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Epidemiological Study on the Prevalence of *Staphylococcus aureus* PVL Gene Among Healthy Community in Al- Karkh and Al -Rusaffa Districts Baghdad, Iraq

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Abstract

Five hundred nasal swabs were taken from normal medical staff and public in the city of Baghdad. Several identification parameters were used to recognize the bacterial isolates. *S. aureus* isolations form nasal swabs were identified using morphology and VITECK 2 system. Polymerase chain reaction (PCR) was employed to determine PVL (Panton–Valentine leukocidin) gene in *S. aureus*. The data showed no significant evidence on the relationship between PVL gene presence and gender and age of the studied groups. There was no relation between the prevalence of PVL gene in the age groups of 21-30 years (p=0.328) and 31-40 years (p=0.682).

The results showed that 38.4% and 37.5% *S. aureus* isolates were isolated from nurses working at Baghdad Teaching Hospital and AL-Yarmouk Teaching Hospital, respectively, while, doctors, pharmacists, and radiology staff were negative (0.00%).

There was no significant difference between hospital staff and community in AL-Karkh (p=0.838) and in AL-Rusafa (p=0.118).

Keywords: Staphylococcus aureus plv gene, nasal swabs and healthy community

دراسة وبائية حول انتشار المكورات العنقوديةالذهبيه ذات جينPVL بين المجتمع الصحي في منطقتي الكرخ والرصافة بغداد ، العراق

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الخلاصه

تم أخذ مسحات الأنف من الفتحات الأمامية من العاملين الاصحاء في المستشفى ومجموعه اخرى من المجتمع. ومن ثم تم التحري عن العزلات البكتيرية بناءً على خصائص العزل والتعريف المختلفة عن طريق التحري من S. aureus باستخدام الكثير من الفحوصات المختبرية. استخدم تفاعل البلمرة المتسلسل (PCR)لتحديد جين pvl في المكورات العنقودية الذهبية.أظهرت البيانات عدم وجود دليل معنوي على العلاقة

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بين وجود الجين pvl والجنس والفئات العمرية لمجموعات الدراسة ، كما لا توجد علاقة بين انتشار جين PVL في الفئة العمرية (21–30 سنة p = 0.328 (و (31–40 سنة.0.682 =p)
أظهرت النتائج (38.4٪) و (37.5٪) المكورات العنقوديات. تم عزلها من الممرضات العاملات في مستشفى بغداد التعليمي ومستشفى اليرموك التعليمي على التوالي ، بينما كان الأطباء والصيادلة ومصورون الاشعه سالبًا بنسبه (0.00٪).
لم يكن هناك فرق معنوي بين طاقم المستشفى والمجتمع ع = 0.838 في الكرخ ، وبين طاقم المستشفى والمجتمع ع = 0.118 في الرصافه.

Introduction

S. aureus bacteria is considered as the most common cause of serious illnesses. It is highly distributed in clinical settings, being normally inhabitant of skin and nose of healthy human beings [1].

Staphylococcus aureus is dominant in nosocomial and community-acquired infections, being generally considered as the cause of post-surgical wound infections [2, 3].

Three percent of people are colonized persistently with *S. aureus*. It has a significant impact, particularly in hospital community. It is also responsible for lethal illnesses, such as those affecting the bones, heart, and blood stream [5].

Once *S. aureus* reaches into the interior tissues or bloodstream, it causes infection. It has a distribution in the cutaneous and mucosal barriers during the long-lasting skin and wounds infections. Lee *et al*. (2018) reported that patients who require invasive medical devices (such as peripheral and central venous catheters), elderly people, and low immunity patients, are mostly susceptible to *S.. aureus* infection [6].

S. aureus is considered as a life threatening pathogenic bacteria which can cause various human diseases. It exerts its activities via the production of many virulence factors, such as extra cellular proteins (e.g. cell surface proteins, adhesion proteins, enzymes, toxins) and various elements that assist bacteria to escape the body innate immunity. In addition, it exerts multi- drug resistance that facilitates bacterial existence and tissue invasion [7].

S. aureus produces many toxins, such as enterotoxin, shock toxin, cytolysins, exfoliate toxins, and super antigens [8, 9]. Panton–Valentine leukocidin (PVL) consists of two separate water-soluble proteins (Luk F-PV and Luk S-PV), which show cytolytic activities against a wide range of cells; however, they particularly affect leukocyte cells [10].

