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Staphylococcal cassette chromosome *mec* (SCC*mec*) typing and Gentamicin resistance in methicillin-resistant *Staphylococcus aureus* among children with atopic dermatitis in Egypt

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Abstract

Children with atopic dermatitis (AD) have a higher prevalence of *Staphylococcus aureus* colonization. Gentamicin is an important topical antibiotic that is used in the treatment of AD lesions. This study aimed to evaluate the prevalence of gentamicin resistance, Panton-Valentine Leukocidin (PVL), and Staphylococcal cassette chromosome *mec* (SCC*mec*) type in community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), which colonizes the skin of the AD children. Gentamicin resistance in CA-MRSA isolates was detected using the E-test. Polymerase Chain Reaction (PCR) was used to test the genes for aminoglycoside modifying enzymes (AMEs), PVL, and SCC*mec* types of CA-MRSA isolates. A total of 29 isolates of CA-MRSA were obtained from the skin lesions of 100 patients, and a high prevalence of gentamicin resistance (79.3 %) was detected among these isolates. The most predominant AME gene among the gentamicin resistant isolates was *aac(6')-Ie-aph(2')-Ia*. However, the *PVL* gene was detected in 14 (48.3 %) of the isolates. Both gentamicin resistance and PVL were significantly associated with a high SCORAD score. SCC*mec* type V (31 %) was most common among the CA-MRSA isolates. This study revealed a high prevalence of gentamicin resistance among MRSA that colonises the skin of AD, with the *aac(6')-Ie-aph(2')-Ia* aminoglycoside-resistant determinant predominating. The most frequently observed cassette was SCC*mec* type V with the emergence of SCC*mec* type III among the MRSA colonizer strains.

Keywords: Atopic dermatitis, Gentamicin resistance, Methicillin-resistant *Staphylococcus aureus*, Panton Valentine Leukocidin, Staphylococcal cassette chromosome *mec*

1. Introduction

Atopic dermatitis (AD) is the most common inflammatory skin disorder that is recorded in children (Das *et al*., 2022). *Staphylococcus aureus* colonization; especially methicillin-resistant *Staphylococcus aureus* (MRSA) strains is more common among the children with AD than in the healthy children (Lo *et al*., 2010). In the skin, *Staphylococcus aureus* colonization may play a major role in the exacerbation of eczema through the secretion of toxins, mainly Panton-Valentine Leukocidin (PVL), and super antigens (Cavalcante *et al*., 2015; Chen *et al*., 2022). According to the previous study conducted by Hiramatsu *et al*., (2001), the *mecA* gene is recorded in the staphylococcal cassette chromosome *mec* (SCC*mec*). Binary combinations of the *mec* complex and the *ccr* allotype can identify several SCC*mec* "types" (Chen *et al*., 2009). MRSA that is relevant to healthcare settings frequently include Types I, II, and III, whereas type IV is predominantly identified in the community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) (Kong *et al*., 2016). Secondary infections in AD patients are very common and are challenging to treat. Gentamicin, fusidic acid, and mupirocin are the most commonly used topical antibiotics to treat secondary bacterial infections (Williamson *et al*., 2017). Inactivation of aminoglycoside antibiotics by the aminoglycoside-modifying enzymes (AMEs) is the main mechanism of resistance to these drugs (Ramirez and Tolmasky, 2010). Fears that the skin of AD patients acts as a reservoir for this *Staphylococcus aureus* bacterium are intensified by the increased prevalence of CA-MRSA in skin and in the soft tissue infections (Chung *et al*., 2008). The objective of this study were to identify the SCC*mec* types, *AMEs*, *PVL* genes among isolates of CA-MRSA that colonises the AD pediatric skin lesions, and to interrelate these variables to the disease extent.

2. Materials and methods

2.1. Samples collection and isolation of *Staphylococcus aureus*

About 100 AD-diagnosed children who attended to the dermatology outpatient clinic at Mansoura University Hospital (M.U.H.) between March 2020 and December 2021, were the subjects of this cross-sectional study. The CA-MRSA infected children had median age of 6 (2-11) with a 1.9:1 male to female ratio. The SCORing Atopic Dermatitis (SCORAD) index was implemented to figure out the extent of AD (Park *et al*., 2016). Exclusion criteria, isolation and identification of the CA-MRSA were carried out as previously described by Ali *et al*., (2019). Briefly, eczematous plaques were sampled using sterile cotton swabs. *Staphylococcus aureus* isolation was conducted on nutrient, blood, and mannitol salt agar media, followed by its identification through colony morphology, Gram-stained smears, release of catalase, free coagulase and DNAse (Forbes *et al*., 2007).

