

# Nasal Carriage and Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* from Healthcare Workers and Community People in Minia City, Upper Egypt

Heba A. Mohamed<sup>1</sup>, Gamal F. Mahmoud<sup>1</sup>, Salah M. Abdalla<sup>2</sup>, Sahar A. Mandour<sup>3</sup>, Fatma Y. Mohamed<sup>3</sup>

<sup>1</sup>Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, Egypt

<sup>2</sup>Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt

<sup>3</sup>Department of Microbiology and Immunology, Faculty of Pharmacy, Deraya University, Minia, Egypt

Corresponding Author

Heba A. Mohamed

Mobile: 01020296850

E-mail:

[Heba.ahmed@mu.edu.eg](mailto:Heba.ahmed@mu.edu.eg)

ORCID: 0000-0002-8454-0738

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**Background and study aim:** Human anterior nares can be colonized by *Staphylococcus aureus*, which represents a great risk of developing invasive infections. This study aimed to assess resistance profiles and the prevalence of resistance genes in *Staph aureus* strains isolated from nasal swabs of healthcare workers and community people. **Patients and Methods:** two hundred and thirty-three nasal specimens were collected from both healthcare workers and community people in Minia City. *S. aureus* was identified by conventional methods. Antibiotic sensitivity testing was done using the Kirby-Bauer Disk Diffusion assay. Screening of virulent genes including (mec A, pvl, hla, seb, fnbA, icaA and tsst-1) was done using polymerase chain reaction (PCR). **Results:** A total of 95 *S. aureus* isolates were recovered, 46 (48.4%) were isolated

from healthcare workers and 49 (51.6%) from community people. Virulence genes mec A, hla, fnbA, and seb were detected in all-positive strains as follows: 14 (48.3%), 10 (34.5%), 4 (13.8%), and 2 (6.9%) respectively. Virulence genes (mec A hla, fnbA, and seb genes) were more detected among *S. aureus* isolates of community people than those nasally harbored by healthcare workers. The Ica A gene was only detected in healthcare workers' pvl-positive strains (8, 28%), while the tsst-1 gene wasn't detected among all *S. aureus* pvl-positive strains. **Conclusion:** Nasal carriage of *S. aureus* were detected in both community people and health care workers, almost equally. Virulence genes (mec A, pvl, hla, fnbA, and seb genes) were more detected in strains isolated from community people than those isolated from healthcare workers.

## INTRODUCTION

*Staphylococcus aureus* can be present in the human body as a member of the body flora [1] or as an opportunistic pathogenic bacteria that causes a variety of nosocomial and community diseases [2,3,4]. The nose (anterior nares) is considered the most common biological niche for *S. aureus* carriage in humans, due to the specific anatomy of this region facilitating the colonization of SA, however, this bacterium can colonize other body regions. These bacteria can live in the nasal cavity in about 20–30% of the human population asymptotically and persistently [1]. However, ongoing *S. aureus* nasal colonization may raise the likelihood of subsequent infections [5], moreover, some studies relate *S. aureus* nasal carriage to some autoimmune diseases[6,7].Methicillin-resistant *S. aureus* (MRSA) infections were initially only

found in hospitals, but they have since become a major source of community-acquired (CA) infections worldwide [8]. Extensive and repeated use of broad-spectrum antibiotics is the mandatory contributing factor to the rise in antimicrobial resistance of MRSA. The spread of multi-drug-resistant *S. aureus* strains, especially MRSA, complicates the treatment of staphylococcal infection and represents a significant economic problem[9].

In addition to antimicrobial resistance, genes involved in virulence are essential for the production of toxins and other substances that exacerbate illness. Increased pathogenicity and the risk of serious infections have been

associated with the presence of particular virulence genes, including panton-valentine leukocidin (*pvl*), alpha-toxin (*hla*), and toxic shock syndrome toxin-1 (*tsst-1*) [10].

This study aimed to screen both healthcare workers and community people for nasal carriage of *staph aureus*, especially MRSA. In addition, this study was designed to investigate antibiotic resistance profiles and virulence genes (*mec A*, *pvl*, *hla*, *seb*, *fnbA*, *tsst-1*, and *icaA*) among the isolated strains.

## METHODS

### Sample Collection:

A total of 233 nasal specimens were taken from community people and healthcare workers of Minia University Hospital during the period between May 2021 to April 2022. All nasal swabs were streaked onto different media and incubated at 37°C for 24 hours [11].

