ORIGINAL ARTICLE

Prevalence of Nasal Colonization with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Among Children in an Egyptian Community

[1]Enas M. Hefzy*, [2]Tharwat E.E. Radwan, [2]Aliaa N. M. Ibrahim, [3]Mohammed M. Hefzy, [4]Mahmoud A. F. Khalil

[1]Medical Microbiology and Immunology, Department, Faculty of Medicine, Fayoum University, Egypt
[2]Botany Department, Faculty of Science, Fayoum University, Egypt
[3]Medcare Hospital, Sharjah, UAE
[4]Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, Egypt

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Corresponding Author:
Enas Mamdouh Hefzy, MBBS, MD; Medical Microbiology and Immunology Department, Faculty of Medicine, Fayoum University, Fayoum, Egypt, 63514;
Tel.: +201092059008;
emh01@fayoum.edu.eg,
ORCID.
0000-0001-9363-3998

ABSTRACT

Background: *Nasal Staphylococcus aureus is asymptomatically and permanently colonizing up to one-third of the population. This may start during the first few days of life and can lead to infections.* ***Objectives:*** *This study aimed to identify MRSA nasal carriage among children in an Egyptian community, detection of antibiotic resistance, biofilm-formation along with the virulence genes found in MRSA isolates.* ***Methodology:*** *In this community-based cross-sectional study, a total of 200 nasal swabs were collected. Cefoxitin susceptibility testing was done for MRSA detection. The MRSA isolates underwent antibiotic susceptibility and biofilm-formation testing. Genes associated with biofilm formation and virulence in MRSA isolates were also explored. From the examined subjects, 133 carriers of S. aureus were identified. About half (51.8) % of these isolats were MRSA. The majority of MRSA isolates [73.9% (n=51)] were found to be positive for clfA gene. The prevalence of icaD, fnbA, icaA, cna, icaC, and icaB genes were found to be 0%, 14.5%, 0%, 33.3%, 0%, and 44.9%, respectively.* ***Conclusion:*** *This study showed that the presence of biofilm-encoding genes positively affects biofilm production activity. According to this study, many healthy children had nasal colonization with S. aureus and MRSA that necessitates developing effective antibiotic usage in the community.*

INTRODUCTION

Mucous membranes and skin carries *Staphylococcus aureus (S. aureus),* the significant human pathogen. About 30% of healthy individuals have nasal colonization by these commensal bacteria. Nasal colonization in humans may start during the first few days of life [1] . Infections including serious conditions like endocarditis, bacteremia, and sepsis are all possible in those who have *S. aureus* nasal colonization [2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for a significant rise in morbidity and mortality from both community- and healthcare-acquired infections[3].

MRSA carries the *mecA* gene, which produces PBP2a, a penicillin-binding protein. PBPs are essential for constructing bacterial cell walls, but beta-lactam antibiotics neutralize them. PBP2a, however, is less affected by these antibiotics, enabling it to support bacterial survival even in their presence [4]. Additionally, the *mecC* gene is a just recently identified type of methicillin resistance genes (*mec*) located in *S. aureus* [5]. Cefoxitin, as recommended by the CLSI (Clinical and Laboratory Standards Institute), has been identified as a distinct penicillin derivative effective against *mecA*-related methicillin resistance due to its significant stimulation of the *mecA* regulative system [6].

S. aureus attaches itself more easily to human tissue when a number of genes encoding microbial surface components, such as fibronectin-binding protein (*fnb*) and collagen-binding protein (*cna*), exist. These genes encodes host cell proteins and sticky matrix molecules [3]. One important component of *S. aureus* pathogenicity, clogging growth factor A (*ClfA*), Furthermore, *ClfA* promotes bacterial adhesion to plasma protein-coated surfaces, allowing for bacterial colonization and the production of biofilms [7].