Grumann *et al.,* (2014) demonstrated a decent epidemiology connection between PVL and chronic or persistent skin and soft tissue infections (SSTIs), as well as necrotizing pneumonia, but the role of PVL in these diseases has remained under discussion [11].

In spite of the predictable value of PVL as a highly alert virulence factor, inadequate data are reported on its occurrence among *S. aureus* isolates from medical staff and un-symptomized individuals, in comparison to virulence strain has been isolated. Scientists are spotting light on the PVL gene–positive *S. aureus* obtained from nasal swabs of un-symptomized carriers in the public.

The aim of this study is to determine *S. aureus* PLV gene positive bacteria that colonize the health care community. We also aimed at comparing the data between the two districts of Baghdad (AL-Karkh and AL-Rusafa).

The findings illustrated no sign of prevalence of *S. aureus* PLV gene positivity in the hospital community. The data propose that PVL is unexpected to be involved in the pathogenesis of hospital nosocomial infect mainly in post-surgical wound infection.

Materials and Methods

Samples collection

Five hundred nasal swabs were taken with normal saline from healthy hospital workers of AL-Yarmouk and Baghdad Hospitals as well as from the general public in Baghdad. Samples were examined for *Staphylococcus aureus* during the period between April 2019 to August

2019 by using sterile cotton swabs. Physicians and others medical staff were examined during this study.

Isolation and identification of *S. aureus*

Nasal swabs were cultured on blood medium and mannitol salt agar medium. Inoculated culture media were incubated at 37 °C for 24 hours. *S. aureus* mannitol-fermenting colonies appeared in a yellow color. Additional macroscopic and microscopic examinations were implemented for suspected species. Biochemical tests were also implemented. All strains were screened and *S. aureus* was identified using several identification tests, such as Gram stain, catalase test, coagulase test, and VITECK 2 system.

Extraction of DNA

Nucleic acid materials were extracted from bacterial isolates according to Lilit [12]. Samples were stored under deep freeze conditions until the day of the experiments.

Polymerase chain reaction (PCR) of 16S *rRNA* Gene

Detection of luk s-luk f pvl gene by PCR

The test was carried out using PCR master mix, Promega), according to a previously described method [12].

Results and discussion

A total of 106 nasal swabs were taken from the staff of Baghdad Teaching Hospital. Among those, 13 (12.26%) were positive for *S. aureus*. Also, amongst 106 nasal swabs collected from the staff of AL-Yarmouk Teaching Hospital, only 8 (7.5%) were positive (Figure 1)

Table 1- Distribution of *S. aureus* bacteria according to gender and age form two sites in Baghdad (Baghdad teaching hospital and Al yarmok hospital)

Female	Male	
No. of isolates	**No. of isolates**	**Age group**
3	**0**	**20**
16	**7**	**30**
10	**7**	**40**
4	**7**	**41**
2	**2**	**50**

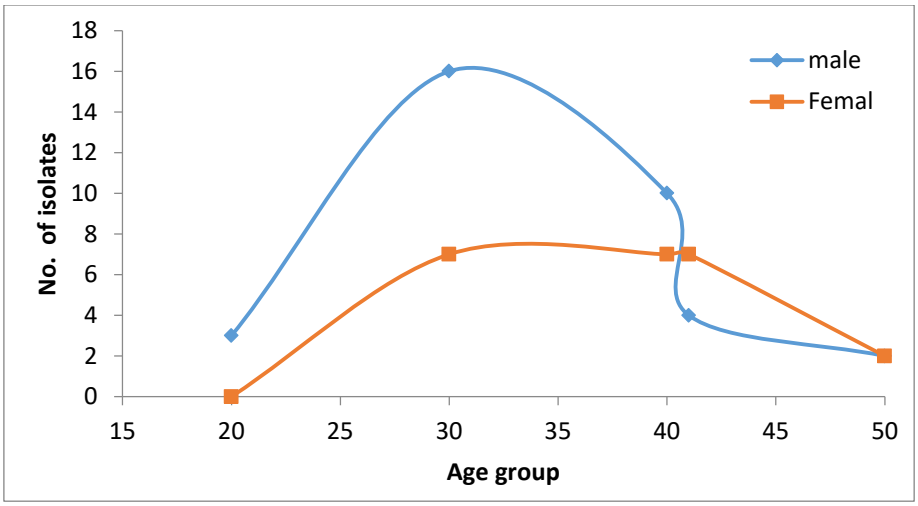

Figure 1-Distribution of *Staph aureus* bacteria according to gender and age form two sites in Baghdad (Baghdad teaching hospital and Al yarmok hospital).