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility of the MRSA isolates was assessed using the disk diffusion panel, according to the CLSI guidelines (Wayne, 2018). The inhibition zone diameter was measured using a calibrated ruler after placing several antibiotics individually on the surface of Mueller-Hinton agar plates followed by incubation at 37°C for 24 h, including erythromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), vancomycin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), linezolid (30 µg), fusidic acid (10 µg), rifampicin (5 µg), tobramycin (10µg), and amikacin (30 µg). Cefoxitin disc (30 g) was used to screen the *Staphylococcus aureus* isolates for methicillin resistance. The gentamicin sensitivity of MRSA isolates was tested using the minimum inhibitory concentration (MIC) Test Strip (Liofilchem, Roseto degli Abruzzi, Italy), according to the CLSI guidelines (Wayne, 2018).

2.3. DNA isolation

The MRSA isolates were sub-cultured overnight at 37 °C on blood agar plates. About 3-5 colonies were picked for genomic DNA extraction utilizing the protocol of i-genomic BYF DNA extraction mini-Kit (Abdulghany and Khairy, 2014).

2.4. Amplification of *mecA*, *PVL* genes and SCC*mec* typing using PCR

All the cefoxitin resistant isolates of *Staphylococcus aureus* were subjected to conventional PCR for amplification of *mecA* and *PVL* genes (Lina *et al*., 1999; Mehrotra *et al*., 2000). Two separate multiplex PCR reactions were performed to screen all the MRSA isolates for *AMEs* genes, and to characterize them according to SCC*mec* types using specific primers for SCC*mec* types III, IV, V (Table 1), in reference to Vakulenko *et al*., (2003); Lu *et al*., (2008). MRSA reference strain (ATCC 25923) obtained from The Naval Medical Research Unit No. 3 (NAMRU-3) was employed as a control. Table (1) lists the primers introduced in this study, the product sizes, and PCR programs of the genes examined.

2.5. Statistical analysis

The PVL and gentamicin resistance of the CA-MRSA isolates were linked to AD severity using the Mann-Whitney U test. A *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1. Identification of MRSA and their resistance to antibiotics

Twenty-nine isolates of CA-MRSA were recovered from the skin lesions of 100 AD children. The MRSA isolates were characterized phenotypically and genotypically. All the CA *Staphylococcus aureus* cefoxitin resistant isolates were found to possess the *mecA* gene. All the CA-MRSA isolates were susceptible to vancomycin and linezolid (100 %), while resistance to erythromycin displayed the highest rate (93.1 %), followed by tetracycline resistance (89.7 %) among the colonizers. Fusidic acid resistance was detected in 72.4 % of the isolates. Fig. (1) demonstrates the antibiotic resistance pattern of CA-MRSA to all the tested antibiotics. The recorded resistance patterns of CA-MRSA isolates' against gentamicin, tobramycin, and amikacin were: 23 (79.3 %); 22 (75.8 %); 17 (58.6 %), respectively. The MIC results for gentamicin ranged between 0.5 and 256 µg/ ml (Fig. 2). The MIC50 and MIC90 values of the gentamicin resistant isolates were 48 µg/ ml and 128 µg/ ml, respectively.

Among CA-MRSA gentamicin resistant isolates, the *aac(6')-Ie-aph(2")-Ia*, *ant(4')-Ia, and aph(3')-IIIa* genes were identified in 19, 17 and 11 isolates; respectively, with the predominance of *ant(4')-Ia* gene in all the CA-MRSA colonizers; moreover, it was additionally detected in 2 tobramycin resistant and 1 amikacin resistant isolates. The three AME genes *(ant(4')-Ia, aac(6')-Ie-aph(2")-Ia*, and *aph(3')-IIIa)* coexisted in 8 of the isolates, while two of these genes coexisted in 10 isolates *(ant(4')-Ia* and *aac(6')-Ie-aph(2")-Ia*, while *aac(6')-Ie-aph(2")-Ia* and *aph(3')-IIIa* existed in 2 isolates, and *ant(4')-Ia, and aph(3')-IIIa* was recorded in a single isolate. Distribution of the *AMEs* genes among the MRSA isolates is summarized in Table (2).