### Isolation and Identification of *S. aureus*:

Specimens were identified microscopically and by conventional biochemical tests including Mannitol salt agar culture, catalase production test, coagulase test, and DNase production test [11].

### Antibiotic susceptibility testing:

Antibiotic susceptibility was done using the Kirby-Bauer disc susceptibility technique [12], using a standard reference strain, *S. aureus* ATCC (6538), and results were interpreted with the guidance of the Clinical Laboratory Standards Institute (CLSI 2020) [13].

The following 17 antibiotic discs obtained from (Oxoid, UK) were used: Ampicillin-Sulbactam (SAM:20 µg), Amoxicillin-Clavulanic (AMC:30 µg), Flucloxacillin (FL:5 µg), Oxacillin (OX:1 µg), Piperacillin-Tazobactam (TZP:110 µg), Vancomycin (VA:30 µg), Azithromycin (AZM:15 µg), Cephalexin (CL:30 µg), Cefoxitin (FOX:30 µg), Cefepime (FEP:30 µg), Cefaclor (CEC:30 µg), Gentamicin (CN:10 µg), Clindamycin (DA:2 µg), Ciprofloxacin (CIP:5 µg), Levofloxacin (LEV:5 µg), Linezolid (LNZ:30 µg), and Rifampicin (RA:5 µg).

### Cefoxitin disc diffusion test:

Using a 30 µg disc, the test was performed on each isolate. The isolate was converted into a 0.5 McFarland standard, and grass culture was

performed on the Mueller Hinton agar (MHA) plate. Zone diameters were measured after plates were incubated for eighteen hours at 37°C. Oxacillin resistance was defined as an inhibition zone diameter of less than 19 mm, whereas sensitivity to oxacillin was defined as a diameter of more than 20 mm [14].

### PCR testing:

**DNA Extraction:** *S. aureus* genomic DNA was extracted using the boiling method [15], one to two colonies from overnight cultures were suspended in 500µl of sterile distilled water, then this mixture was boiled at 100 °C for fifteen minutes. After 5 minutes of 14,000 rpm centrifugation, 2µl of the supernatant was used as the genomic template for subsequent testing.

### Polymerase Chain Reaction:

Table (1) listed the primers used in that study for the determination of genes encoding methicillin and penicillin-like antibiotics resistance (*mec A*), panton valentine leukocidin (*pvl*), toxic shock syndrome toxin ( *tst-1*), staphylococcal enterotoxin b (*seb*), fibronectin-binding protein A (*FnbA*), α-hemolysin (*hla*), and intracellular adhesion A (*icaA*). Table (2) illustrates the polymerase chain reaction (PCR) conditions using The DNA thermocycler.

### Gel electrophoresis:

For visualizing the PCR products, 5 µl of the PCR amplicons were loaded in a (1.5) percent agarose gel containing fluorescent ethidium bromide dye (0.5 mg/ml, Medox Biotech India Pvt Ltd) and a molecular weight ladder (1500bp DNA marker; H3 ready to use, Gene Direx). After two hours of electrophoresis at 80 V, different amplified DNA samples were examined using a UV lamp set at 264 nm.

### Statistical analysis:

The data were analyzed using  $\chi^2$  using SPSS version25 (SPSS, Inc., Chicago, IL, USA). The results were considered significant when  $P \leq 0.05$ .

## RESULTS

Ninety-five (40.77%) *S. aureus* isolates were recovered from 233 collected nasal specimens, including 46 (48.4%) isolates from healthcare workers and 49 (51.6%) from community people.

*S. aureus* was more prevalent in female patients than male ones.

It was observed that *S. aureus* and MRSA were highly detected in the age group of (21-30) years, while the lowest percentage of their isolation was detected in the age group between (0-10) as shown in (table 3).

#### Antibiotic susceptibility test:

Seventeen antibiotics were tested in this study on 95 (40.77%) *S. aureus* isolates, it was found that the highest resistance percentage of *S. aureus* isolates was to flucloxacillin for community isolates and to oxacillin for healthcare personnel staph isolates while the least resistance percentage was to clindamycin for all isolates. Table (4) and Fig. (1) showed the resistance pattern of *S. aureus* isolates, there was no significant difference between *S. aureus* resistance percentages from healthcare workers and community people for all tested antibiotics except for oxacillin.