An important virulence factor that allows *S. aureus* to withstand the host immune response is its capacity to produce biofilm. Biofilm formation leads to an increase in antimicrobial drug resistance [8]. In *S. aureus*, cell-to-cell adhesion is regulated by the polysaccharide intercellular adhesin (PIA), also known as poly-(1-6)-polymeric N-acetyl-glucosamine (PNAG), which is produced by the *icaADBC* operon, facilitating the formation of biofilms in *Staphylococcus* species [9]. A

substantial function in the production of biofilms has been found for the *ica* genes: *icaA* and *icaD*. Hence, in *S. aureus* isolates, the *ica* locus must be detected combined with the phenotypic detection of biofilm to improve the diagnostic choice of the appropriate treatment [8].

A research study performed in Egypt has exposed that greater than 70% of healthcare-associated *S. aureus* infections and also 11.5% of community-acquired infections are credited to MRSA [10]. Furthermore, a study have examined the nasal colonization of MRSA among healthcare employees in Egypt found that 13.5% were colonized by MRSA [11].

Children had significantly greater rates of MRSA carriage with community-acquired MRSA (CA-MRSA) genotypes than adults. Living with small children was also linked to a higher incidence of adult MRSA colonization. When taken together, these findings strongly suggested that young children were the community's main MRSA reservoir and the primary demographic responsible for the accelerated spread of CA-MRSA [12]. Therefore, this work aimed to explore the prevalence of nasal colonization with MRSA among children in one Egyptian community, as well as its virulence traits and resistance to antibiotics.

METHODOLOGY

The purpose of the investigation was to determine the frequency of MRSA nasal colonization in children within a specific Egyptian governorate and to examine the characteristics of virulence and antibiotic resistance in isolates that tested positive for MRSA.

Participants and place of study:

This investigation was done in the period from February 2019 to February 2020, This investigation was performed in Fayoum Governorate which is located less than a hundred kilometers to the west of Cairo. Two hundred healthy children aged ≤ 18 years old were included in the study. Both boys and girls were randomly selected for the study. The participants were recruited from daycare centers and local schools. Children who had taken antimicrobial drugs within the previous two weeks or had a history of illness were not allowed to participate in the trial. The microbiology lab at Fayoum University's, Faculty of Medicine was the place where the collected specimens were processed.

The research was conducted in accordance with the principles of the original Declaration of Helsinki released in 1964 and its subsequent amendments. The research plan was approved by the Research Ethics Committee at Fayoum University's Faculty of Medicine in Egypt (Identifier: R487). Comprehensive measures have been implemented to ensure the confidentiality of data throughout its gathering, safekeeping, examination, and distribution phases. Careful explanation of the study, including its purpose, risks, benefits; was provided and informed consent was secured from caregivers.

Nasal swabbing and transport:

Sampling was performed by double rotating a pre-wetted sterile cotton swab with sterile peptone water in the vestibule of both anterior nares. The collected swabs were transported in sterile peptone water to the lab within two hours of collection.

***Methicillin resistance detection and S. aureus genotyping*:**

S. aureus was identified by conventional microbiological methods [13]. To identify MRSA, among isolates identified as *S. aureus*, cefoxitin discs (30 µg/mL) diffusion assay (In vitro diagnostics, HIMEDIA laboratories, Mumbai, India) was performed [6]. In this assay, S. aureus ATCC 25923 and ATCC 43300 served as a negative control, and as a positive control, respectively. Isolates identified as MRSA were inoculated in sterile tubes (Eppendorf) containing 20% glycerol and stored at −70ºC for further investigations [14]. Molecular testing was used to validate the identity of the MRSA isolates [15].

Testing for antibiotic resistance:

The Kirby-Bauer disc diffusion technique was utilized to assess antibiotics resistance of isolates that were identified as MRSA. Antibiotics, as shown in table 2, were selected and results were interpreted in compliance with CLSI recommendations. *S. aureus* ATCC 25923 used as the test organism for assurance of quality [6].

Assessing biofilm production:

The previously reported microtiter plate test (MtP) was utilized to detect the production of biofilms [16]. The isolates that produced biofilm were categorized into four groups: non-producers, weak producers, moderate producers, and strong producers. Each experiment was performed in triplicate to ensure reliability. *S. aureus* ATCC 35556 and *S. aureus* ATCC 25923 were employed in the biofilm experiment as negative and positive controls, respectively [17].

