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OCCURRENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG THE HEALTHY HOSPITAL WORKERS, COMMUNITY AND CHARACTERIZATION FOR THE PRESENCE OF *luks-lukf* PVL GENE

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ABSTRACT
This was across-sectional study in which cultures from anterior nares were obtained from healthy hospital worker and community. Isolates were identified as *S. aureus* based on morphological and biochemical tests. Antibiotic susceptibility profiles were determined by the disk diffusion method. All isolates were further analyzed by PCR to determine the presence of PVL gene. Overall *S. aureus* nasal colonization prevalence was 10.8%, with the highest rate in healthy hospital workers (12.8%) while the lowest was detected in community (8.7%).The luks-lukf panton –valentine leukocidin in virulence gene was present in 11.1% of the 42 MRSA isolated.

KEYWORDS: luks-lukf PVL gene, Nasal carriage, Methicillin-resistant *Staphylococcus aureua*, Hospital health worker.

INTRODUCTION

Staphylococcus aureus is the most clinically significant species of staphylococci. It is responsible for a number of infections both relatively mild and life-threatening. *Staphylococcus aureus* can be recovered from almost any clinical specimen and is an important cause of nosocomial or hospital-acquired infection (Beilman *et al*., 2009; Mahon *et al*., 2011 Coates *et al.,* 2014). *Staphylococcus aureus* is frequent carried by healthy individuals on the skin and mucous membranes. Carriers serve as a source of infection to themselves and others by direct contact or contamination of food which can then result in food poisoning (Gopireddy, 2011; cornelissen *et al*., 2013).The fast increase in the spread of antibiotic resistant *Staphylococcus aureus* worldwide through the years has become a major public health problem and the main resistance developed by *Staphylococcus aureus* is to Methicillin. This is currently becoming a serious problem because of the related high morbidity and mortality indices, as well as a high prevalence in community-acquired infections (CA) (Baddour *et al*., 2006; Marquis, 2008; Cooke and Brown, 2010; David and Daum, 2010). Pathogenicity of *Staphylococcus aureus* depends on various bacterial surface components and extracellular proteins. However, the precise role of single virulence determinants in relation to infection is hard to establish. The frequent recovery of staphylococcal isolates that produce leukocidal toxins from patients with deep skin and soft tissue infections and severe necrotizing pneumonia, suggests that the Panton-Valentine leukocidin (PVL) is virulence factor that has a major role in pathogenicity (Pérez-Roth *et al*., 2010; Stevens *et al*., 2014). CA-MRSA strains frequently produce panton-valentine leukocidin. PVL pore forming exotoxin is encoded by two co-transcribed genes, lukS-PV and lukF- PV, carried on a prophage integrated in the *Staphylococcus aureus* chromosome (Alonzo, and Torres, 2014, DuMont and Torres, 2014).

MATERIALS & METHODS

Nasal swabs from 502 health hospital personnel of Baghdad teaching hospital, ALyarmok teaching hospital and community, Baghdad, Iraq were collected during the period of April to August 2015. Doctors, laboratory worker, pharmacist, radial photograph, physiotherapist, nurses, sub staff, medical assistant were included in the study. Nasal swabs were collected from anterior nares by using sterile cotton swabs, the specimens were inoculated onto blood agar and mannitol salt agar and inocubated at 37°Cfor 48 hours, mannitol fermenting colonies that were yellow or golden yellow. Colonies suggestive of *S. aureus* were identified using gram stain, catalase and coagulase tests.
Inoculation was then carried out on Mueller-hinton agar using asterile swab, after disk antibiotics were applied Oxacillin, Methicillin, Amikacin, Vancomycin, Gentamycin, Erythromycin, Tetracyclin , Chloramphenicol, Clindamycin.
Antibiotic susceptibility testing of all isolates was performed by modified Kirby bauer disc diffusion method as recommended by CLSI guidelines (CLSI, 2008).