Detection of PLV gene using PCR

Out of 206 *S. aurus* isolates, only 13 were confirmed to be *S. aureus*, which were then examined for their PVL gene. The data showed a significant prevalence of PVL gene in the males with the age of 21-30 years, with 16 *S. aureus* bacteria, while a limited isolated only 2

were positive for PVL gene with 12.5%.Hwever, only 7 isolates were recorded in females, all of which being negative for PLV gene.

The frequency of gene occurrence was different according to gender and age. PVL gene expression value in age 21-30 years in male was 2 (12.5%) and 2 (28.6%) female in age 41-50 years, while male 1(10%) and female 1(14.3%) in age 31-40 years (Table 2). These data are compatible with other previous findings which showed that 56% of 24 years old female patients carried pvl positive Methicillin-resistant *S. aureus* MRSA strains [13].

These findings agreed with those of earlier studies [14, 15] which illustrated that *luk-pv* gene appeared with in 40.62% of clinical samples from younger age group (less than 15 years old). In addition , a significant presence of PVL age group was detected in subjects with age of less than 15 years in comparison to older patients. Related results were reported in India [16]. Epidemiological studied reported that *pvl* gene has a noticeable impact on the pathogenesis of MRSA, although only limited evidence was published on the relation of pathogenicity of community acquired MRSA with the existence of PVL gene [17]. The role of PVL is to enhance pathogenicity of *S. aureus* via multiple biological processes, such as necrosis and programmed cell death [18]. However, the worldwide patterns of PVL among MRSA isolates are different. A limited incidence of PVL was reported from different regions around the world. For example , in Europe, the incidence was a low as 5 % , while it was 8.1 % in Saudi Arabia [15] and 14.3% in Bangladesh [19]. Another work [20] detected *pvl* gene in a single bacterial isolate (1%) among 65 bacteremia patients, 2 (2.19%) isolates from 91 patients with cutaneous infections, and 4 (7.27%) isolates from 55 patients with respiratory tract infections.

Table 2- Distribution of pvl gene positivity in study subjects according to the gender and age.

Age			PVL gene		Total	p- value
			Positive	Negative		
<=20	Male	Count		3	3	
		% within Gender		100.0%	100.0%	
	Total	Count		3	3	
		% within Gender		100.0%	100.0%	
21-30	Male	Count	2	14	16	
		% within Gender	12.5%	87.5%	100.0%	0.328
	Female	Count	0	7	7	
		% within Gender	.0%	100.0%	100.0%	
	Total	Count	2	21	23	
		% within Gender	8.7%	91.3%	100.0%	
31-40	Male	Count	1	9	10	
		% within Gender	10.0%	90.0%	100.0%	0.682
	Female	Count	1	6	7	
		% within Gender	14.3%	85.7%	100.0%	
	Total	Count	2	15	17	
		% within Gender	11.7%	88.3%	100.0%	
41-50	Male	Count	0	4	4	
		% within Gender	0.0%	100.0%	100.0%	

	Female	Count	2	5	7	
		% within Gender	28.6%	71.4%	100.0%	
	Total	Count	2	9	11	
		% within Gender	18.2%	81.8%	100.0%	
>50	Female	Count		2	2	
		% within Gender		100.0%	100.0%	
	Total	Count		2	2	
		% within Gender		100.0%	100.0%	