Molecular identification and characterization of isolates of MRSA:

DNA extraction

DNA extraction from all identified MRSA isolates was performed by Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) (Thermo Fisher Scientific Baltics UAB Company, Vilnius, Lithuania) according to the Gram-Positive Bacteria Genomic DNA Purification Protocol. Quantification of DNA extracted was performed spectrophotometrically (NanoPhotometer P-Class® USB P-330 & P-360 Technologies 2013, Munich, Germany).

PCR Assays

Recognition of S. aureus and MRSA by PCR technique All of the collected DNA samples underwent

multiplex PCR for detection of the *nuc* (nuclease) gene, unique to *S. aureus*, which identify coagulase-negative *Staphylococci* (CoNS) and *S.aureus* [18]. Differentiating MRSA from MSSA isolates was done by detection and the *mecA* (methicillin resistance determinant) gene [15]. The PCR amplification conditions were as follows: an initial denaturation of 5 min at 95°C; 10 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 45s; 25 cycles of 94°C for 30s, 50°C for 30s, and 72°C for 45s; and a final extension at 72°C for 7 min [19]. The primer sequences, annealing temperatures, and PCR product molecular sizes for all PCR reactions are reported in **(Table 1)**

Detection of virulence genes among MRSA isolates: The PCR tests used to identify *fnbA* [20], *clfA* [21], and *cna* genes were performed. The PCR amplification conditions were: initial denaturation at 95ºC for 3 min, followed by 35 cycles of denaturation at 95ºC for 30s, then annealing for 30s (annealing temperature for each gene is shown in **Table 1)**, extension at 72ºC for 60s, followed by a final extension aimed at 5 min at 72ºC**.**

Identification of intercellular adhesion genes (icaA, icaB, icaC, and icaD): Amplification of *icaA, icaB, icaC, and icaD* genes using specific primers **(Table 1)** was conducted by multiplex PCR under the following conditions: initial denaturation at 95ºC for 6 min, followed by 35 cycles of denaturation at 95ºC for 50s, annealing temperature at 53ºC for 50s and extension at 72ºC for 60s with a final extension at 72ºC for 7 minutes [3]**.**

All oligonucleotide primers were synthesized at Thermo Fisher Scientific Company, United Kingdom.

Table 1: Oligonucleotide primers for PCR amplification of genes used in the study

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing Temperature (^{0}C)	Amplicon Size (bp)	References
nuc	GCGATTGATGGTGATACGGTT	AGCCAAGCCTTGACGAACTAAAGC	55	279	[23]
mecA	GTGAAGATATACCAAGTGATT	ATCAGTATTTCACCTTGTCCG	55	112	(23]
icaA	ACACTTGCTGGCGCAGTCAA	TCTGGAACCAACATCCAACA	53	188	[3]
icaD	ATGGTCAAGCCCAGACAGAG	AGTATTTTCAATGTTTAAAGCA	53	198	[3]
icaB	CCCAACGCTAAAATCATCGC	ATTGGAGTTCGGAGTGACTGC	53	1080	[3]
icaC	CATGAAAATATGGAGGGTGG	TCAAACTGATTTCGCCCACCG	50	1000	[3]
fnbA	ATCAGCAGATGTAGCGGAAG	TTTAGTACCGCTCGTTGTCC	55	198	[24]
clfA	ATTGGCGTGGCTTCAGTGCT	CGTTTCTTCCGTAGTTGCATTTG	55	292	[25]
cna	AAAGCGTTGCCTAGTGGAGA	AGTGCCTTCCCAAACCTTTT	55	192	[3]

nuc (nuclease gene; *mecA* (methicillin resistance determinant gene); *icaA, B, C, D* (intercellular adhesion gene *A, B, C, D*); ; *fnbA* (fibronectin-binding protein A;*clfA* (clumping factor A; *clfA* (clumping factor A; *cna* (collagen- binding protein)

Statistical Analysis

Collected data were processed and analyzed using SPSS 16 (Statistical Package for the Social Sciences; SPSS Inc. Chicago, IL, USA). Categorical data were presented using frequency percentages. The Chi-square test was used to express the relation between quantitative variables. Fisher–Exact test was used instead for two by two tables with expected cell frequency in any cell less than five. P value <0.05 was considered significant.