3.2. Screening for *PVL* gene and SCC*mec* typing

The *PVL* gene was detected in 14 isolates (48.3 %) of the CA-MRSA (Fig. 3). About 9 (31 %) isolates harbored SCC*mec* type V, 8 (27.6 %) harbored SCC*mec* type III, 6 (20.7 %) harbored SCC*mec* type IV, and 6 (20.7 %) isolates were non typeable, which means that they did not express any of the screened SCC*mec* types.

3.3. Correlation between *PVL* gene and gentamicin resistance to AD severity

Regarding SCORAD index, the CA-MRSA colonized patients that carry the *PVL* gene had significant high SCORAD index of 27.8 %, compared to the CA-MRSA patients that had no PVL gene (17.7 %) (p =0.016), as demonstrated in Fig. (4).

Table 1. List of primers used in this study to screen the MRSA isolates for *AMEs* genes, and to characterize them according to SCC*mec* types

Gene	Primer sequence (5'-3')	Product size (bp)	PCR program	References
mecA	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	163	5 min. at 94°C, 35 cycles (2 min. at 94°C, 2 min. at 57°C, 1 min. at 72°C); 7 min. at 72°C.	(Mehrotra *et al*., 2000)
aac(6´)-Ie-aph(2)-Ia	CAGAGCCTTGGGAAGATGAAG CCTCGTGTAATTCATGTTCTGGC	348	3 min. at 94°C; 35 cycles (40 sec at 94°C, 40 sec at 55°C, and 40 sec at 72°C); 2 min. at 72°C	(Vakulenko *et al*., 2003)
aph(2˝)-Ib	CTTGGACGCTGAGATATATGAGCAC GTTTGTAGCAATTCAGAAACACCCTT	867		
aph(2´)-Ic	CCACAATGATAATGACTCAGTTCCC CCACAGCTTCCGATAGCAAGAG	444		
aph(2´)-Id	GTGGTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	641		
aph(3´)-IIIa	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATACAGCTCGCG	523		
ant(4´)-Ia	CAAACTGCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT	294		
luk-PV	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	433	30 cycles (30 sec at 94°C, 30 sec at 55°C, and 1 min. at 72°C)	(Lina *et al*., 1999)
Typing for				
***Mec* complex** **-Universal (mA6)** **-Class A** **-Class B** **-Class C (IS2)**	TATACCAAACCCGACAAC GTGCAGATAGTAACGGTGCTTG GGTTTCACTCGGATGTCTGT TGAGGTTATTCAGATATTTCGATGT	1164 (Type II Or 998 (Type III 1592 804	2 min. at 95°C, 30 cycles (30 sec at 95°C, 30 sec at 58°C, 1.5 min. at 72°C); 4 min. at 72°C.	(Lu *et al*., 2008)
***Ccr* complex** ***Ccr* Type 2**	ATCATTACGTCAACAACCGC ACTATAACCTTCTGTGCTTTGC	315		

Table 2. Distribution of the *AMEs* genes and SCC*mec* type among the CA-MRSA isolates

***AME*s genes**			**Type of SCC*mec***				**Resistance**			**Total No.**
ant(4′)-Ia	*aac(6′)-Ie-aph(2″)-Ia*	*aph(3′)-IIIa*	III No.	V No.	IV No.	NT* No.	CN	TOB	AK	
+	+	+	5	2		1	8	7	6	8
+	+	-	2		2	3	7	7	4	7
-	+	-		1		1	2	2	2	2
+	-	-			3	1	1	3	2	4
+	-	+		1			1	1	1	1
-	+	+	1	1			2	2	2	2
-	-	-		4	1		2	0	0	5
20	**19**	**11**	**8**	**9**	**6**	**6**	**23**	**22**	**17**	**29**

