ORIGINAL ARTICLE

The Prevalence of Panton-Valentine Leukocidin (*PVL*) gene among *Staphylococcus aureus* Strains Isolated from Both Medical staff and Community People in Minia Governorate, Egypt

[1]Sahar A. Mandour*, [2]Gamal F. Mahmoud, [3] Salah M. Abdalla, [1]Fatma Y. Mohamed, [2] Heba A. Mohamed

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Deraya University, Minia, Egypt
[2]Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, Egypt
[3]Department of Microbiology and Immunology, Faculty of Pharmacy Suez Canal University, Ismailia, Egypt

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Corresponding Author:
Sahar A. Mandour, PhD
Department of Microbiology and Immunology, Faculty of Pharmacy, Deraya University, Minia, Egypt
Tel.: +20-102-665-7199
sahar.mandour@deraya.edu.eg

ABSTRACT

Background: *Staphylococcus aureus (S. aureus) is a major virulent human pathogen that is currently the most common cause of infections in hospitalized patients and community people. One of the key virulence factors of S. aureus that causes necrosis, apoptosis, and destruction of leucocytes is known as the Panton-Valentine Leukocidin (PVL) gene.* ***Objective:*** *was to study the distribution of PVL gene among the isolated S. aureus strains from nasal specimens of both medical and community people and compare their resistance profiles.* ***Methodology:*** *in our study, 233 nasal specimens were collected from both the medical staff of Minia University Hospital and the community people. S. aureus was identified by microscopical examination and conventional culture media. The antibiotic sensitivity test was done using a disc diffusion assay. Screening of PVL genes was done using the polymerase chain reaction (PCR).* ***Results:*** *Forty-six (48.4%) and 49 (51.6%) S. aureus isolates from medical staff and the community people were detected respectively. Among 29 PVL-positive S. aureus isolates, there were 8 (17.4%) isolates from medical staff and 21 (42.9%) isolates from community people. It was found that antibiotic resistance percentage among all PVL-negative S. aureus isolates was higher when compared to PVL- positive isolates.* ***Conclusion:*** *This study detected a statistically significant higher percentage of PVL-positive strains among community-isolated S. aureus than medical staff isolates. Therefore broader surveillance of PVL spread is recommended.*

INTRODUCTION

Staphylococcus aureus is a major and common medically important human pathogen, linked to hospital and community-acquired infections worldwide[1, 2]. *S. aureus* can be found on healthy people's skin and mucous membranes, most frequently in the nasal region[3]. Before the development of antibiotics, *S. aureus*-related invasive infections were frequently fatal[2].

Although the introduction of penicillin significantly improved the prognosis for patients with severe Staphylococcal infections, resistance developed after a few years of clinical usage as a result of the synthesis of β-lactamases [4].

Methicillin was designed to be resistant to β-Lactamase, but Methicillin-Resistant *S. aureus* (MRSA) strains were discovered shortly after methicillin was introduced into clinical practice[2]. MRSA belongs to the most dangerous bacteria, causing nearly half of all antibiotic-resistant organism-related deaths [5]. The advent of MRSA, which causes infections of the sinuses and ears, is caused by different risk factors. Widespread use of broad-spectrum antibiotics and prior nose operations are the main contributing factors [6,7].

The majority of infections are caused by *S. aureus* and MRSA and appear as skin infections or soft-tissue infections. However, life-threatening invasive infections have been concluded, including necrotizing pneumonia, necrotizing fasciitis, and sepsis-like syndromes[8]. Methicillin resistance is directed by a modified protein known as low-affinity PBP (penicillin-binding protein) which is encoded by the *mec A* gene and is located in the mec chromosomal mobile genetic elements of Staphylococcal cassette chromosome (*SCC mec*) [5]. *S. aureus* pathogenicity is linked to an extensive arsenal of virulence factors and toxins[8].

In addition to antimicrobial resistance, *S. aureus* can produce a wide range of virulence factors, including toxins and enzymes, hemolysins, and *PVL*[9]. *PVL*, a cytotoxin that causes white blood cells destruction, tissue necrosis, and apoptosis, *PVL* is known to be a stable marker of community-acquired MRSA strains[2,10].