RESULTS

Demographic characters of participants:

Among the studied children (n=200), 106 (53%) were girls and 94 (47%) were boys. Age ranged from 1-14 years old, 41 (20.5%) were aged < 6 years old, 122 (61%) were 6-12 years old and 37 (18.5%) were >12-14 years old (**Table 2).** The mean ± SD (standard deviation) was 8.7 ± 3.5 years

Table 2: Age and sex of participants

	N=200	
Age	**Boys (N=94)**	**Girls (N=106)**
<6	24(25.5%)	17(16%)
6-12	53(56.3%)	69(65.1%)
>12	17(18%)	20(18.87%)

Age and sex distribution of MRSA cases:

Out of 200 samples, six had no growth, Among the 194 samples with growth, 189 were gram-positive cocci while five were gram-negative bacilli . Out of the gram-positive cocci, 133 were identified as *S. aureus* **(Figure 1; Table 3)**. Using the cefoxitin diffusion on disk test, sixty-nine (51.9%) of the 133 isolates that were examined were found to be MRSA **(Table 4).** This indicates that 34.5% (69 out of 200) of the children sampled were colonized with MRSA in their nasal passages. Among the initial sample (n=200), MRSA colonization was observed in 27.7% (n=26/94) of the

boys and 40.6% (n=43/106) of the girls (*p-value* = 0.055). Among the MRSA-colonized subjects (69 in total), 62.3% (43 cases) were identified in females, while 37.7% (26 cases) were found in males **(Figure 1).**

Among the initially sampled children (n=200), MRSA colonization (n=69) was observed in 16/41 (39%) of children aged< 6 years; 43/122 (35.2%) in children aged 6-12 years, 10/37 (14.5%) in children aged >12 years, *p* value=0.518