Where; *NT: non-typeable, CN: Gentamicin, TOB: Tobramycin, AK: Amikacin

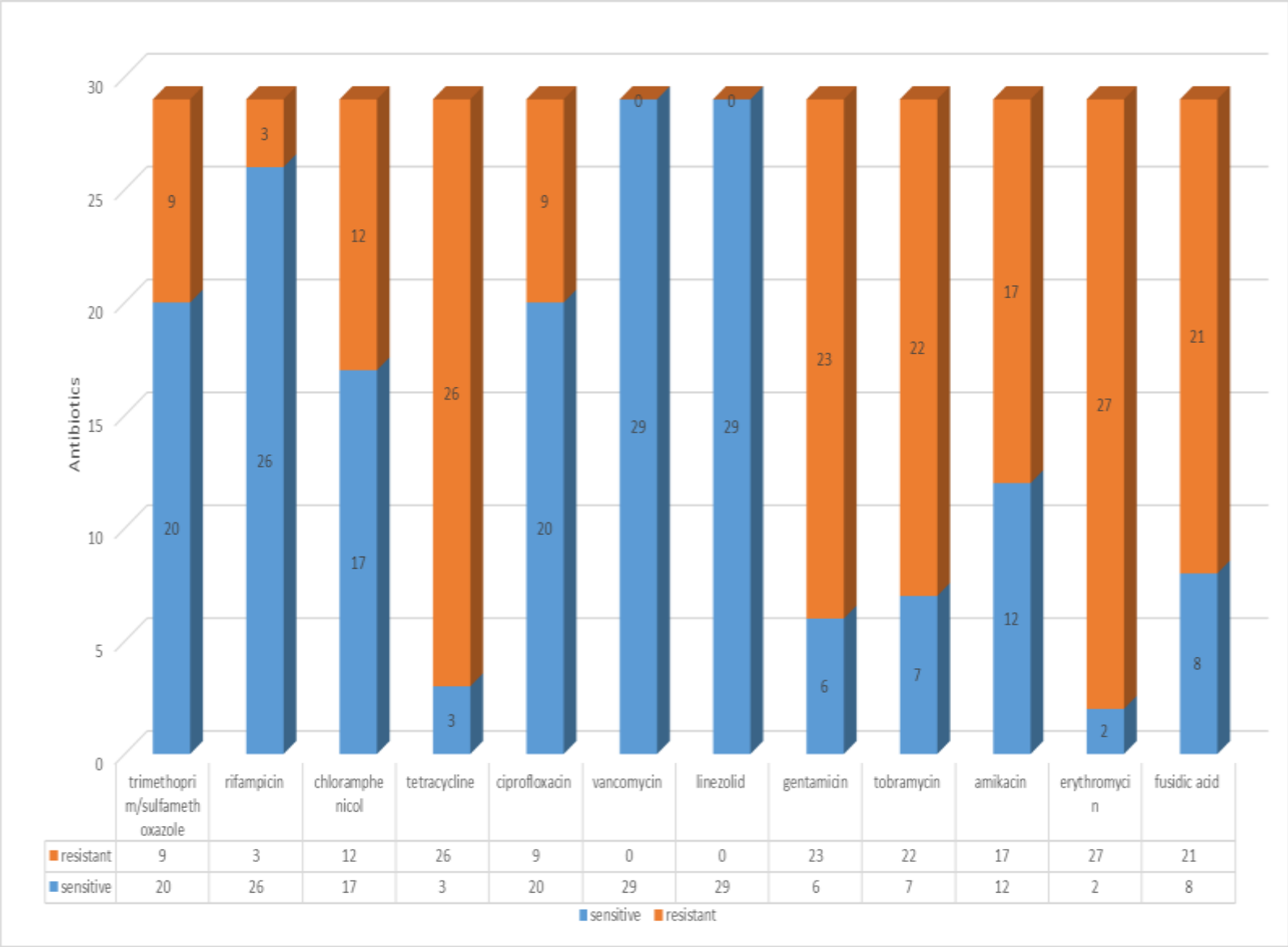

Fig. 1: Antibacterial resistance patterns of the CA-MRSA isolates among the AD children, where sensitivity is represented by blue columns and resistance by orange columns

Fig. 2: Gentamicin susceptibility testing using the MIC Test Strip according to CLSI guidelines. Gentamicin resistant CA-MRSA isolate (MIC_{50} = 48 µg/ ml, MIC_{90} = 128 µg/ ml)