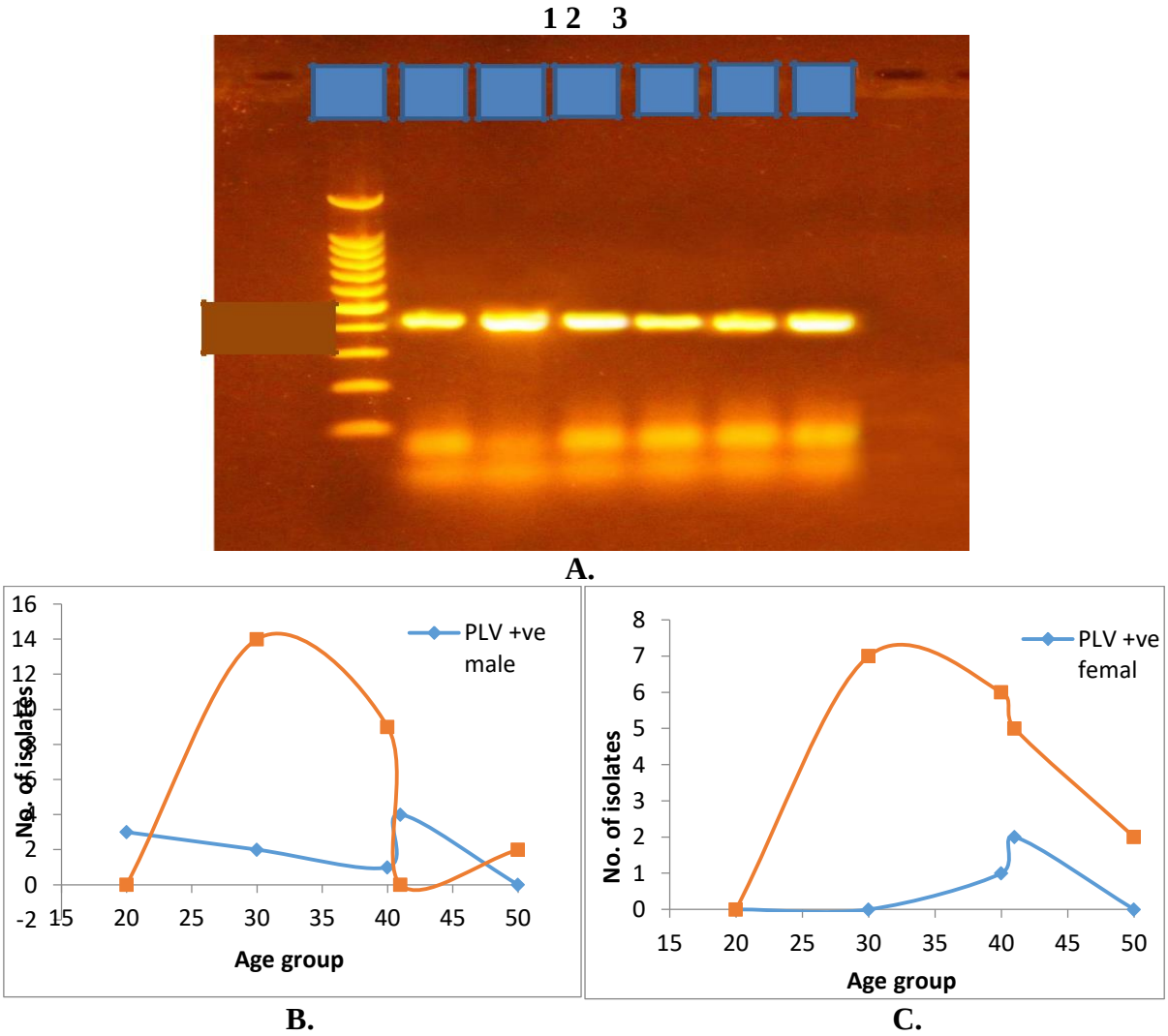

Figure 2 - A. distribution of *S. aureus* according to gender and age in males. B. distribution of *S. aureus* according to gender and age in females in subjects from Baghdad teaching hospital and Al yarmok hospital in Baghdad. C. Agarose gel showing amplification of LUK S-LUK f PVL gene (433bp), Lane 1: 100bp ladder. Lanes 2,3,4,5,6,7: positive *luk s-luk f* pvl gene.

Staphylococcus aureus isolates distribution among nasal swabs from staff of two Baghdad Hospitals

A total of 106 nasal swabs were taken from the staff of Baghdad Teaching Hospital, among which 13 were positive for *S. aureus*. Also, 106 nasal swabs were collected from the staff of AL-Yarmouk Teaching Hospital, of which 8 were *S. aureus* positive.

There was no significant association between the number of *S. aureus* isolates collected from the two hospitals (p=0. 251). The highest percentage of *S. aureus* was isolated from nurses of Baghdad Teaching Hospital (38.4%) and AL-Yarmouk Teaching Hospital (37.5%), while is the value recoded from doctors, pharmacists, and radiology staff was (0.00%).