#### Detection of MRSA:

Out of 95 *S. aureus* isolates, 59 (62.1%) strains were detected as methicillin-resistant staph aureus (MRSA) while 36 (37.9 %) isolates were methicillin-susceptible staph aureus (MSSA), this result was obtained by cefoxitin-resistance testing then confirmed by PCR test for *mecA*

gene. It was detected in healthcare workers (30, 50.8%) more than in community people (29, 49.2%), table (5). In addition, MRSA was found in females (34, 57.6%) more than in males (20, 42.4%).

#### Virulence genes:

It was found that 29 (30.52%) *S. aureus* strains harbored *pvl* gene. Pantone valentine leucocidin-positive *S. aureus* isolates were detected more detected in community people, 21(72.4%) than in healthcare workers, 8 (27.6%). Out of 29 community MRSA isolates in this study, there was a high prevalence of *pvl* (14, 48.27%), however, the prevalence of *pvl* among 30 MRSA isolated from healthcare workers was 5 (16.6%). The percentage of the *pvl*-positive MRSA was higher than the *pvl*-positive MSSA.

It was observed that the distribution of virulence genes among *pvl* positive strains was as follows: *mecA*, *hla*, *fnbA*, and *seb* genes were more detected in community people, they were detected in 14 (48.3%), 10 (34.5%), 4 (13.8%) and 2 (6.9%) respectively. *IcaA* gene was only detected in healthcare workers' *pvl* positive strains (8, 28%), while the *tsst-1* gene wasn't detected among all *S. aureus pvl* positive strains as shown in Fig (2) and Table (6).

**Table (1).** sequences of primers

Genes	Primer sequence (5' to 3')	Amplicon size (bp)	References
<i>Mec A1</i> (F)	GTA GAA ATG ACT GAA CGT CCG ATA A	310	(16)
<i>Mec A2</i> (R)	CCA ATT CCA CAT TGT TTC GGT CTA A		
<i>Luk-PV-1</i> (F)	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	(16) & (17)
<i>Luk-PV-2</i> (R)	GCATCAAGTGTATTGGATAGCAAAAGC		
<i>hla</i> (F)	CTGATT ACT ATC CAA GAA ATT CGA TTG	209	(18)
<i>hla</i> (R)	CTTTCC AGC CTA CTT TTT TAT CAG T		
<i>seb</i> (F)	ACATGTAATTTTGATATTCGCACTG	667	(19)
<i>seb</i> (R)	TGCAGGCATCATGTCATACCA		
<i>fnbA</i> (F)	GCG GAG ATC AAA GAC AA	1279	(20)
<i>fnbA</i> (R)	CCATCTATAGCTGTG TGG		
<i>tst</i> (F)	ACCCCTGTTCCCTTATCATC	326	(21)
<i>tst</i> (R)	TTTTCAGTATTTGTAACGCC		
<i>icaA</i> (F)	GATTATGTAATG TGCTTGGA	770	(18)
<i>icaA</i> (R)	ACTACT GCT GCG TTAATAAT		

**Table (2).** PCR Amplification conditions

Genes	Denaturation initially	Denaturation	Annealing	Extension	Extension Finally	Cycles (n)	Product (base pair)	References
<i>Luk-PV</i>	94 °C for 4 min	94 °C for 45 s	56 °C for 45 sec	72 °C for 30 sec	72 °C for 2 min	30	433 bp	(16)
<i>Mec A</i>	94 °C for 4 min	at 94 °C for 45 s	56 °C for 45 s	72 °C for 30 s	72 °C for 2 min	30	310 bp	(16 & (17))
<i>Hla</i>	95 °C for 5 min	95 °C for 50 s	58 °C for 30 sec	72 °C for 60 sec	72 °C for 10 min	35	209 bp	(18)
<i>Seb</i>	95 °C for 5 min	95 °C for 60 sec	55 °C for 45 sec	72 °C for 60 sec	72 °C for 10 min	35	667 bp	(19)
<i>FnbA</i>	95 °C for 5 min	95 °C for 60 sec	47 °C for 60 sec	72 °C for 90 sec	72 °C for 5 min	40	1279 bp	(20)
<i>Tst</i>	94 °C for 5 min	94 °C for 60 sec	54 °C for 60 sec	72 °C for 60 sec	72 °C for 7 min	35	326 bp	(21)
<i>ica A</i>	95 °C for 5 min	95 °C for 60 sec	50 °C for 1 min	72 °C for 1.5 min	72 °C for 5 min	30	770 bp	(18)