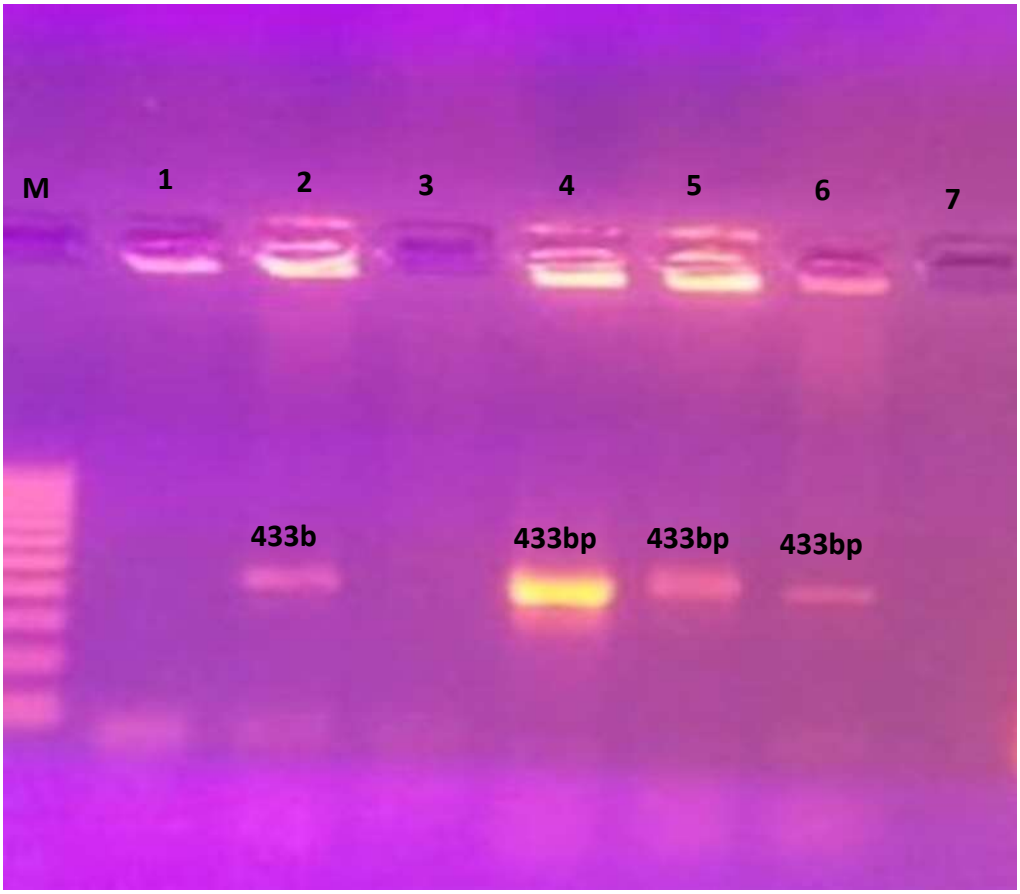

Fig. 3: Gel electrophoresis of PCR amplified *PVL* gene (433 bp) among CA-MRSA isolated from AD children. Where; Lane (M): DNA ladder (100 bp). Lanes 4, 5, 6: positive results for *PVL* (433 bp). Lane 2: MRSA ATCC 25923 positive control for PVL (433 bp). Lane 1: negative control. Lanes 3, 7: negative results for *PVL*