PVL is regarded as a hallmark of community-acquired germs since it is frequently present in community-associated MRSA strains and infrequently in hospital isolates [11].

This study aimed to investigate the distribution of the *PVL* gene in nasal carriage *S. aureus* bacteria isolated from both medical staff in Minia University Hospital and community people in addition to the correlation between *PVL* gene carriage and antimicrobial resistance profile of *S. aureus* in these isolates.

METHODOLOGY

Collection of sample

Two hundred and thirty-three fresh nasal swabs were collected randomly from medical staff in Minia University Hospitals and community people during the period between May 2018 to April 2019, including 104 and 129 nasal specimens from medical staff and community people respectively. Patients were between (0-60) years old. These samples were handled following clinical specimen collection guidelines (CLSI, 2020)[12]. A personal questionnaire was filled in and all recorded data were entered into a Microsoft Excel spreadsheet.

Ethical approval:

Written patient consent was obtained prior to registration from all participants in this study. The study protocol was in agreement with the Helsinki Declaration and was approved by the Ethics Committee in Human Research of the Faculty of Pharmacy, Deraya University, Egypt (Approval No 1/2018).

Isolation of *S. aureus* and Identification:

Each clinical specimen was microscopically and phenotypically identified by routine culture and biochemical methods including Mannitol salt agar, coagulase test, catalase test, and DNase test, and aerobically incubated at 37℃ for 24 hours [13].

Antibiotic Susceptibility Tests:

Antibiotic Susceptibility was tested using the Kirby-Bauer disc diffusion technique as explained by the Clinical Laboratory Standards Institute (CLSI 2020)[12] using reference strain, standard *S. aureus* ATCC (6538). The following 17 commonly used antibiotic discs obtained from (Oxoid, UK) were used: Amoxicillin-Clavulanic (AMC:30 µg), Ampicillin-Sulbactam (SAM:20 µg), Azithromycin (AZM:15 µg), Cefaclor (CEC:30 µg), Cefepime (FEP:30 µg), Cefoxitin (FOX:30 µg), Cephalexin (CL:30 µg), Ciprofloxacin (CIP:5 µg), Clindamycin (DA:2 µg), Flucloxacillin (FL:5 µg), Gentamicin (CN:10 µg), Levofloxacin (LEV:5 µg), Linezolid (LNZ:30 µg), Oxacillin (OX:1 µg), Piperacillin-Tazobactam (TZP:110 µg), Rifampicin (RA:5 µg) and Vancomycin (VA:30 µg). The results were interpreted according to the (CLSI, 2020)[12].

Detection of *PVL* gene using PCR:

a. DNA extraction procedures:

Extraction of *S. aureus* genomic DNA template was done using the boiling method [14]. One to two colonies from fresh cultures were suspended in 500 µl of sterile distilled water, and the suspension was boiled at 100°C for 15 minutes. Then centrifuged at 14,000 rpm to separate the debris for 5 minutes, 2 µl of the clean supernatant was utilized as the template for PCR amplification, it can be stored at 20℃.

b. Screening *S. aureus* isolates for the *PVL* gene: Using the following primers in table (1).

Table 1. Primers and amplicon size of the *PVL* gene

Genes	Primer sequence (5' to 3')	Amplicon size (bp)	References
Luk-PV-1 **(F)**	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	11;15;16
Luk-PV-2 **(R)**	GCATCAAGTGTATTGGATAGCAAAAGC		

The DNA thermocycler was set up to do 35 cycles of amplification (denaturation for 45 s. at 94°C, annealing for 45 s. at 56°C, and extension for one min. at 72°C) and 35 cycles of initial denaturation for 5 min at 95°C, and a final extension for 10 min at 72°C.

Amplified PCR products were visualized by electrophoresis using agarose gel (1.5%) containing ethidium bromide (0.5 mg/ml, Medox biotech. India Pvt Ltd) and a ladder (M.Wt 100 bp DNA; H3 ready to use, 5x FIREPOL® Master Mix, Solis Biodyne, Estonia) and using primers as shown in Table (1). After 2 hrs. of electrophoresis at 80 V and multiple amplified DNA was analyzed by 264 nm wavelength UV Amplification of a fragment of DNA 433 bp corresponding amplification of a fragment of *PVL* genes [15]; [11].