Table 3- Comparison of *Staphylococcus aureus* isolates among nasal swabs from staff of Baghdad Teaching hospital and Al-Yarmouk Teaching hospital.

Job		Hospital				Total
		Baghdad		Al-Yarmouk		
		Nasal swab	positive	Nasal swab	positive	
doctors	Count	13	0	13	1	26
	% within Hospital	12.2%	0.00%	12.2%	12.5%	12.3 %
lab workers	Count	19	4	23	2	42
	% within Hospital	17.9%	30.7%	21.6%	25%	19.9 %
Medical assistants	Count	14	1	2	0	16
	% within Hospital	13.2%	7.6%	1.8%	0.00%	7.5%
nurses	Count	27	5	13	3	40
	% within Hospital	25.5%	38.4%	21.8%	37.5%	18.9 %
pharmac -ists	Count	12	0	21	0	33
	% within Hospital	11.4%	0.00%	12.2%	0.00%	15.5 %
physioth -erapists	Count	4	1	1	0	5
	% within Hospital	3.7%	7.6%	4%	0.00%	2.4%
radiology staff	Count	5	0	10	0	15
	% within Hospital	4.7%	0.00%	9.4%	0.00%	7%
sub staff	Count	12	2	23	2	35
	% within Hospital	11.4%	15.3%	21.6%	25%	16.5 %
Total	Count	106	13	106	8	212
	% within Hospital	100.0 %	100.0%	100.0 %	100.0%	100.0 %

No significant difference, Chi-Square Test p=0.251.

A comparison between community and hospital staff according to district in Baghdad

A total of 295 nasal swabs were taken from community and hospital staff in AL-Karkh. The results showed that 59.1% of community and 62.5% of hospital staff were positive for *S. aureus*. However, among 205 nasal swabs collected in AL-Rusafa, 40.9% of community and 37.5% of hospital staff were positive for *Staph aureus* . There was no significant difference between hospital staff and community in AL-Karkh (p=0.838) and AL-Rusafa (p=0.118) (Table 4).

The nasal swabs taken from medical workers showed a noticeable percentage of *staphylococcus aureus* isolates in nurses (20%) and physiotherapists (20%)) in comparison to doctors (3.8%), medical assistants (6.2%), sub staff (11.4%), and lab workers (14.2%), while no *S. aureus* was isolated from pharmacists and radiology staff.

The total percentage of *S. aureus* isolates collected from Baghdad Hospital (12.2%) was higher than that from AL-Yarmouk Hospital (7.5%).

These findings are in consistence with those from a previous work [21], which demonstrate that percentages of *S. aureus* of 25% , 22.8%, and 21% in doctors , nurses and nursing assistant staff, respectively. Moreover, Egwuatu *et al* (2013) stated that 4.6% of doctors and nurses did not wear gloves during clinical examination, while 6.9% did not replace their gloves in-between patients observations [22].

The World Health Organization (WHO) claims that hand sensitization is an essential key point to prevent the spread of the infections among health care workers.

Another investigation [21] demonstrated that poor sanitation regulations within the hospital structural scheme, such as improper hand sensitizer and inadequate water cycling, may compromise the capability to prevent the distribution and explain cross-transmission between medical staff and patients.

The carriage rate of *S. aureus* isolated from community (59.1%) and hospital staff (62.5%) in AL-Karkh was higher than that from community (40.9%) and hospital staff (37.5%) in AL-Rusafa.

Table 4- A comparison between community and hospital staff within AL-Karkh and AL-Rusafa based on *S. aureus* positivity.

			Community		Hospital staff		Total	P- value
			Nasal swab	positive	Nasal swab	positive		
Residence	Karkh	Count	125	13	170	20	295	P=0.838
		% within residence	50%	59.1%	57.5%	62.5%	58.9%	
	Rusafa	Count	125	9	80	12	205	P=0.118
		% within residence	50%	40.9%		37.5%	41.1%	
	Total	Count	250	22	250	32	500	
		% within residence	50.1%	40.7%	49.9%	59.3	100.0 %	

Conclusions

In conclusion, this study showed that *S. aurues* plv gene positivity that colonized the hospital community is similar in both districts of Baghdad, while there was no sign prevalence of *S. aureus* plv gene in hospital community. This proposes that PVL is not an essential reason to be involved in the pathogenesis of hospital nosocomial infection mainly in post-surgical wound.

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