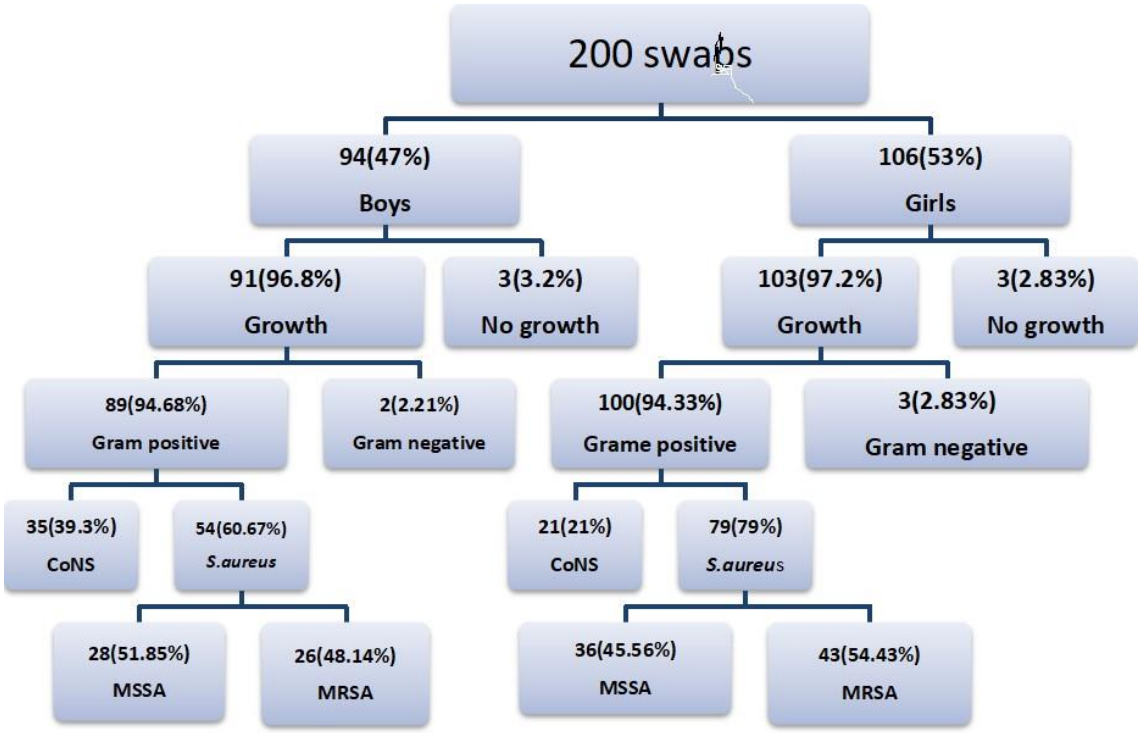

Fig. 1: Distribution of *Staphylococcus aureus* and MRSA isolates among boys and girls

Table 3: Age and sex distribution of *S. aureus*-colonized cases

(N=133)		N	%
Sex	**Girls**	79	59.4
	Boys	54	40.6
		Boys	**Girls**
Age	< 6	10(18.5%)	11(13.9%)
	6-12	30(55.6%)	55(69.6%)
	>12	14(25.9%)	13(16.5%)

Table 4: Age and sex distribution of MRSA-colonized cases (N=69)

(N=69)			%
Sex	**Female**	43	62.3
	Male	26	37.7
Age	< 6	16	23.2
	6-12	43	62.3
	>12	10	14.5

Antibiotic susceptibility test for MRSA isolates.

The antibiotic susceptibility pattern of MRSA isolates is presented in **Table 5.** It was susceptible to the majority of tested antibiotics. The MRSA strains showed high rates of susceptibility (>80%) to chloramphenicol, ciprofloxacin, clindamycin, and teicoplanin, but high resistance to fusidic acid. Almost all isolates (98.6%) were susceptible to rifampicin and tigecycline. Seven isolates (10.1%) showed resistance to mupirocin.

Table 5: Antibiotic susceptibility testing of MRSA isolates.

	Sensitive		Intermediate		Resistant	
	N	%	N	%	N	%
Teicoplanin (30 μg)	64	92.8	5	7.2	0	0
Gentamycin (10 μg)	42	60.9	2	2.9	25	36.2
Erythromycin (15 μg)	27	39.1	30	43.5	12	17.4
Tetracycline (30 μg)	33	47.8	14	20.3	22	31.9
Ciprofloxacin (5 μg)	62	89.9	4	5.8	3	4.3
Clindamycin (2 μg)	57	82.6	7	10.1	5	7.2
Trimethoxazole (25 μg)	40	58.0	6	8.7	23	33.3
Chloramphenicol (30 μg)	62	89.9	2	2.9	5	7.2
Mupriocin (5μg)	62	89.9	0	0	7	10.1
Rifampicin (5 μg)	68	98.6	0	0	1	1.4
Tigecyclin (15 μg)	65	94.2	2	2.9	2	2.9
Ceftraiaxone (30 μg)	23	33.3	39	56.5	7	10.1
Fusidic acid (10 μg)	19	27.5	0	0	50	72.5

Biofilm formation activity

The quantitative MtP method was used for the detection of biofilm produced by MRSA on a 96-well plate. About half (44.9%) of the tested MRSA isolates were found to be biofilm-producers at varying levels. Among 69 MRSA isolates studied, one (1.4%) isolate was a strong biofilm-producer, 21 (30.4%) isolates were moderately biofilm-producers, nine (13.4%) isolates were weak biofilm-producers, and 38 (55.1%) isolates were non- biofilm producers **(Table 6)**.