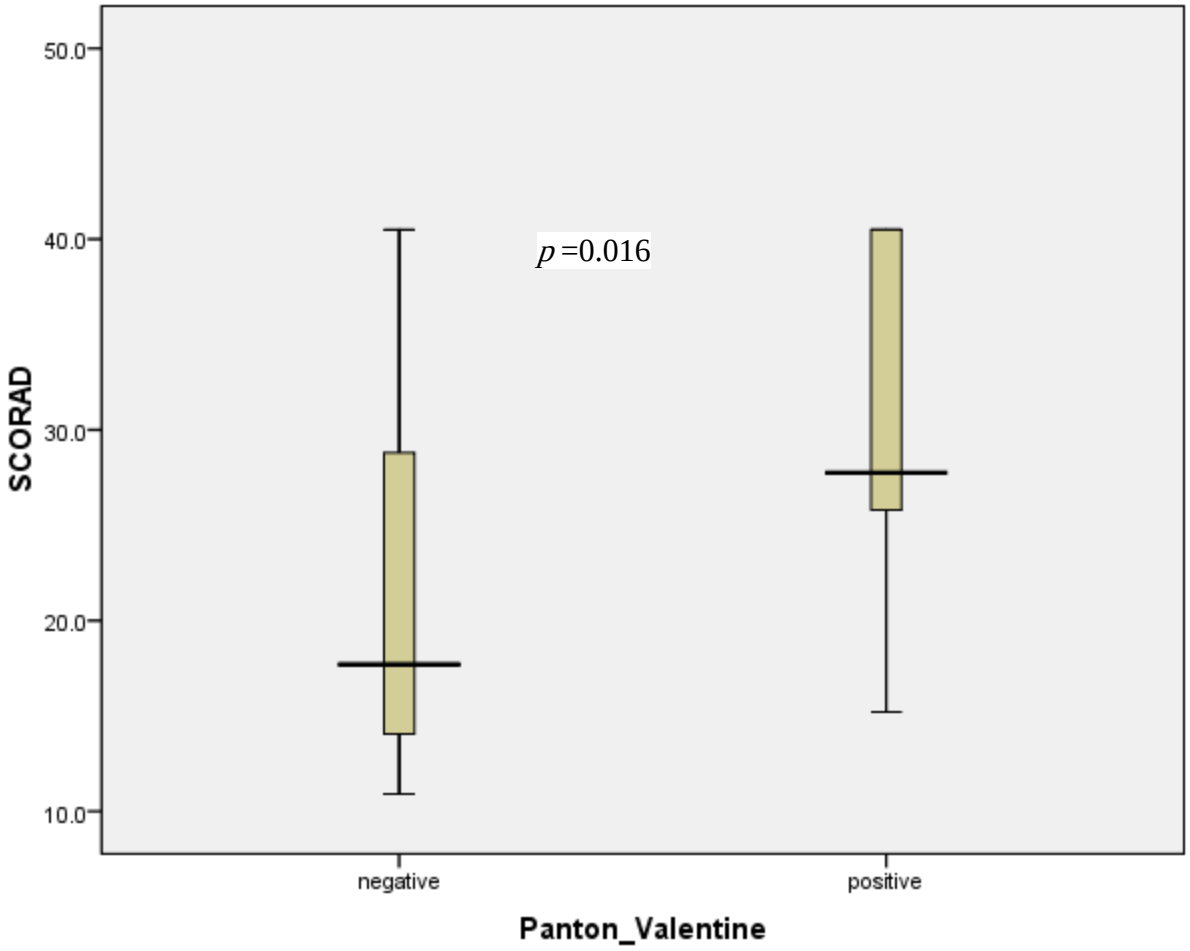

Fig. 4: The SCORAD score of children with AD colonized with 14 *PVL* positive CA-MRSA (median 27.8) versus AD patients colonized with 15 *PVL* negative CA-MRSA (median 17.7). The error bars represent SCORAD Score values in both *PVL* Positive and negative MRSA. The *p*-value was 0.016

Moreover, the SCORAD index in patients colonized with gentamicin resistant MRSA (26.5 %) was significantly higher than that in the gentamicin susceptible MRSA isolates (15.2 %), recording p =0.009 (Fig. 5).

