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ORIGINAL ARTICLE

Skin and Nasal Colonization with Methicillin Resistant Staphylococcus Aureus in Children with Atopic Dermatitis

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ABSTRACT

Background: *Atopic dermatitis (AD) is a chronic inflammatory skin disease with multifactorial etiologies, Staphylococcus aureus (S.aureus) and methicillin-resistant S.aureus (MRSA) that naturally colonize skin and nose are prevalent among children with AD.* ***Objectives:*** *was to determine the prevalence of S.aureus and MRSA colonization of skin lesions and nose of AD children.* ***Methodology:*** *40 children diagnosed as AD from Dermatology Clinic of Najran Armed Forces Hospital, Saudi Arabia, were included in the study; separate swabs from skin lesions & nose of each AD patient were tested for S.aureus and MRSA colonization using the conventional culture based Vitek 2 system and the molecular BD Max MRSA XT assay.* ***Results:*** *Using the conventional Vitek 2 system, the prevalence of skin and nasal colonization with S.aureus in AD patients were 25% and 30% respectively while skin and nasal colonization with MRSA were 7.5% and 7.5% respectively, the BD Max MRSA XT assay identified correctly S.aureus with overall 96 % sensitivity, 100 % specificity and 98 % diagnostic accuracy and identified 100 % of MRSA strains.* ***Conclusion:*** *The increase in prevalence of skin and nasal colonization with S.aureus and MRSA among AD children raises the concern about importance of the accurate and rapid molecular diagnostic techniques for preventing the potential risk of MRSA transmission*

INTRODUCTION

Atopic dermatitis (AD) is a wide spread chronic inflammatory skin disease, affecting 15 to 20% of children and 2 to 10 % of adults [1], The pathogenesis of AD is multifactorial including genetic immunological, environmental factors and skin barrier defects [2].

Atopic dermatitis (AD), also known as atopic eczema results in itchy, red, swollen, and cracked skin, clear fluid may come from the affected areas, which often thickens over time, approximately 90% of patients develop disease within the first 5 years of life, but it can occur at any age and commonly resolves by adulthood; however, 10% to 30% of patients will continue to have symptoms of disease, in children under one year of age, much of the body may be affected but as children get older, the areas on the insides of the knees and elbows are most commonly affected [3].

There are several scoring systems for evaluating and monitoring AD: "Severity Scoring Index of Atopic Dermatitis" (SCORAD), "Eczema Area and Severity Index" (EASI), and "Six Area Six Sign Atopic Dermatitis Score" (SASSAD), in addition, the disease can vary from mild to severe, and requires effective therapeutic strategies and support [4].

Staphylococcus aureus (*S.aureus*) which is a gram-positive bacterium that naturally colonizes the nose and surface of the skin is highly prevalent among patients with AD, and may contribute to the onset or aggravation of AD lesions[5], Methicillin-resistant *S. aureus* (MRSA) are strains of *S.aureus* that have acquired mechanisms of resistance against β-lactam antibiotics though production of an altered penicillin-binding protein known as PBP2a, a product of the mecA gene. MRSA is an important cause of community-associated and healthcare-associated infections [6].

Overall carrier rates of *S.aureus* in healthy humans range from 20 to 50% [7], *S.aureus* colonization has been implicated in the pathogenesis or persistence of many skin diseases through decreased level of barrier lipids, increased local proteases, reduced antimicrobial peptides, such as beta-defensin and production of superantigens, which disrupt the skin barrier by increasing proinflammatory cytokines production by keratinocytes [8].

Individuals with AD have a greater concentration of *S. aureus* colonization of their skin [9], and their Eczematous skin lesions are known to be a source of *S.aureus* transmission, and might be a favorable reservoir for MRSA, strong associations exist between recurrent skin infections, disease severity and *S. aureus* colonization [10]; therefore, early identification of *S.aureus* and MRSA colonization is important for managing patients with AD and preventing the spread of MRSA

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Conventional culture-based identification of MRSA takes an average time to result about 48 hours but different PCR-based molecular diagnostic technologies, including end-point PCR, real-time PCR and multiplex PCR, have been successfully used for rapid detection of *S.aureus* and MRSA for surveillance purposes and testing of clinical samples [1]

One of these molecular technologies is BD Max MRSA XT assay which is an automated qualitative multiplex PCR assay used for the rapid detection of S. *aureus* and MRSA within 3 hrs through the amplification of bacterial DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA [11].