Table 6: Biofilm activity of MRSA isolates

	Frequency (N=69)	Percent (%)
No biofilm	38	55.2
Weak biofilm	9	13.0
Moderate biofilm	21	30.4
Strong biofilm	1	1.4
Total	69	100.0

PCR molecular identification

Molecular identification of S. aureus and MRSA by PCR technique

PCR molecular characterization confirmed the preliminary MRSA identification by cefoxitin resistance as all were positive for *nuc* and *mecA* genes**.**

Prevalence of adhesion and virulence genes in MRSA isolates

One of the objectives of the present study is the detection of biofilm-associated genes among MRSA isolates. **(Table 7)** summarizes the distribution of these genes in MRSA isolates. The majority of MRSA isolates, comprising 73.9% (n= 51/69), were found to be positive for *clfA* gene. The prevalence of *fnbA*, *cna*, and *icaB* genes was found to be 14.5%, 33.3%, and 44.9%, respectively. *icaA*, *icaC*, and *icaD* genes were not detected.

Table 7: Prevalence of virulence genes and biofilm-encoding genes among MRSA isolates causing nasal colonization

Genes	N== (69)			
	Positive		Negative	
	N	%	N	%
icaA	0	0	69	100.0
icaB	31	44.9	38	55.1
icaC	0	0	69	100.0
icaD	0	0	69	100.0
cna	23	33.3	46	66.7
clfA	51	73.9	18	26.1
fnbA	10	14.5	59	85.5

The relationship between biofilm production and the presence of biofilm-encoding genes.

There was a significant association between the presence of *icaB* and *clfA* and moderate or strong biofilm production; the *p*-values were <0.001 and 0.002, respectively. Also, the absence of *cna* and *fnbA* was significantly associated with the absence of biofilm-forming activity, the *p*-values were <0.001 and 0.02, respectively **(Table 8).**

Table 8: The relationship between biofilm-productionand the presence of biofilm-encoding genes

			Biofilm			P value
			No biofilm	Weak biofilm	Moderate or strong biofilm	
icaB	**Negative**	N	32	5	1	<0.001*****
		%	84.2%	55.6%	4.5%	
	Positive	N	6	4	21	
		%	15.8%	44.4%	95.5%	
cna	**Negative**	N	33	4	9	<0.001*****
		%	86.8%	44.4%	40.9%	
	Positive	N	5	5	13	
		%	13.2%	55.6%	59.1%	
clfA	**Negative**	N	16	2	0	0.002*****
		%	42.1%	22.2%	. 0%	
	Positive	N	22	7	22	
		%	57.9%	77.8%	100.0%	
fnbA	**Negative**	N	35	5	19	0.02*****
		%	92.1%	55.6%	86.4%	
	Positive	N	3	4	3	
		%	7.9%	44.4%	13.6%	

*****Significant; **P value (< 0.05).**

When investigating the relationship between biofilm production and the number of virulence genes detected, the data revealed that most of the strongly and moderately biofilm-producing isolates were found to have more than one virulence gene. There was a significant relation between the number of genes in isolates and biofilm activity, *p-value* <0.001 **(Table 9).**

Table 9: Relation of biofilm-forming activity and the number of virulence genes

Number of detected gene		Biofilm Production			P value
		No Biofilm	Weak Biofilm	Moderate or strong Biofilm	
Zero	N	6	0	0	
	%	15.8%	0%	. 0%	
One	N	29	0	0	
	%	76.3%	0%	. 0%	
Two	N	3	8	8	
	%	7.9%	88.9%	36.4%	
Three	N	0	1	13	
	%	0%	11.1%	59.1%	
Four	N	0	0	1	
	%	0%	0%	4.5%	
	Median	1	2	3	<0.001*
	IQR	1-1	2-2	2-3	

* Significant

DISCUSSION

About 30% of people may have *S. aureus* nasal colonization [1]. Our study detects the prevalence of MRSA among *S. aureus* isolates nasally-colonizing healthy Egyptian children as well as their virulence and resistance to different antibiotics.