**Table (3).** prevalence of Staph. aureus and MRSA according to age group.

Age group (years)	<i>S. aureus</i> isolates	MRSA
0-10	2 (2.1%)	1 (1.7%)
11-20	17 (17.9%)	14 (23.7%)
21-30	42 (44.2%)	26 (44.1%)
31-40	14 (14.7%)	8 (13.5%)
41-50	13 (13.7%)	9 (15.3%)
51-60	7 (7.4%)	1 (1.7%)

**Table (4):** Antibiotic susceptibility testing.

Antibiotic	Healthcare workers N=46			community N=49			P value
	S	I	R	S	I	R	
Amox-Clavulanic	18(39.1%)	0(0%)	28(60.9%)	18(36.7%)	0(0%)	31(63.3%)	0.810
Ampicillin-Sulbactam	20(43.5%)	0(0%)	26(56.5%)	24(49%)	0(0%)	25(51%)	0.591
Azithromycin	39(84.8%)	5(10.9%)	2(4.3%)	41(83.7%)	2(4.1%)	6(12.2%)	0.197
Cefaclor	7(15.2%)	6(13%)	33(71.7%)	8(16.3%)	4(8.2%)	37(75.5%)	0.740
Cefepim	30(65.2%)	0(0%)	16(34.8%)	27(55.1%)	0(0%)	22(44.9%)	0.315
Cefoxitin	17(37%)	0(0%)	29(63%)	19(38.8%)	0(0%)	30(61.2%)	0.855

Cephalexin	7(15.2%)	3(6.5%)	36(78.3%)	14(28.6%)	5(10.2%)	30(61.2%)	0.193
Ciprofloxacin	37(80.4%)	2(4.3%)	7(15.2%)	38(77.6%)	1(2%)	10(20.4%)	0.676
Clindamycin	42(91.3%)	4(8.7%)	0(0%)	40(81.6%)	8(16.3%)	1(2%)	0.318
Flucloxacillin	5(10.9%)	0(0%)	41(89.1%)	3(6.1%)	0(0%)	46(93.9%)	0.405
Gentamycin	38(82.6%)	1(2.2%)	7(15.2%)	40(81.6%)	4(8.2%)	5(10.2%)	0.351
Levofloxacin	40(87%)	1(2.2%)	5(10.9%)	40(81.6%)	2(4.1%)	7(14.3%)	0.751
Linezolid	44(95.7%)	0(0%)	2(4.3%)	42(85.7%)	0(0%)	7(14.3%)	0.098
Oxacillin	2(4.3%)	0(0%)	44(95.7%)	11(22.4%)	0(0%)	38(77.6%)	0.010*
Pipracillin-Tazopactam	35(76.1%)	0(0%)	11(23.9%)	36(73.5%)	0(0%)	13(26.5%)	0.769
Rifampin	30(65.2%)	6(13%)	10(21.7%)	33(67.3%)	6(12.2%)	10(20.4%)	0.976
Vancomycin	37(80.4%)	5(10.9%)	4(8.7%)	43(87.8%)	0(0%)	6(12.2%)	0.056

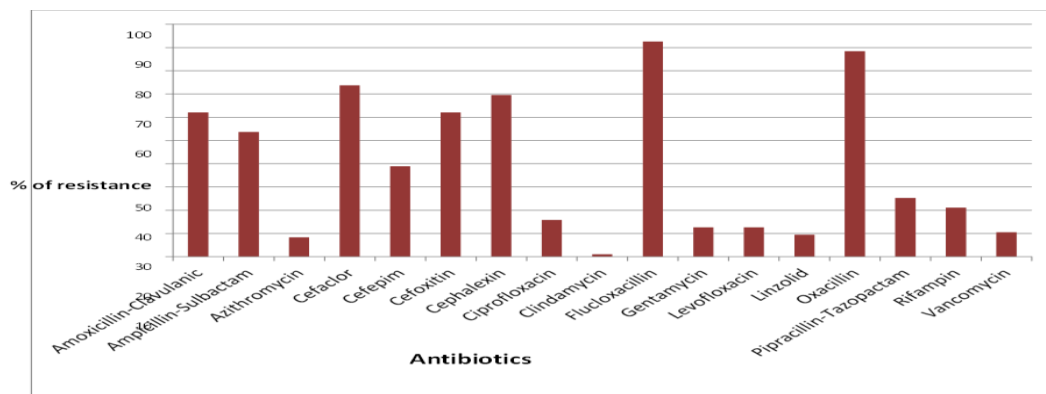
Chi-Square test, \*: Significance at P value < 0.05, I: intermediate, S: sensitive R: resistant