DNA Extraction

Genomic DNA from all isolates was extracted using the following protocol.
1500µl of broth culture were added to microcentrifuge tube and centrifuged at 13,000xg for 1 minutes, 200 µl lysozyme were added to the pellet. The tube was left to incubate at 37°C in water bath for 60 minutes, then centrifuge for 1 minute. 600µl nucli lysis solution was added to the pellet. Gently pipet the cells are resuspended, incubate at 80°C for 5 minutes then cool to room temperature, 30µl of RNase solution were added to the cell lysate, invert the tube 2-5

times to mix, place in a 37°C water bath for 15 minutes, cool to room temperature . 300 µl of protein precipitation solution were added to the RNase-treated cell lysate, vortex at high speed for 20 seconds. Freezing for 5 minutes and finally centrifuge at 13,000 xg for 4 minutes. The supernatant was used as the source of DNA.

Detecting luks-lukf pvl gene by PCR

DNA was amplified in a 25µl PCR mix (PCR master mix, promega), 12.5µl gotaq green master mix 1µl of each primer, 1.5µl DNA template and 9µl H2O. The amplification conditions were initial denaturation at 95°C for 4 min, followed by 30 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds and one cycle of final extension at 72°C for 8 minutes, then cooled at 4°C.PCR amplicons were visualized using UV transilluminator after electrophoresis in 1µg/ml agarose at 100 volts for 90 minutes and stained using ethidium bromide (figure 1).

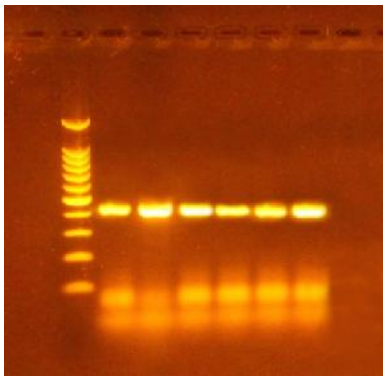

FIGURE 1: Agarose gel showing amplification of LUKS-LUKS PVL gene (433bp), Lane 1: reference 100bp ladder

RESULTS

a- clinical and prevalence features of all groups

The control *S. aureus* isolates was distributed in (8.7%) *S. aureus* positive and (91.3%) negative, (4.5%) pvl gene positive and (95.5%) negative, while patient (12.8%) *S. aureus* positive and (87.2%) negative, (15.6%) PVL gene positive and (84.4%) pvl negative. There was significant difference between control & patient among pvl gene positive p=0.006, and no significant difference between control & patient among *Staphylococcus aureus* positive p=0.548(Table1).

TABLE1: Distribution of *staphylococcus aureus* isolates from study subjects according to Patient, control with biochemical S. *aureus* positive and pvl gene

	Staphylococcus aureus		Total	PVL gene	
	Positive	Negative		Positive	Negative
Control	22	230	252	1	21
	8.7%	91.3%	100.0%	4.5%	95.5%
Patient	32	218	250	5	27
	12.8%	87.2%	100.0%	15.6%	84.4%
Total	54	448	502	6	48
	10.8%	89.2%	100.0%	11.1%	88.9%

TABLE 2: Distribution of *Staphylococcus aureus* isolates from study subjects according to the *staphylococcus aureus* gender, PVL.

	Staphylococcus aureus		Total	PVL gene		Total
	Positive	Negative		Positive	Negative	
Male	32	185	217	3	29	32
	14.7%	85.3%	100.0%	9.4%	90.6%	100.0%
Female	22	263	285	3	19	22
	7.7%	92.3%	100.0%	13.6%	86.4%	100.0%
Total	54	448	502	6	48	54
	10.8%	89.2%	100.0%	11.1%	88.9%	100.0%

b. Distribution of *Staphylococcus aureus* isolates from study subjects according to the biochemical *staphylococcus aureus* gender, PVL.

Staphylococcus aureus isolates was distributed in (14.7%) male and (7.7%) female, pvl gene isolated included (9.4%) male and (13.6%) female, as shown in table(1.2).There was

significant difference between *S. aureus* & gender p=0.012, and no significant between pvl gene & gender p=0.624 in table(2).

c- Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates.

