BACTERIOLOGICAL AND PCR DETECTION OF PVL, MSRA AND MECA GENES AMONG STAPHYLOCOCCUS AUREUS ISOLATED FROM BURN WOUNDS

Shno Jalal Muhamad [a], Khanda Abdulateef Anwar [b], and
Sherko Ali Omer [b]

Submitted: 25/3/2019; Accepted: 6/11/2019; Published: 21/12/2019

ABSTRACT

Background

Methicillin-resistant *Staphylococcus aureus* is a pathogen that is associated with nosocomial and community-burn wound infection. *S aureus* produces Panton-Valentine -Leukocidin which results in the destruction of leukocytes. Resistance of *S. aureus* to macrolides, lincosamides, and streptogramin B is associated with the presence of an efflux pump, encoded by Methionine Sulfoxide Reductase A (*msrA* or *msrB)* genes.

Objectives

To isolate, determine the antibiotic susceptibility pattern, and to detect the presence of *pvl* and *msrA* genes from *Staphylococcus aureus*, isolated from burn wounds.

Materials and Methods

A total of 423 burn wound samples (218 from hospitalized and 205 from outpatients) were cultivated on different bacteriological media. Isolates were identified and *S. aureus* were further subjected to antibiotic susceptibility testing using disk diffusion method. Susceptibility to methicillin, oxacillin or cefoxitin, were used to determine methicillin-resistant *Staphylococcus aureus* strains. Polymerase chain reaction was used to detect *mecA, pvl,* and *msrA* genes in *S. aureus* isolates.

Results

Bacterial growth was detected from 170 (77.9%) of hospital samples and from 183 (89.26%) community-burn wounds. The predominant isolates were Gram-negative bacilli (71.76%) among hospitalized patients followed by *S. aureus* (22.35%). From the community samples, S*taphylococcus epidermidis* was the predominant isolate (86.9%), while few species of other Gram-positive organisms were also detected but no Gram-negatives were isolated. Among the 41 *S. aureus* isolates, the prevalence of methicillin-resistant *S. aureus* strains determined by oxacillin disk diffusion method was 58.53%, 65.85% by cefoxitin, whereas, 87.8% were positive for *mecA* gene by PCR. *Pvl* was detected in 3 (7.31%), while *mrsA* gene was detected among 17 (41.46%) of *S. aureus* isolates.

Conclusions

Infection with Methicillin-resistant *Staphylococcus aureus* was common in burn wounds. The prevalence of *msrA* gene among nosocomial and community-burn wound isolates of *S. aureus* was high, while few *S. aureus* isolates were found to carry *pvl* gene.

Keywords: *Burn wound, Methicillin-resistant Staphylococcus aureus, Panton-Valentine-Leukocidin, Methionine Sulfoxide Reductase .*

---

[a] Surgical Teaching Hospital, Sulaimani Directorate of Health, Kurdistan Region, Iraq.

[b] Department of Microbiology, College of Medicine, University of Sulaimani, Kurdistan Region, Iraq.
Correspondence: khanda.anwar@univsul.edu.iq

https://doi.org/10.17656/jsmc.10230

INTRODUCTION

Burn injury can cause considerable damage to the skin and break its defensive role. The injury destructs the cutaneous barrier and the vascular supply, which will lead to impairment of the local immune system (1). Burn wound infections are one of the most serious complications that occur in the acute period following burn injury (2). The probability of invasive burn wound infection will be determined by many factors including the immunocompromising effects of burns, prolonged hospital stay, intensive diagnostic and therapeutic procedures, patient factors such as age, extent of injury and depth of burn in combination with microbial factors such as type and number of organisms, enzyme and toxin production and motility (3, 4).

There is a moderately predictable time course of the microbial species that infect burn wounds; Gram-positive bacteria, such as staphylococci located deep within sweat glands and hair follicles can survive the thermal insult can colonize the wound surface at the first 48 hours (5). After an average of 5-7 days, burn wounds are colonized with Gram-negative bacteria derived from the host's normal gastrointestinal and upper respiratory flora, or from the hospital environment. Infection with yeasts and fungi tend to occur in the later stages (6).