**Table (5).** MRSA and MSSA distribution in community and health care workers.

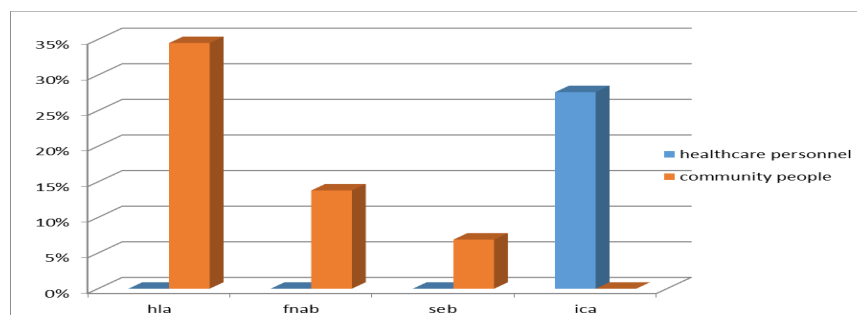
Total <i>Staph aureus</i> isolates		Community		Healthcare workers	
95		49 (51.6%)		46 (48.4%)	
MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
59 (62.1%)	36 (37.9%)	29 (59.2%)	20 (40.8%)	30 (65.2%)	16 (34.8%)

**Table (6):** Distribution of virulence genes in MRSA and MSSA strains harboring *pvl* gene isolated from healthcare workers and community people.

Virulence gene	<i>Pvl</i> + <i>S. aureus</i> isolates (29)			
	Healthcare workers (8)		Community (21)	
	MRSA	MSSA	MRSA	MSSA
<i>mec A</i>	5	3	14	7
<i>Hla</i>	-	-	8	2
<i>fnbA</i>	-	-	4	-
<i>Seb</i>	-	-	2	-
<i>Ica</i>	5	3	-	-
<i>tsst-1</i>	-	-	-	-



**Figure (1).** Antibiotic-resistance pattern of *S. aureus* is isolated from healthcare workers and community people.



**Figure (2).** Distribution of virulence genes among pvl positive healthcare workers and community *S. aureus* isolates.

## DISCUSSION

*Staph. Aureus* is considered one of the leading causes of serious infections with bad prognosis and increased medical care costs. Compared to infections produced by (MSSA), MRSA infections are associated with higher rates of hospitalization, death, and morbidity. Virulence factors such as hemolysins (*Hla* and *Hlb*), Panton-Valentine leucocidin (*pvl*), fibronectin-binding proteins A (*FnBPA*) and B (*FnBPB*), and toxic shock syndrome toxin-1 (*TSST-1*), almost all of them contribute to the pathogen's capacity for adhesion, colonization, and tissue invasion, thus enhancing pathogenicity [22].

As far as we know, this is the first study to determine the nasal carriage of *S. aureus* as well as MRSA, and *pvl* gene rate among healthcare workers and community people in Egypt's Minia governorate.

In our findings, *S. aureus* was detected in 95 (40.8%) nasal specimens consistent with that of Samsudin et al., [23] who revealed that 50.8% of isolates were *S. aureus*. On the other hand, our

findings showed that the overall prevalence of nasal *S. aureus* was higher than the results revealed by some previous studies [24], [25], [26] which showed that the distribution of *S. aureus* in nasal samples was (11%), (25.3%) and (26%) respectively.

Regarding gender, the overall incidence of *staph aureus*, especially MRSA nasal carriage was found higher in female swabs (57.6%) than in male swabs (42.4%). This finding is in line with the result obtained by Hogan et al., [24] who revealed that the incidence of MRSA was more common in women (12.7 %) than in men (8.0 %). A different result was obtained by Olsen et al., [26] and Abdel-Maksoud et al., [27] who noticed that MRSA nasal carriage was more prevalent in men than in women.

This study revealed that the distribution of *S. aureus* was (48.4%) and (51.6%) among healthcare workers and community people respectively, these results are in agreement with the results of Hogan et al., [24] who revealed that the distribution of *S. aureus* was (46.8%) and (53.2%) and among healthcare workers and



community samples respectively. In contrast, Abdel-Maksoud et al., [27] reported that the incidence of *Staphylococcus aureus* was higher among healthcare workers (71%) than that in the community (29%). Bettin et al., [28] found that *S. aureus* isolates among medical student samples were (22.1%).

The antimicrobial sensitivity test in this study showed that *S. aureus* isolates showed the least resistance to clindamycin (1.1%), this result is close to the results obtained by previous studies [28, 29, 30] that reported the high efficiency of clindamycin against *S. aureus* isolates.