The aim of this study was to determine the prevalence of *S.aureus* and MRSA colonization of skin lesions and nose of AD children using conventional method and a rapid molecular method

METHODOLOGY

This study was carried in the Dermatology Outpatients Clinic of Najran Armed Forces Hospital, Najran, Saudi Arabia, in the period from July 2019 to August 2020 on 40 children diagnosed as AD, 10 children without dermatological symptoms or signs were included as controls, informed consents were taken, detailed history, full general and dermatological examinations were done for all the participants, Severity Scoring Index of Atopic Dermatitis (SCORAD) scoring system was used to evaluate the severity of AD, the study was approved by the ethical committee of Najran Armed Forces Hospital.

***Inclusion criteria*:** AD children aged upto 18 years.

Exclusion criteria:

- Chronic skin diseases as psoriasis, Seborrheic Dermatitis and Contact Dermatitis.
- Clinical evidence of 2ry bacterial infection of skin lesions

Identification of S. aureus:

A total of 100 sterile amies gel transport swabs were collected, 2 swabs from each AD patient (Separate swabs for skin lesions & nose) and 2 swabs from each control (Separate swabs for skin of cubital fossa or axilla & nose), the swabs were transported within 2 hours to the Microbiology Laboratory, the swabs were firstly placed in sample buffer tubes of the BD Max MRSA XT assay then used to inoculate the culture media including 5% sheep blood agar and mannitol salt agar (*Oxoid, Victoria, Australia*), incubated aerobically at 37°C for 24 h, Colony morphology, gram stain, catalase test and coagulase test using the Microgen™ Staph latex agglutination test (*Microgen Bioproducts Ltd, UK*) were used to identify *S.aureus* [23].

Vitek 2 system identification of MRSA:

Using Vitek 2 automated identification system (*bioMerieux, Marcy l'E'toile, France*), pure subcultures were suspended in sterile saline to achieve a turbidity of 0.5 McFarland standards and used to inoculate the colorimetric gram positive identification cards (GP ID) containing the biochemical substrates, automated susceptibility testing was performed with the gram positive susceptibility cards (AST P580) according to the manufacturer's instructions, S.*aureus* is interpreted as MRSA if oxacillin resistant (MIC > 0.5 µg/mL) or cefoxitin screen positive (MIC > 6 µg/ml), *S. aureus* ATCC 29213 strain was used as quality control[12, 28].

The BD Max MRSA XT assay system:

The swabs were placed in sample buffer tubes then the tubes vortexed and placed into the BD Max System (*BD Diagnostics, Sparks, MD, USA*) according to each manufacturer's instructions and the automated procedures occur: the bacterial cells were lysed, DNA was extracted on magnetic beads and concentrated, and then eluted DNA was added to PCR reagents which contain the MRSA-specific primers used to amplify the DNA targets which are staphylococcal cassette chromosome mec (SCCmec) right-extremity junction (MREJ), and methicillin resistance (mecA/mecC), detection of MREJ and mecA/mecC genes was required for the result "MRSA positive", while detection of MREJ without mecA/mecC was interpreted as "*S.aureus* positive & MRSA negative", The BD Max MRSA XT assay provided rapid results within 3 hr [11, 29].

Statistical analysis:

Statistical analysis was carried out using SPSS, version 16, the sensitivity, specificity and diagnostic accuracy were calculated considering the culture based method as the gold standard reference test, values were presented as number and percentages, for all tests P value < 0.05 was considered significant.

RESULTS

The study included 40 AD patients aged upto18 years; 57.5% males and 42.5 % females, , according to SCORAD score; 47.5%, 40%, 12.5% had mild, moderate or sever AD manifestations respectively, 85% manifested at age < 6 years and 40% had family history of atopic disease (table 1),

There were no significant differences of characteristics data between the AD patients and the controls except for family history of atopic diseases, previous hospitalization within the previous year and previous steroids or antihistaminic intake (table 1)

Table 1: Characteristics of AD Patients and Controls

Characteristics	AD Patients (n=40) n (%)	Controls (n=10) n(%)	P value
Age (y): Infant (< 1 y)	11(27.5)	1(10)	
Preschool(1-6 y)	16(40)	5(50)	NS
School (7-12 y)	10(25)	2(20)	
Adolescent (13-18 y)	3(7.5)	2(20)	
Sex: Male	23 (57.5)	5(50)	NS
Female	17 (42.5)	5(50)	
Age of onset: < 6 years	34(85)	-	-
> 6 years	6(15)	-	-
SCORAD score: Mild	19(47.5)	-	-
Moderate	16(40)	-	-
Sever	5(12.5)	-	-
Family history of atopy	16(40)	1(10)	0.02
Risk factors:			
Previous hospitalization	3(7.5)	0(0)	0.009
Previous antibiotics	22(55)	5(50)	NS
Steroids or antihistaminic	31(77.5)	0(0)	0.001

Abbreviations: AD; Atopic dermatitis, SCORAD; SCORing Atopic Dermatitis NS: Not significant
Data are expressed as number (percentage); P value > 0.05 is not significant

By using culture and vitek 2 system identification; for the AD patients, 25% (10/40) of skin swabs & 30% (12/40) of nasal swabs were S.*aureus* positive, 7.5% (3/40) of both skin and nasal swabs were MRSA positive and for the healthy controls, only 1/10 (10%) and 2/10 (20%) of skin & nasal swabs respectively were S.*aureus* positive with no MRSA isolated (table 2).