Statistical analysis:

The data were analyzed using $\chi 2$ using SPSS version 25 (SPSS, Inc., Chicago, IL, USA). The results were considered significant when $P \leq 0.05$.

RESULTS

Incidence of *S. aureus* isolated from medical staff and community people:

Ninety-five (40.8%) *S. aureus* strains were recovered from 233 specimens. Out of them, 46 (48.4%) strains were found in medical members and 49 (51.6%) isolated strains were found among the community people.

Prevalence of *S. aureus* according to gender and age:

Our finding observed that *S. aureus* appears in a higher percentage in females (63%) than in males (37%) represented in fig (1). While the most percentage was in the age group harboring *S. aureus* was between 21-30 years old as shown in fig. (2).

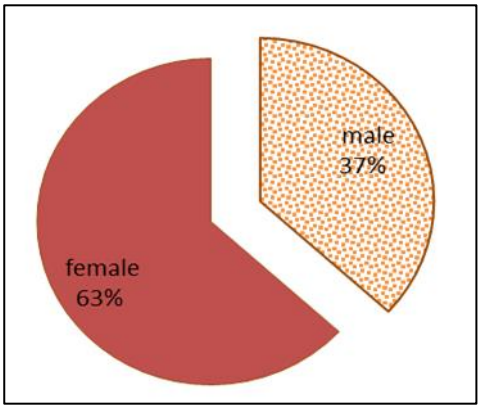

Fig. 1: Prevalence of *S. aureus* nasal isolates according to gender

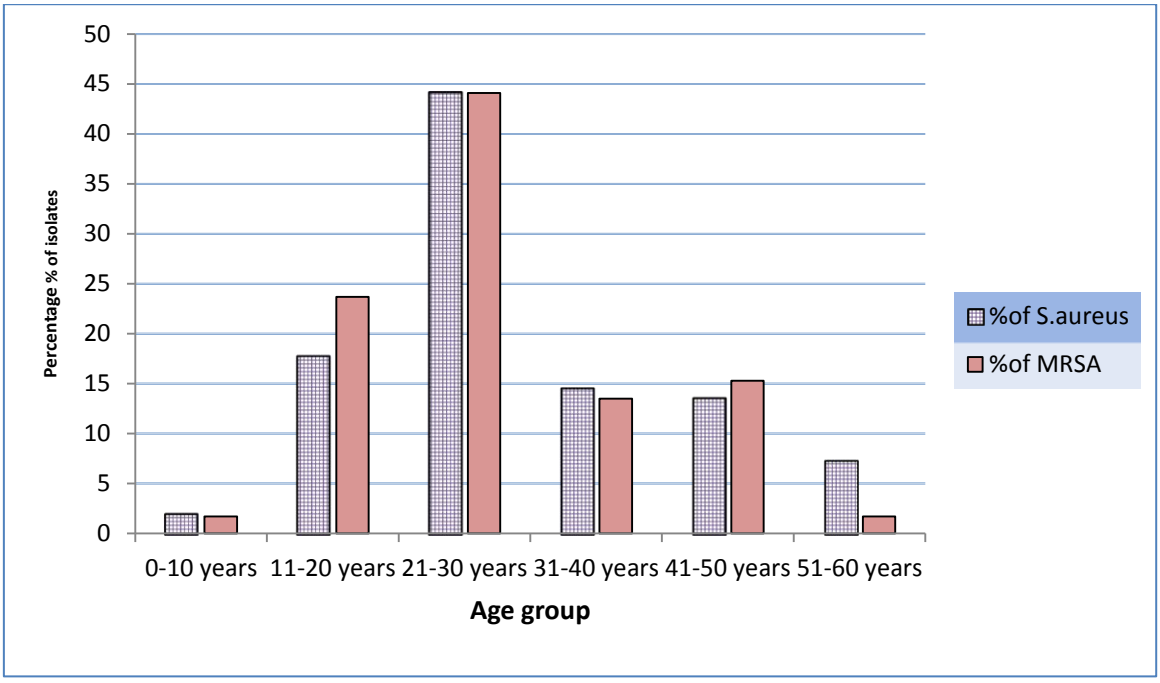

Fig. 2: Prevalence of ***S .aureus*** among age groups

Antibiotic susceptibility test:

In our study,17 commonly used antibiotics were tested against *S. aureus* isolates, and it was observed that the highest resistance *S. aureus* isolates to Flucloxacillin was (92.6%), and the lowest resistance was to clindamycin (1.1%) as shown in fig. (3). The resistance pattern of *S. aureus* was isolated from medical staff and community people observed that there were no significant differences between isolated *S. aureus* from medical staff and community people for all antibiotics except oxacillin.

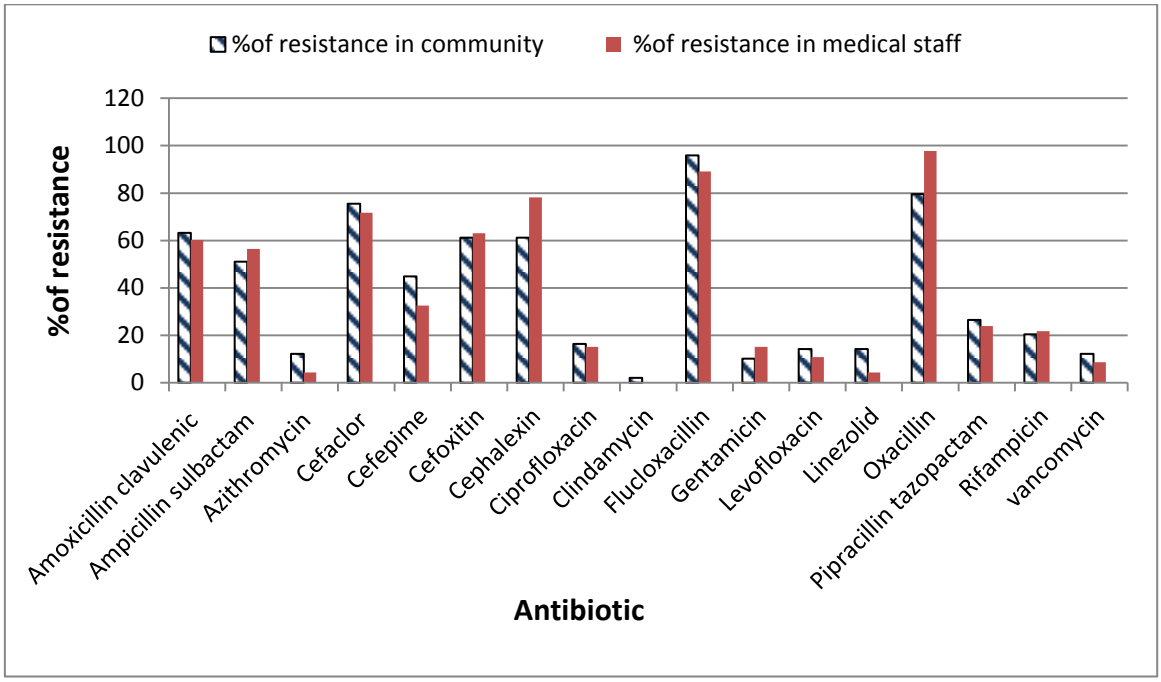

Fig. 3: Antibiotic-resistant pattern of *S. aureus* isolated from medical staff and community

PCR results:

The *S. aureus* isolates that harbored *PVL* genes were observed in 29 (30.52%) of the total *S. aureus* isolates. *PVL* genes were detected by PCR at 433 bp as shown in fig. (4).