All isolates of *S. aureus* were subjected for antibiotic susceptibility test. The resistance rate to methicillin was (77.7%) followed by oxacillin (75.9%) tetracycline (74.1%), gentamycin (40.7%), erythromycin (24.1%), clindamycin (7.4%), vancomycin and amikacin were found to be the most effective antibiotic with resistance rate of (3.7%), (0%) respectively(table 3).

TABLE 3: Antibiotic susceptibility pattern of *staphylococcus aureus*

antibiotic	Resistant	sensitive	p-value
methicillin	42(77.7%)	12(22.3%)	0.00
oxacillin	41(75.9%)	13(24.1%)	0.00
vancomycin	2(3.7%)	52(96.3%)	0.00
gentamycin	22(40.7%)	32(59.3%)	0.22
amikacin	0(0%)	54(100.0%)	0.00
chloramphenicol	5(9.3%)	49(90.7%)	0.00
tetracycline	40(74.1%)	14(25.9%)	0.001
erythromycin	13(24.1%)	41(75.9%)	0.00
clindamycin	4(7.4%)	50(92.6%)	0.00

d-Nasal carriage among hospital staff

There is no significant difference between the staff for positive nasal swab and MRSA P>0.05, the high percentage of MRSA isolated from physiotherapist &Medical assistant was (20%) followed by nurses (87.5%), Lab.work (66.6%),Sub staff(25%),while no MRSA isolated from doctor, pharmacist & Radial photograph in table (4).

TABLE 4: Incidence rat of MRSA among hospital staff

Staff	Nasal swab	positive	MRSA
doctor	26	1(3.8%)	0(0%)
Lab.work	42	6(14.2%)	4(66.6%)
nurses	40	8(20%)	7(87.5%)
Sub staff	35	4(11.4%)	1(25%)
pharmacist	33	0(0%)	0(0%)
Radial photograph	15	0(0%)	0(0%)
physiotherapist	5	1(20%)	1(100.0%)
Medical assistant	16	1(6.2%)	1(100.0%)
p-value		0.079	0.206
total	212	21(9.9%)	14(66.6%)

DISCUSSION

The nasal carriage rate of *S. aureus* was found to be 10.8% among the sample. 502, (12.8%) from the patients (hospital personnel) and 8.7% from the control (community). The rate of *S. aureus* isolated from patients was higher than those isolated from control (Table 1). In a similar study conducted by Shakya *et al*. (2010) they found that nasal carriage rate of 12.5% among patients, visitors and HCWs with nasal carriage rate among HCWs being 25% which is higher than our finding. These results agree with other studies which showed lower MRSA 2.1% in Nigeria ,7.7% in Serbia, 9.7% in Ukarine, other studies which ranged between 5.9%-15.6% (Nahimana *et al*., 2006; Wang *et al*., 2010; Adelowo *et al*., 2014; Cirkovic *et al*., 2014; Netsvyetayeva *et al*., 2014), while disagree with other studies which have high MRSA level, 21.2% in Sao Tome and Principle, 36.8% in Iran, 50% in Libya, 50.5% in Ireland, 66.4% in Scotland (Huggins *et al*., 2011 ;Matheson *et al*., 2012 ; Conceicao *et al*., 2013 ; Mohajari *et al*., 2013; El-Bouri & El-Bouri, 2013). Adelowo *et al*. (2014) observed differences in MRSA colonisation level between studies. These differences could be attributed to diversity in countries and hospitals along with differences in microbiological methods (from sampling technique to culture media), type of antibiotic used and periods of treatment. Furthermore, these differences might be noticed according to the laboratory methods that employed to detect and screen MRSA (Jacobus *et al*., 2007; Adesida *et al*., 2007).The carriage rate was higher among males (14.7%) than females (7.7%) (Table 2). These results agree with several studies (Klevens *et al*., 2007; Laupland *et al*., 2008; Mathanraj *et al*., 2009). Mathanraj *et al*. (2009) suggest that the explanation of the gender effect needs to conduct further studies, to investigate the possible role of hormones. The percentage of pvl gene was (11.1% included 15.6% pvl gene isolated from hospital personnel and 4.5% isolated from community (Table 1). This result is consistent with other studies in which pvl gene ranged between, 4.9% to 14.3% (Souza *et al*., 2006; san juan *et al*., 2012). On the other hand, the result of the present study was lower than estimates obtained by several researches (Souse *et al*., 2006; Ruimy *et*