Our study of 200 samples from healthy children revealed that 66.5% of the children were colonized with *S. aureus* and 34.5% of them were colonized with MRSA, These findings are comparable to a study of healthy children conducted in a major Brazilian city, where the prevalence of *S. aureus* nasal colonization was48% [22]. The high probability of MRSA carriage in

our research's community youngsters may be attributed to several factors, including the overuse of antibiotics by means of no national antibiotic stewardship is employed in Egypt and parents can easily get variable antibiotics as over-the-counter drugs which accounts for massive misuse and overuse of antibiotics in the Egyptian community. Also, poor hygiene practices and poor sanitation can contribute to this condition as Fayoum, where is study was based, is mostly a rural governorate with limited sanitation in some areas. Furthermore, the deficiency of robust infection control measures in both healthcare facilities and the community exacerbates the situation.

After a thorough search of the literature, we have found that this is the first study to investigate nasal colonization of Egyptian healthy children. Only one previous study has searched colonization of children in one pediatric hepatology unit and found that *24.2* % of ward patients, and 36.4 % and 16.7 % of NICU and PICU, respectively, were colonized with MRSA [23].

Egypt has established its very own national activity strategy to take on resistance to antibiotics, integrating administration devices as well as efforts to increase public understanding. Many research studies as well as programs in Egypt have concentrated on handling antibiotic resistance and also executing antibiotic stewardship. The influence of COVID-19 on antibiotic resistance in Egypt has increased problems especially noted by a rise in antibiotic use throughout the pandemic [24]. The current study highlights the importance of dealing with antibiotic resistance via extensive plans as well as public recognition campaigns in the nation.

Our MRSA colonization prevalence surpasses that reported by Sunette et al [25]. in Namibia, which was 17.6% , and mirrors the 35.9% documented by Erami et al [26]. among school children in Iran. Additionally, our findings align with the prevalence of MRSA colonization observed in healthy children attending public daycare centers in Brazil [27], and are similar to the MRSA colonization prevalence of 29.3% among healthy children in southern Taiwan [26] .

Nasal colonization has been associated with younger age and male sex in a number of studies [28]. In the present study, we did not find any significant difference between both sexes regarding MRSA colonization, while the percentages of isolated MRSA were higher in females than males, which contrasts the studies by Erami et al [26], and Reta et al [29].

In general, several different factors contribute to *S. aureus* carriage including those associated with the host, the pathogen itself, and the environment [30], and differences in geographical distribution and population characteristics [29]. In our study, the distribution of MRSA among healthy children was higher in the age group 6-12 years than in the group aged <6 years or aged > 12 years, this difference was not significant. This difference may be due to their over-activity, their immature immune system, and interaction with asymptomatic MRSA carriers. These findings parallels those of Sunette et al [25]. They found that there were no statistically significant variations in nasal MRSA species between age groups.

In our study, of the isolates of *S. aureus*, 52% were determined to be methicillin-resistant by cefoxitin disc diffusion test and detection of the *mecA* gene. This was slightly less than previous Iranian study that reported MRSA colonization in children [26].

Consistent with previous studies, our MRSA isolates demonstrated notable susceptibility to teicoplanin (92.8%), clindamycin (89.6%), ciprofloxacin (89.9%), and chloramphenicol (89.9%), mirroring findings from a study in Jordan [31]. MRSA species showed significant sensitivity to the majority of tested antibiotics, maybe because of the frequency of CA-MRSA bacteria that are genetically driven by the *SCCmec* type IV genetic element that is not as likely to develop multidrug resistance [29]. A small number of MRSA species exhibited resistance, which could result from the potential blending of hospital-acquired MRSA as well as CA-MRSA isolates. The high rates of resistance that were recorded against fusidic acid (72.5%) are consistent with those reported in other studies [32]. That is because it has been used more frequently throughout time.

The issue of mupirocin resistance in *S. aureus*, including MRSA strains, has been a concern. About a tenth of MRSA isolates in this research had mupirocin resistance, which is in line with a previous comprehensive analysis. The emergence of mupirocin resistance presents a threat to invasive infections, emphasizing the importance of continued monitoring of its spread [33].