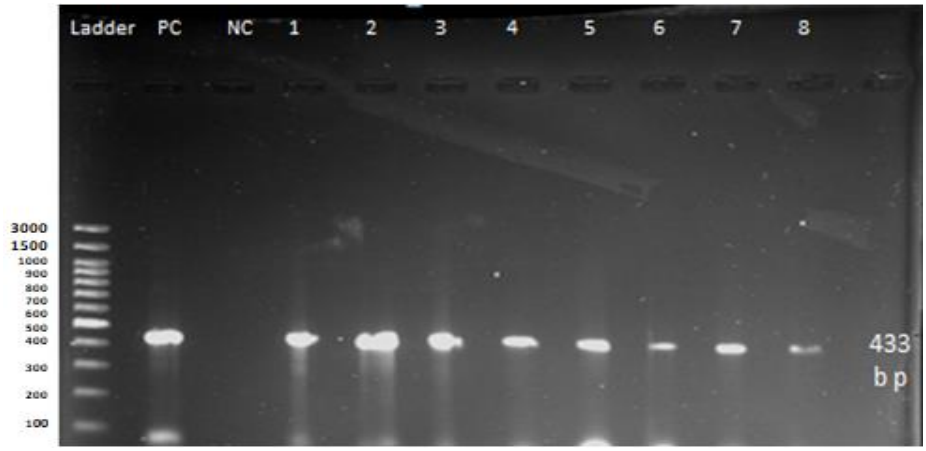

Fig. 4: Agarose gel electrophoresis showing amplification products of *PVL* gene by PCR; lane Pc: positive control lane Nc: negative control and lanes 1 to 8: positive PCR products (433bp)

The majority of *PVL*-positive *S. aureus* isolates were detected in 21 (42.9%) community people while 8 (17.4%) were found in medical staff as shown in table (2) and Fig. (5).

Table 2: Relation of *PVL* gene between different samples

		Community	Medical	*P value*
		N=49	N=46	
***PVL* gene**	**-ve**	28 (57.1%)	38 (82.6%)	***0.007****
	+ve	21 (42.9%)	8 (17.4%)	

*Chi Square test, *: Significant level at P value < 0.05*

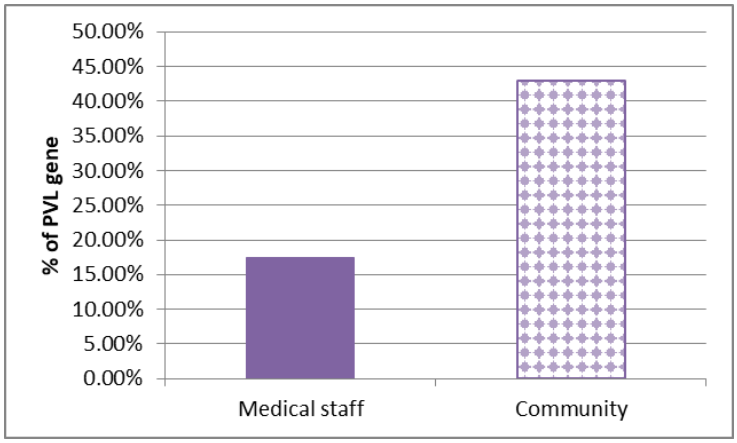

Fig. 5: Prevalence of *PVL* gene in medical staff and community

It was observed that the amoxicillin-clavulanic, cefepime, and oxacillin represent the high incidence of resistance pattern among *S. aureus PVL* harbored gene isolated from medical staff, while the high resistance rate for *PVL* harbored *S. aureus* isolated from the community was to flucloxacillin, oxacillin, amoxicillin-clavulanic, cephalexin, and cefaclor antibiotics.

Antibiotic resistance among *PVL* negative *S. aureus* isolates was found higher percentage when compared to *PVL*- positive *S. aureus* isolates as shown in table (3).

Table 3. Correlation of antibiotic sensitivity pattern with *PVL* gene among all *S. aureus* isolates