al., 2008; Souza *et al*., 2010 ; Schaunburg *et al*., 2011; Kaur *et al*., 2012). Kaur *et al*. (2012) explained the high percentage of PVL producing MRSA as well as MSSA strains could be due to the carriage of large parts of SCC *mec* including the *luk-F* and *luk-S* genes to other resistant strains of *S. aureus* by various ways of recombination & also be due to misuse of antibiotics in the country causing selective pressure for development of resistant strains along with the virulence factor *i.e.* PVL. In this study it was found that the pvl gene was positive in hospital personnel higher than in community, while in other study pvl gene present in 83% of MRSA isolated ,are consistent with the characteristics of CA-MRSA reported in other studies (Huang *et al*., 2006;Dumitrescu *et al*., 2007; Bhattacharya *et al*., 2007). PVL gene occurrence in MRSA strains, typical of CA-MRSA, is known to cause diseases such as necrotizing pneumonia and necrotizing skin infections even in previously healthy individuals. It has relatively faster growth rate compared to HA-MRSA and is also highly transmissible (Milstone *et al.*, 2010), and is becoming more prevalent in the hospital settings. These characteristics would further increase the risk of MRSA infections in the hospital, and would further compound the problem of having few options in treating MRSA infections. The nasal swabs taken from hospital staff show the higher percentage of *staphylococcus aureus* isolated from nurus (20%) & physiotherapist (20%) & less percentage in doctor (3.8%) followed by medical assistant (6.2%) sub staff (11.4%), lab work (14.2%), while no *S. aureus* isolated from pharmacist & radial photograph (table 4). This result agreed with other study done by Conceicao *et al*. (2013) in which *S. aureus* carriage among physicians (25%) ,nurses (22.8%) & anurse aids (21%) and Egwuatu *et al*. (2013) who found 4.6% of doctors and nurses did not wear gloves. Carrier rates amongst clinical staff were 12.8% which is much higher than the results obtained by Kesah *et al*. (2003). Egwuatu *et al*. (2013) explain the high carriage rate among healthcare worker is an indication of poor infection control practices in the hospital and observed that workers with greater than 10 years of service were more likely to harbour MRSA. The World Health Organization claims that hand hygiene among HCW were the leading measure to prevent the spread of health care-associated infections. All isolated of *S. aureus* from nasal swabs were subject for antibiotic susceptibility test. The highest percent of antibiotic resistance to methicillin (77.7%) (Table (1.3), this result agrees with the result obtained by AL-Hasani *et al*. (2012) who demonstrated that the rate of resistance was (81%) & and with ALmaliki (2009) who showed that the rate of methicillin resistance *S. aureus* was (80.3%). Other studies found that *S. aureus* isolated show 100% methicillin resistance which was higher than our result (ALmaliki,2009; Bayat *et al*., 2011; Al-Hassnawi, 2012). The lowest over all MRSA proportion was found in Tunisia (18%) & morocco (at ibn rochd hospital, Casablanca) (19%) (Borg *et al.*, 2007).The relatively high percentage of resistance to these antibiotics was not attributed only to production of β-lactamase enzyme, but could be due to the decreased affinity of the target PBPs or decreased permeability of the drug into the cell (Jacoby & Munoz-Price, 2005). The lowest rate of resistance was detected for amikacin (0%) and vancomycin (3.7%) (Table 3).

These results agree with the results obtained by Kaup *et al*., (2013) who showed that *S. aureus* isolates from skin and wound infections were highly sensitive to vancomycin (15.7%). However, Sankarankutty, (2014) reported that the rates increased to 44.7% for the same antibiotic.

CONCLUSION

In this study we established a low percentage of pvl gene and nasal carriage of *S.aureus* in the healthy hospital workers and community but high percentage of MRSA. However, as there's only a small group of participants included in this study, larger studies are needed to further determine the carriage rate of *S. aureus* in this population.

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