Out of the 95 *Staph. Aureus* isolates, there were 59 (62.1%) isolates detected as MRSA while 36 (37.9 %) isolates were Methicillin-Susceptible (MSSA), which agrees with Song et al., [31], who recorded the prevalence of MRSA and MSSA by 58.1%, and 41.9% respectively. While Akhtar et al. [32] found that the incidence of MRSA was (45.3%).

Our study reported that *S. aureus* harboring *pvl* genes were present in 29 (30.52%) of total *S. aureus* isolates, this result is similar to Alli et al., [33] who reported (34.6%) *pvl* gene prevalence. Lower percentages (4.4%, 12.3%, and 8%) of *S. aureus* carrying the *pvl* gene were detected by Samsudin et al., [23], Darboe et al., [34] and Bhatta et al., [35] respectively. However, Hussein et al., [36] reported a higher incidence of the *S. aureus* harbored *pvl* genes (61.4%).

Our findings showed that 8 (17.4%) and 21 (42.9%) were the percentages of *pvl* gene incidence among healthcare workers and community people *S. aureus* isolates, these results conflict with the results obtained by Hogan et al., [24] and Akhtar et al., [32] who reported that the existence of *pvl* gene among healthcare workers and community people was (7.5%), (26%) and (11%), (13.8%) respectively.

The *pvl* gene prevalence among MRSA strains (32.2%) more than in MSSA strains (27.7%) and this result agree with the result found by Bettin et al., [28], Bhatta et al., [35] and Shrestha et al., [37] and opposite to the result found by Samsudin et al., [23], Pany et al., [38] and Tristan et al., [39] who reported that MSSA made up the high percentage of the *pvl*-positive isolates.

A high prevalence of *pvl* among CA MRSA isolates was detected at 48.27% (14/29) more

than *pvl* in MRSA isolates from healthcare workers at 16.6% (5/30) and this result was somewhat similar to the result found by Bhatta et al., [35] who reported that existence of *pvl* gene within community-acquired MRSA strains (16.7%) was higher than existence of *pvl* gene among healthcare workers MRSA strains (7.3%).

Our study reported that the virulence genes *mec A*, *hla*, *fnbA*, and *seb* genes commonly distributed among community people-*pvl* harboring strains as 14 (48.3%), 10 (34.5%), 4 (13.8%), and 2 (6.9%) respectively, while *ica A* gene was more prevalent in healthcare workers *pvl* positive strains (28%). Akhtar et al., [32] reported a high prevalence of the *fnb A* gene. Moazen et al., [40] reported a low prevalence of the *seb* gene among *S. aureus* strains and this result is similar to our finding.

In this study, the *tsst-1* gene was not detected among both healthcare workers and community people, this agrees with Moazen et al., [40], Machado et al., [41], and Li et al., [42], who reported a low prevalence of this gene.

Finally, our results revealed that the studied virulence genes were commonly distributed in MRSA harboring *pvl* gene more than MSSA-*pvl* positive strains, This is consistent with studies done by Song et al., [31] and Darboe et al., [34]. However, Machado et al., [41] found that the *hla* gene prevalence is more common in MSSA than in MRSA.

The differences between our results and other studies may be attributed to differences in geographical distribution.

## CONCLUSION:

Nasal carriage of *S. aureus* were detected in both community people and health care workers. Virulence genes (*mec A*, *pvl*, *hla*, *fnbA*, and *seb* genes) were more detected in strains isolated from community people than those isolated from healthcare workers.

## Ethical approval:

The Human Research Ethics Committee at the Faculty of Pharmacy at the University of Deraya, Minia, Egypt, approved the study (Approval No 1/2018).

**Conflict of Interest:** The authors declare no conflict of interest.

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#### Availability of data and materials

The datasets are available from the corresponding author upon reasonable request.

**Author Contributions:** Each author contributed to the project's planning, patient research, sample collection, data processing, or data interpretation. Each contributor contributed to the drafting and editing of the manuscript.

## HIGHLIGHTS

- Human anterior nares is colonized by *Staphylococcus aureus*, this represents a great risk of developing invasive infections.
- This study aimed to screen community people and healthcare workers for nasal carriage of *staphylococcus aureus* and to assess the incidence of virulence genes.
- This study detected higher percentages of virulence genes in strains isolated from community people than those isolated from healthcare workers, which represents a public health problem.

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