Antibiotics	***PVL*-negative** isolates **(N= 66)**			***PVL*-positive** isolates **(N= *29*)**			***P value***
	S	I	R	S	I	R	
Amoxicillin-Clavulanic	(25) 37.9%	(0) 0%	(41) 62.1%	(11) 37.9%	(0) 0%	(18) 62.1%	0.996
Ampicillin-Sulbactam	(34) 51.5%	(0) 0%	(32) 48.5%	(10) 34.5%	(0) 0%	(19) 65.5%	0.125
Azithromycin	(55) 83.3%	(6) 9.1%	(5) 7.6%	(25) 86.2%	(1) 3.4%	(3) 10.3%	0.585
Cefaclor	(8) 12.1%	(9) 13.6%	(49) 74.2%	(7) 24.1%	(1) 3.4%	(21) 72.4%	0.147
Cefepime	(38) 57.6%	(0) 0%	(28) 42.4%	(19) 65.5%	(0) 0%	(10) 34.5%	0.467
Cefoxitin	(26) 39.4%	(0) 0%	(40) 60.6%	(10) 34.5%	(0) 0%	(19) 65.5%	0.650
Cephalexin	(13) 19.7%	(6) 9.1%	(47) 71.2%	(8) 27.6%	(2) 6.9%	(19) 65.5%	0.678
Ciprofloxacin	(56) 84.8%	(2) 3%	(8) 12.1%	(19) 65.5%	(1) 3.4%	(9) 31%	0.082
Clindamycin	(56) 84.8%	(10) 15.2%	(0) 0%	(26) 89.7%	(2) 6.9%	(1) 3.4%	0.181
Flucloxacillin	(5) 7.6%	(0) 0%	(61) 92.4%	(3) 10.3%	(0) 0%	(26) 89.7%	0.654
Gentamycin	(56) 84.8%	(3) 4.5%	(7) 10.6%	(22) 75.9%	(2) 6.9%	(5) 17.2%	0.573
Levofloxacin	(57) 86.4%	(3) 4.5%	(6) 9.1%	(23) 79.3%	(0) 0%	(6) 20.7%	0.167
Linezolid	(60) 90.9%	(0) 0%	(6) 9.1%	(26) 89.7%	(0) 0%	(3) 10.3%	0.848
Oxacillin	(7) 10.6%	(0) 0%	(59) 89.4%	(6) 20.7%	(0) 0%	(23) 79.3%	0.188
Pipracillin-Tazobactam	(52) 78.8%	(0) 0%	(14) 21.2%	(19) 65.5%	(0) 0%	(10) 34.5%	0.170
Rifampin	(46) 69.7%	(6) 9.1%	(14) 21.2%	(17) 58.6%	(60) 20.7%	(6) 20.7%	0.283
Vancomycin	(53) 80.3%	(5) 7.6%	(8) 12.1%	(27) 93.1%	(0) 0%	(2) 6.9%	0.211

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

The multi-drug resistance (MDR) is noticed in 14 (48.3%) *S. aureus* isolates harboring the *PVL* gene, while extensive drug resistance (XDR) noticed in only one *PVL*-positive isolate The total results are discussed as shown in Fig.(6).

