people and this was known to be correlated with disease severity, regardless of the site of carriage [(4)].

The age of the studied patients ranged from 4 to 33 years with a mean±SD of (10.2 ± 6.54), and the male to female ratio was 52/56. This was in agreement with **Tanei**[(6)] and **Raznatovic *et al.***[(7)] who declared that atopic eczema rashes are common in all ages and the onset is usually common in the first few years of life and also is common in adolescents and adults**.** Moreover, **Chu *et al.*** [(8)] reported no difference in the male-to-female ratio in their studied group. Also, **Atar-Snir** [(9)] reported that gender had no effect on the frequency of atopic dermatitis up to the age of ten years, but from ten to eighteen years, atopic dermatitis became more common in females (16.3 percent for females versus 8.3 percent for males), which might suggest a role for gender-specific pubertal variables. Regarding the severity of atopic dermatitis, 50% of our patients had mild disease, 37% had moderate disease and 13% had severe disease. This comes in accordance with **Celakovska and Bukac** [(10)] who reported about 9% out of the total 283 patients examined, had severe AD.

In this study, we had chosen the site for swabbing with prominent lesion and the most common sites of swabbing in our studied patients were leg and thigh (37%) followed by arm and forearm (31.5%) then abdomen (19.4%) and the least common site of swabbing was the neck (12%).

However, **Chu *et al.***[(8)] found that the head and neck area was implicated in 35% of the patients, then the upper extremities (33%), the lower extremities (25%), and finally the trunk (16%). This could be explained by the combination with other possible allergic diseases as they did not exclude other allergic diseases in that study.

The present study revealed that atopic dermatitis skin lesions seem to be specifically prone to *staph aureus* colonization. Especially, out of 108 AD patients, 66 (61.1%) were colonized by *staph aureus*. This comes in accordance with **Di Domenico *et al.*** [(11)] who found 54.3% (44/81) of AD patients were colonized by *staph aureus*. In addition, **Pascolini *et al.*** [(12)] supported our findings and reported 66 out of 117 (57%) patients harbouring *staph aureus* on their skin lesions.

Less than our results, a study in Tehran carried out by **Rezaei *et al.*** [(13)] found in 38 AD patients only 18 patients were colonized by *staph aureus* (47%). This could be attributed to different patient's age group included in that study. Also, **Kennedy *et al.*** [(14)] had found that *staphylococci* were significantly less abundant in one-year-old affected infants in a study that included only 10 AD patients.

In the present study, we detected pathogens other than *staph aureus* colonizing the skin of AD patients; 4.6% of cultures showed *candida* growth, 3.7% were *streptococci*, 3.7% were coagulase-negative staphylococci (*CONS*), 4.6% were lactose fermenting gram-negative bacilli, and 9.3% were mixed pathogens while 13% of cultures gave no growth.

This finding goes with the fact declared by **Buda and Miedzobrodzki**[15] who stated that there were changes in the microflora of the skin in atopic dermatitis with an increase in the incidence of the presence of *CONS, staph aureus, klebsiella,* and *streptococci*.

When we studied the differences between positive and negative *staph aureus* culture groups regarding age, sex, and atopic dermatitis severity, we found no statistically significant differences between both groups. Despite 85.7% of our severe cases were positive for *staph aureus* versus only 53.7 % of mild cases, but unfortunately, this did not reach the significant level. This comes in agreement with **Hill *et al.*** [16] who found that there was no significant association between *staph aureus* colonization and either age, or sex. Also, **Di Domenico *et al.*** [11] who found that the prevalence of *staph aureus* among severe patients of AD was 76.9%. Moreover, **Pascolini *et al.*** [12] found that *staph aureus* colonization was related to the severe clinical expression of AD.

The current study illustrated that our isolates were all resistant to penicillin (100%), 98.5% were resistant to fusidic acid, 60.6% were resistant to levofloxacin, 47% were resistant to doxycycline, 100% were susceptible to linezolid, 93.9% were susceptible to azithromycin, 90.9% were susceptible to vancomycin, 86.4% were susceptible to sulphamethoxazole trimethoprim, 68.2% were susceptible to clindamycin, and 51.5% were susceptible to gentamicin. Concordant with our results, **Jung *et al.*** [17] reported that benzylpenicillin was the lowest susceptible antibiotic followed by fusidic acid. Meanwhile, all their *staph aureus* isolates were susceptible to linezolid, sulphamethoxazole- trimethoprim, and vancomycin. In addition, **Alenizi**[18] reported in a Saudi Arabian study that 89.75% of the tested strains were resistant to penicillin while 74.6% were susceptible to gentamicin and 94.9% were susceptible to vancomycin. Also, **Blazewicz *et al.*** [19] found that the antimicrobial resistance patterns of *staph aureus* in AD patients were 0% for vancomycin, 2% for linezolid, and 58.5% for ampicillin.

In the same concern, **Ali *et al.*** [20] reported in a study conducted in Mansoura University, Egypt that 100% of isolated *staph aureus* colonizing AD patients were susceptible to vancomycin, 100% were resistant to ampicillin. Moreover, **Buda and Miedzobrodzki**[15] reported high resistance to fusidic acid and advised limiting its use. Also, due to the excellent response to macrolides, they recommend 2nd generation macrolides as azithromycin and clarithromycin in widespread or severe secondary staphylococcal infection.

However, a Brazilian study conducted by **Bessa *et al.*** [21] found only 4.4% of their *staphylococcal* isolates from atopic eczema patients were resistant to fusidic acid and they attributed this low percentage in that study to infrequent usage of topical fusidic acid because these products are considerably high-cost and scarce in the Brazilian public health system.

The present study was concerned about identifying methicillin-resistant *staph aureus* among AD patients and reported that 56 isolates out of 66 were resistant to cefoxitin (30 µg); 84.8% were phenotypically identified as MRSA. That percentage was a little bit more than **Lo *et al.*** [22]**,** who found 12 MRSA isolates among 20 AD patients (60%).

The difference in the prevalence of MRSA could be explained by a longitudinal study that was performed by **Chaptini *et al.*** [23] during the period from 1999 to 2014 and declared that the prevalence of MRSA in AD patients had significantly increased among the previous 15 years and between 2011 and 2014, patients were almost 24 times more prone to test positive for MRSA.

CONCLUSIONS

Staphylococcus aureus colonization was detected with a high percentage among atopic dermatitis patients with an extremely higher incidence in severe forms of atopic dermatitis than mild forms.

RECOMMENDATIONS

We recommend adding *antistaphylococcal* antibiotics to the treatment regimen of AD patients according to results of culture and antibiotic sensitivity testing with follow up for remission and frequency of relapses among those patients.

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