The capacity of *S. aureus* to produce biofilms significantly contributes to its pathogenicity, providing protection against immune responses and antibiotics. It is associated with chronic infections such as sepsis, endocarditis, and osteomyelitis, and other serious conditions, especially hospital-associated infections [34]. In our study, data from a quantitative biofilm formation assay using MtP showed an isolate with high adhesion capacity; 21/69 isolates (30.4%) had moderate adhesion; and 9/69 strains (13.4%) with weak adhesion ability; 38/69 isolates (55.1%) had no adhesion ability. The results of this study align with those of investigations conducted by Nourbakhsh et al [35], who reported that there is no direct relationship between the methicillin resistance characteristics and the more or less biofilm-forming ability of the strains. Although some are weak or do not form biofilms, this does not rule out the possibility that they cause infections in people with weakened immune systems.

Cna and FnbA, and FnbB proteins are surface elements that *S. aureus* uses to cling to human skin [36].

MRSA isolates, in this study, underwent detection of specific adhesion genes such as *clfA*, *cna*, and *fnbA* as well as biofilm-related genes such as *icaA, icaD, icaC and, icaB* by PCR. In a total of 69 MRSA isolates, the highest frequency was observed for the *clfA* gene with only 26% of the isolates lacking this gene which was the same as the previous report by Kohn et al. suggesting that 89% of the test isolates were *clfA* positive [37]. Also, the *cna* gene was found in one third of the isolates, which was similar to other studies that reported the prevalence of *the cna* gene as 46% [38].

In the present study, the data of PCR analysis indicated that 44.9% of the MRSA isolates were found to harbor *the icaB* gene only. The *icaA*, *icaC*, and *icaD* genes were not detected in any MRSA isolates. Namvar et al [39]. reported that *S. aureus* isolates could not form biofilm unless they were positive for the *icaD* gene**.** This opposed our results that reported biofilm formation without the existence of *icaA/D* genes.

Our findings indicate that healthy school children have the potential to carry *S. aureus*, especially MRSA. Overall, our results support the analysis conducted in 2016 by Arali et al [40]**,** that MRSA and multi-drug resistant strains can potentially spread by healthy school children under the age of 16 . This might be due to several risk factors, including taking antibiotics in the three months before sample collection, coming into contact with animals, being around hospitals, and geographic variation. Nevertheless, none of these characteristics were evaluated in the current work and this was a limitations of the current study.

CONCLUSION & RECOMMENDATIONS

Conclusively, a high percentage of healthy children in the studied community had nasal colonization with *S. aureus* and MRSA. Therefore, we should pay attention to the hygiene of children at schools and nurseries and adhere to frequent hand washing. Antibiotics should not be used unless prescribed by a doctor, and awareness should be given to the appropriate usage of antibiotics. Therefore, it is crucial to intensify antimicrobial stewardship initiatives in our nation. Future research should target the molecular characterization and classification of MRSA. Young children and newborns who may act as reservoirs for MRSA should be included in future research.

Declarations:

Author contribution:

E.M.H.: Conceptualization, Methodology, Validation, Formal analysis, Data Curation, Manuscript writing. Review & Editing, Visualization. Supervision Project administration; **T.E.E.R.:** Conceptualization, Methodology, Formal analysis, Supervision, Project administration; **A.N.M.I.:** Methodology, Formal analysis, Investigation, Data Curation, Original Draft Writing; **M.M.H.:** Analysis, Original Draft Writing, Manuscript revision, Manuscript editing; **M.A.F.K:** Methodology, Validation, Investigation, Supervision. All authors read and approved the final manuscript.

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Abbreviations

CA-MRSA: Community-acquired MRSA
Cna: Collagen-binding protein
ClfA: Clumping factor A
CLSI: Clinical and Laboratory Standards Institute
Cons: Coagulase-negative *Staphylococci*
Fnb: Fibronectin-binding protein
MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-sensitive *S. aureus*
MtP: Microtiter plate assay
Nuc: Nuclease gene
OD: Optical density
PBPs: Penicillin-binding proteins
PBS: Phosphate-buffered saline
PIA: Polysaccharide intercellular adhesin
PNAG: Poly-(1-6)- polymeric N-acetyl-glucosamine
S. Aureus: Staphylococcus aureus
SD: standard deviation
TSB: Trypticase Soy Broth

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