Table2: Prevalence of Skin and Nasal colonization with *S.aureus* and MRSA in AD patients& Controls

	AD Patients (n=40)		Controls (n=10)	
	Skin n (%)	**Nose n (%)**	**Skin n (%)**	**Nose n (%)**
S.aureus	10(25)	12(30)	1(10)	2(20)
MRSA	3(7.5)	3(7.5)	0(0)	0(0)

Abbreviations: AD; Atopic dermatitis, *S.aureus; Staphylococcus aureus* ,
MRSA; Methicillin resistant *staphylococcus aureus*, Data are expressed as number (percentage)

As regards the relationship between the AD severity and rate for MRSA colonization, it was found that 66.6 % (2/3) of MRSA colonized patients (both in the skin and nose) were with severe AD degree and 33.3% (1/3) were with moderate AD degree

Antibiotic susceptibility patterns of MRSA isolated from skin & nose of AD patients were not reported in our study due to the small number of isolated MRSA (only 3 isolates)

Compared with the conventional culture based and vitek 2 system method, the BD Max MRSA XT assay within 3 hours identified correctly 9/10 of *S.aureus* isolates from skin swabs & 12/12 of *S.aureus* isolates from nasal swabs of patients & the 3 *S.aureus* isolates from skin and nose of controls with overall 96% sensitivity, 100% specificity, 100% positive predictive value, 96.15% negative predictive value and 98% diagnostic accuracy, also this assay identified correctly the 3 MRSA strains isolated from skin and nasal swabs with 100% sensitivity, specificity and diagnostic accuracy (figure 1)

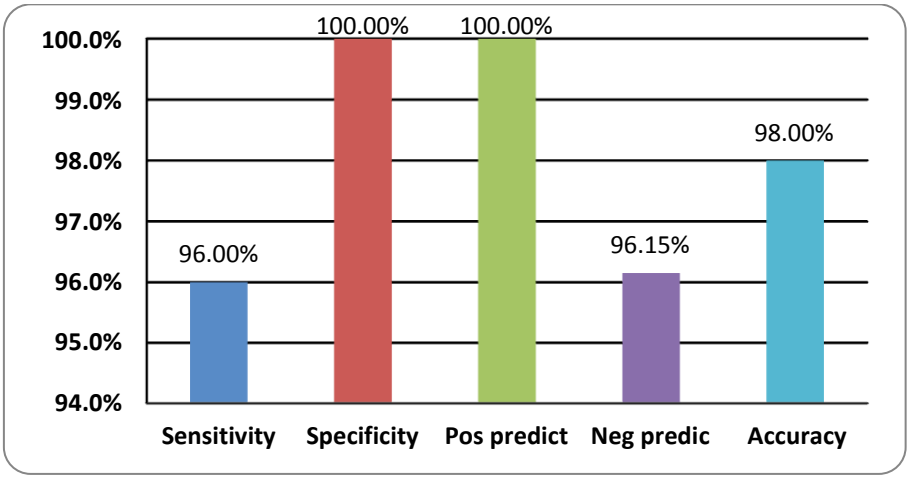

Fig 1: Performance data of the BD Max MRSA XT assay

DISCUSSION

Atopic dermatitis is a wide spread chronic inflammatory skin disease with multifactorial etiologies including genetic, immunological and environmental factors [2], although many body sites are frequently colonized with *S.aureus* for varying time periods, the main colonization site of *S.aureus* is the anterior nares, among nasal *S.aureus*, approximately half also carry the organism on their skin, patients with skin disease like AD are at increased risk for skin and nasal colonization with *S.aureus* and MRSA compared with the general population [12,13], However, there is a few data regarding the pattern and prevalence of skin and nasal colonization with *S.aureus* in children with AD, especially with reference to MRSA and also whether this has a significant association with the severity of the disease [14]

As regards the relationship between the AD severity and the rate for MRSA colonization, we found in our study that 66.6 % of MRSA colonized patients (both in the skin and nose) were with sever AD degree and 33.3 % were with moderate degree and none with mild degree, these results were similar to another study that found that 50% of the patients with severe AD acquired MRSA colonization[15], and another study showed that 56.4% of MRSA cases were moderate grade and 10.9% of cases were severe grade[14], but the study by Dae et al. [16] reported a different data that 67.9% of MRSA cases were in the mild AD degree.