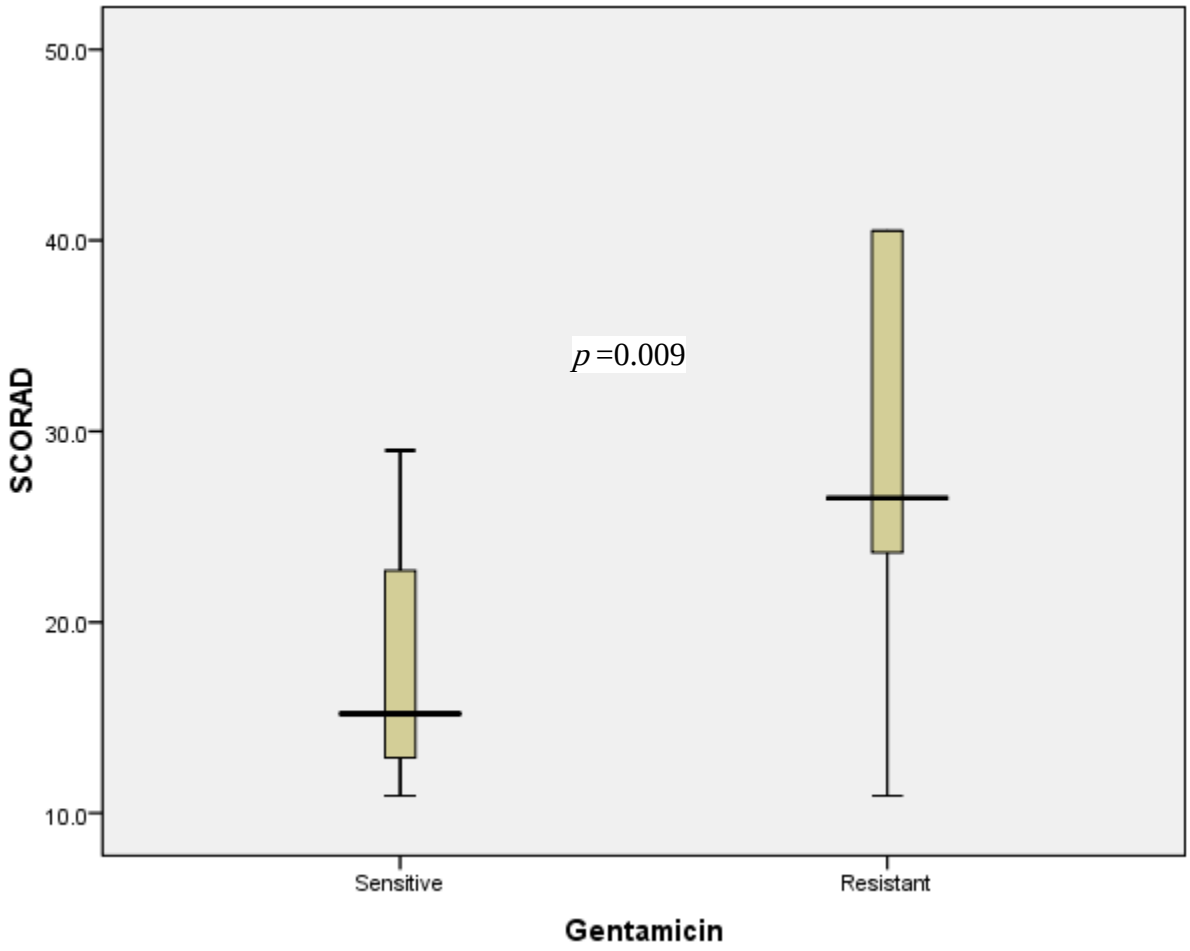

Fig. 5: The SCORAD score of children with AD colonized with 23 gentamicin resistant CA-MRSA (median 26.5), versus AD children colonized with 6 gentamicin sensitive CA-MRSA (median 15.2). The error bars represent SCORAD Score values in both gentamicin sensitive and resistant MRSA. The *p*-value was 0.009

4. Discussion

Atopic dermatitis patients are at growing threat of emerging microbiological resistance, and will become subsequently colonized with MRSA, due to of the repeated usage of topical and systemic antibiotics (Lipnharski *et al*., 213; Ogonowska *et al*., 2021). In several previous studies, the rate of MRSA colonization of the skin of AD pediatric patients varied between 0.5 % to 41.8 % (Balma-Mena *et al*., 2011; Jagadeesan *et al*., 2014; Park *et al*., 2016; Cavalcante *et al*., 2021). In this study, colonization of skin lesions with CA-MRSA was detected in 29 % of the cases. This high-level rate may be attributed to the low socioeconomic conditions, hot humid weather, overcrowding, nutritional status, prevalence of parasitic infections, and to the difference in geographical area, in accordance with the results of Jagadeesan *et al*., (2014).