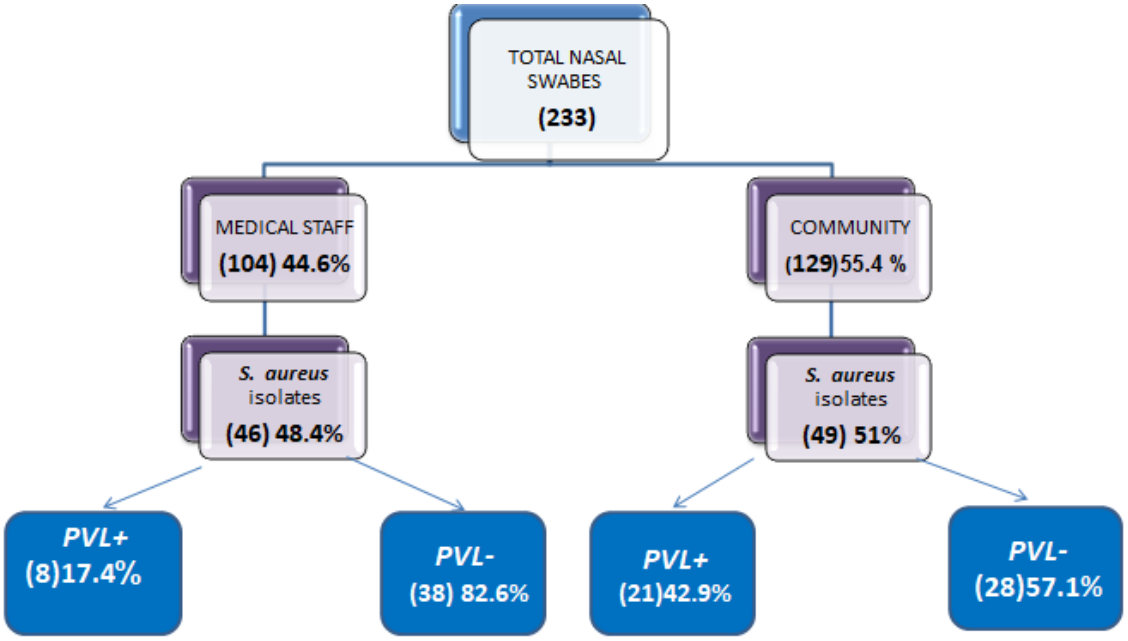

Fig. 6: Flow chart showing total study results

DISCUSSION

Staphylococcus aureus is considered one of the most researched microorganisms that cause serious infections with significant morbidity, mortality, and high health services costs. The major virulence factor of *S. aureus* is *PVL* gene, which induces lysis and apoptosis in leukocytes [1].

In our finding, the *S .aureus* isolates were demonstrated in 95 (40. 8%) nasal carriage specimens, in coincide with Samsudin et al. [1] who found that the (50.8%) isolates were distributed among total isolates. In disagreement with the study of Bettin et al.[7] who reported that the percentage of *S. aureus* isolates was (79.2%). The elevation in the percentage of *S. aureus* dissemination may be an alarm to increase care and hygiene [1].

Our findings revealed the distribution of *S. aureus* which were (48.4%) and (51.6%) among medical staff and community people respectively, these results are in contrast with the result discussed by Abdel-Maksoud et al. [17] who found the incidence of *S. aureus* was higher among medical staff (71%) than that found in the community (29%).

The antimicrobial sensitivity test in our study showed the lowest resistance pattern of clindamycin against (1.1%) *S. aureus* isolate, this result is similar to the results obtained by other reports which observed that the most efficient drug was clindamycin with a low resistance percentage [8; 18; 19].

Concerning the prevalence of MRSA in both sexes, the overall incidence of MRSA in this study was higher in females (57.6%) than in males (42.4%), this result is in agreement with the result obtained by El-Bouseary [7] who detected that the high prevalence of MRSA was observed in (62%), (38%) in female and male respectively.

As previewed in fig. (3) the most common age group that carried *S. aureus* and MRSA was between 21-30 years old, this finding is supported by El-Bouseary [7] who recognize that the age group between 21-30 years old as the most age groups that carry *S. aureus* and MRSA, while in this study the least group carrying *S. aureus* and MRSA, was between 0-10 years old this results in contrast with that obtained by the researchers who found that the age group between 0-10 years old was the most group carrying *S. aureus* and MRSA [7].

Our finding reported that *S. aureus* harbored *PVL* genes were detected in 29 (30.52%) of *S. aureus* isolates. In contrast Darboe et al. [20] concluded that the higher incidence of *S. aureus* harbored *PVL* genes were (61.4%). This percentage is also higher than that found

by Samsudin et al. [1] who showed that *S. aureus* harbored *PVL* genes (4.4%) of total *S. aureus* isolates.

The prevalence of *S. aureus* harbored *PVL* gene among medical staff and community people was (17.4%) and (42.9%) respectively in this finding, these results were nearly similar to results discussed by Hussein et al. [21] who found that the percentage of *S. aureus* harbored *PVL* gene in medical staff and community were (7.3%) and (26.1%) respectively, also it is less than another report which showed that PVL gene was found in (9.8%) of medical staff isolates [22].

CONCLUSION

This study detected a significantly higher percentage (*P value* =0.007) of *PVL*-positive strains among community isolates than in medical staff isolates. *S. aureus* - infected females were found more than males so gender should be incorporated when treating patients and risk categorizing *S. aureus* –patients. The alarming prevalence of *PVL* gene should be treated by a coordinated approach and continued surveillance for its dissemination.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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