In our study, we found that the prevalence of skin and nasal colonization with *S.aureus* in AD patients were 25% and 30% respectively while skin and nasal colonization with MRSA were 7.5% and 7.5% respectively, our results for the colonization rates of *S.aureus* and MRSA were different from that reported by Heba et al. [27] in Mansoura, Egypt who found that *S.aureus* was present in 90% of AD patients and 48.3% were colonized in both lesional skin and nose and 46.7% were colonized with MRSA.

Also our results for the colonization rates of *S.aureus* and MRSA were lower than those reported in other countries around the world such as a recent study that found that *S.aureus* colonization rate was 75.7% in pediatric AD skin lesions, with MRSA accounting for 18.4% of *S.aureus* isolates[17] and the study reported by *Suh et al*.[18] who observed that *S.aureus* colonization occurred in 80% of AD cases and 16% of them were MRSA and also lower than the result performed by Eliane et al.[15] that reported high prevalence of AD patients colonized with *S.aureus* (82.9%) and also a high prevalence of MRSA colonization (22.2%), also In New Zealand, 75% of AD pediatric patients were colonized with *S.aureus* but only 2% with MRSA[19] and in Brazil, Petry et al. [20] found 68.8% of AD patients colonized by *S.aureus* isolates but did not detect MRSA in the nasal sites, but in the opposite side, Pascolini et al.[22] in Italy identified that only 4.5% of atopic children were colonized by *S.aureus*.

A recent systematic review pooled 95 studies and concluded that 70% of AD lesional skin of AD patients were colonized with *S.aureus*, compared to 39% of non lesional skin, and that 62% have nasal colonization [21].

Several studies performed on health care workers (HCWs) as that performed in Egypt by Alaa Eldeen et al.[23] to detect the prevalence of nasal MRSA among medical residents of Al-Azhar university hospital, Damietta branch and found that the carriage rate of *S.aureus* and MRSA were 22.6 % and 17.8% respectively, and the study published by Abdel Rahman et al.[24] who reported 20% nasal MRSA carriage among HCWs in ICUs at Ain Shams university hospital and those reported in Nigeria by Fadeyi et al.[25] and India by Vinodhkumaradithyaa et al.[26] these authors reported an overall nasal carriage of *S.aureus* among HCWs were 85%, 52.5% and 13% respectively.

These differences in the prevalence of *S.aureus* and MRSA in AD patients reported in different studies might be due to variable methods of sampling and diagnosis,

intermittent colonization, sample size and the hygienic status of patients.

In our study, the BD Max MRSA XT assay identified correctly *S.aureus* strains isolated from skin and nasal swabs with overall 96% sensitivity, 100% specificity and 98% diagnostic accuracy, also identified correctly MRSA strains isolated from skin and nasal swabs with 100% sensitivity, specificity and diagnostic accuracy, our results were nearby the results of a study that validated two PCR assays performed on the BD Max system showed that the identification of *S. aureus* had sensitivity and specificity of 96.4% and 93.6%, respectively [29], and another study evaluating the BD Max MRSA XT assay that identified *S. aureus* with a sensitivity, specificity and agreement 87.5%, 97.1% and 94.0 %respectively [11]

Previous two studies evaluating the use of different swab types as liquid Stuart, liquid amies, amies gel, and ESwab with the BD Max assay showed statistically insignificant variable sensitivity of the different swab types [29, 30]

the BD Max MRSA XT assay in addition to its high performance data, the total turnaround time to detect *S.aureus* and MRSA is around 3 hours and varies depending on the number of samples processed, this accurate and rapid detection method is important not only to rapid choice the appropriate antibiotic therapy but also to control the spread of MRSA , in addition, this assay detects mec A/mec C genes, so can identify MRSA strains with the newly discovered mec C gene that cannot be detected by assays that do not detect mec C, these false-negative MRSA results can lead to the uncontrolled transmission of undetected MRSA [30]

The limitation of our study is the relatively small sample size and that the study was carried out in outpatient clinic with most of the AD patient were with mild or moderate SCORAD score; therefore, further larger studies are needed to clarify the actual prevalence of *S.aureus* and MRSA colonization of skin lesions and nose of AD children

CONCLUSION

The current study highlights the increase in prevalence of skin and nasal colonization with *S.aureus* and MRSA among children with AD and raises the concern about the clinical importance of the accurate and rapid molecular diagnostic techniques like the BD Max MRSA XT assay for rapid managing patients with AD and preventing the potential risk of transmission of MRSA

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- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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