Currently, 82.8 % of the CA-MRSA isolates carried the *AMEs* genes. Moreover, a high rate of gentamicin resistance was reported in this study (79.3 %). Aminoglycoside resistance is intimately linked to methicillin-resistance, due to proximity of the *mecA* and *AME* genes (Choi *et al*., 2003; Fathi *et al*., 2022). Similarly, a high frequency of gentamicin resistance (58.8 %) was recorded in the previous study conducted in Korea by Chung *et al*., (2008). On the contrary, lower rate was reported in Brazil (Cavalcante *et al*., 2015). Distribution of the aminoglycoside resistance determinants varied among the different regions; even in those isolates recovered from the same city, and is also linked to type of the used aminoglycoside (Ida *et al*., 2001; Ardic *et al*., 2006). In this study, although *ant(4')-Ia* was the most common detected gene in the isolates of CA-MRSA; however, *aac(6')-aph(2")* was the most common gene detected in the gentamicin resistant isolates, which was also reported recently by Al-Mayahi and Srhan (2021). Similarly, several studies conducted in Kuwait and Japan reported that *ant(4')-Ia* was the most common aminoglycoside-resistant determinant (Udo and Dashti, 2000; Ida *et al*., 2001). Meanwhile, *aac(6')-aph(2")* was the predominant gene in other previous reports (Choi *et al*., 2003; Ardic *et al*., 2006; Fathi *et al*., 2022). In this study, about 2-3 *AMEs* genes coexisted in the gentamicin resistant isolates. We observed that combination of the *aac(6')-aph(2")* and *ant(4')-Ia* , in addition to *aac(6')-aph(2")*, *ant(4')-Ia* and *aph(3')-IIIa* genes, were the most common genotypes. Combination of *AMEs* genes was also reported in another previous study conducted by Udo and Dashti, (2000).

In accordance with results of the preceding studies conducted by Chung *et al*., (2008); Tang *et al*., (2011) on CA-MRSA in the AD children; all the current MRSA isolates were susceptible to vancomycin, while resistance to erythromycin displayed the highest rates followed by tetracycline resistance among the colonizer isolates. Fusidic acid resistance was detected in 72.4 % of the isolates, which was the matter of Danish AD patients that previously exhibited 41 % resistance to fusidic acid (Edslev *et al*., 2018). On the contrary, lower rate of fusidic acid resistance (11.8 %) was documented among the MRSA, which were isolated from Children with Eczematous AD Lesions (Chung *et al*., 2008). High prevalence of fusidic acid resistance may be attributed to the frequent use of fusidic acid treatment, which likely results in the emergence of more resistant strains.

Panton-Valentine leukocidin (PVL) is a cytotoxin that is responsible for destruction of the leukocytes, and causes tissue necrosis by destruction of the cell membranes. PVL is frequently associated with community-acquired necrotic lesions of the skin and subcutaneous tissues (Lina *et al*., 1999). PVL was detected in about half of the current CA-MRSA isolates, and was significantly associated with high SCORAD score. In accordance, similar rate of PVL prevalence among the MRSA isolates was documented in the pediatric AD patients (Lo *et al*., 2010). However, contribution of PVL in recrudescence of

severity of AD in the children is controversial (Edslev *et al*., 2018; Cavalcante *et al*., 2021).

Among the MRSA isolates, SCC*mec* V was the most common type. Also, SCC*mec* V was the most common cassette previously reported by Lo *et al*., (2010) in pediatric AD. Meanwhile, other studies in the same group of patients reported SCC*mec* IV as the predominant cassette (Chung *et al*., 2008; Cavalcante *et al*., 2015; Abad *et al*., 2020). Although, type IV SCC*mec* is the most common element in CA-MRSA; however, SCC*mec* I–III elements were common in the nosocomial MRSA (Sadeghi Moghaddam *et al*., 2022). In this study, type III was detected in 27.6 % of the CA-MRSA isolates. A study conducted in Iran by Fasihi *et al*., (2017) reported that the most common cassette types in the community were SCC*mec* types I and III. This establishes the idea of transmission of CA-MRSA from population to the hospital settings, and transmission of hospital-acquired MRSA from hospital settings to the community (Yeung *et al*., 2011). Another study conducted in Brazil revealed the association of SCC*mec* type III with the multidrug-resistant isolates, which supports the hypotheses that hospital is the origin of these MRSA strains, and that these strains are currently increasing as colonizing pathogens in the pediatric community (Lamaro-Cardoso *et al*., 2009).

Conclusion

Current data revealed a high frequency of gentamicin resistance among the CA-MRSA that colonise the skin of the atopic dermatitis children; with the predominance of *aac(6')-Ie-aph(2')-Ia* aminoglycoside-resistant determinant, followed by *ant(4')-Ia*. Moreover, SCC*mec* type V was the most common cassette with the possible emergence of SCC*mec* type III among the CA-MRSA colonizers.

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None to declare.

Conflict of interest

All authors declare non-existence of conflict of interests.

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Ethical approval

This work was ethically approved by the Faculty of Medicine's Institutional Research Board (IRB) (Code number: R/22.11.1932), Mansoura University. An informed consents were provided by the participants and parents that shared in this study.

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