Staphylococcus aureus has been recognized historically as a virulent and important human pathogen. It is still one of the most frequently encountered single bacterial species in hospitals and continues to be a frequent cause of burns and wound sepsis (7). Methicillin-resistant *Staphylococcus aureus* (MRSA) have become increasingly prevalent as nosocomial pathogens especially in burn units which are the leading cause of their death (8). Resistance to methicillin is determined by acquisition of a mobile genetic element, the staphylococcal cassette chromosome (SCC*mec*) that carries the *mecA* gene responsible for resistance to β-lactams in MRSA. According to the Clinical and Laboratory Standards Institute, (CLSI), screening with cefoxitin instead of oxacillin is recommended for MRSA detection (9).

Staphylococcus aureus produces a numbers of virulence factors such as Panton-Valentine-Leucocidin (PVL) and Methionine sulfoxide reductases (Msr). PVL is a cytolytic toxin encoded on a *S. aureus* bacteriophage integrated into the bacterial genome (10). PVL is expressed by *S. aureus* during human infection and it is demonstrated that PVL have a significant role in the pathogenesis of MRSA by targeting polymorphonuclear cells, monocytes and macrophages (11). PVL has been detected in large percentages from isolates that caused necrotic skin lesions, severe necrotizing pneumonia (12, 13), and furunculosis, especially in young and previously healthy adults (14).

Methionine sulfoxide reductases are enzymes that catalyze the reduction of methionine sulfoxide to methionine; these enzymes participates in the defense against oxidative stress by reducing methionine sulfoxide residues in proteins back to methionine (15). Resistance of *S. aureus* to macrolides, lincosamides, and streptogramin B is associated with presence of an efflux pump, encoded by Methionine Sulfoxide Reductase A (*msrA* or *msrB)* genes (16).

The aim of this study is to isolate, determine the antibiotic susceptibility pattern, and to detect the presence of *mecA, pvl*, and *msrA* genes from *Staphylococcus aureus*, isolated from burn wounds.

MATERIALS AND METHODS

This observational study was carried out in Emergency and Plastic Surgery Hospital in Sulaimani city from a period of January to October 2016. Two hundred and five burned patients from outpatient clinic (who not used antibiotics) and two hundred and eighteen burn patients who were admitted to different wards were included in this study.

Tissue samples from hospitalized patients were immediately processed by vortexing for 10 second in 5 mL of sterile normal physiological saline. One piece of tissue was inoculated into 10 mL of Sodium thioglycolate broth and incubated for 24 hours at 37°C. Subcultures were done on blood agar, mannitol agar, and MacConkey agar, all incubated at 37 °C for 18-24 hours. Swabs were used to inoculate the surface of different media that were processed as mentioned. Identification of the isolates were based on culture characteristics, Gram staining featuers, biochemical tests, API® 32 Staph system (BioMérieux, France) and Vitek® 2 system (BioMérieux, France), (17, 18).

Antimicrobial susceptibility test was performed using disk diffusion method according to the Kirby-Bauer method (19). The susceptibility was interpreted according to CLSI (9).

For polymerase chain reaction (PCR), *S. aureus* genomic DNA was extracted using Genomic DNA bacteria kit (Geneaid, USA) according to manufacturer's instructions. Three sets of primers were used for amplification of *mecA, luk-PV* and *msrA* genes (Table 1). The PCR mixture was prepared as following: 5 µL of template DNA 50 ng, 0.5 µL of each of forward and reverse primer (10 pmol/ µL), 12.5 µL of 2X Prime Taq Premix (GeNet Bio, Korea) and completed with DNase, RNase free H_2O to a final volume of 25 µL. The mixtures were amplified as shown in Table 2 (20, 21).

Electrophoresis was carried in 1.5% agarose using 5 µL of PCR product and a 100 bp DNA ladder (Geneaid, USA). DNA was visualized and documented under UV light (22).

Two samples from *msrA* PCR products and one samples from *pvl* PCR product were sent for DNA sequencing. Sequencing was performed by macrogen (http://dna.macrogen.com/eng/index.jsp) using BigDye®Terminator V3.1 Cycle Sequencing Kit (Applied Biosystem). DNAMAN (Lynnon Corporation, version 4.13) was used for the analysis of sequenced data. Nucleotide BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to query for the sequenced products.

Table 1. The primers used for amplification of *mecA, luk-PV* and *msrA* genes.

Primer name	Primer sequence	Amplicon size bp	Reference
mecA- For	5′-GTAGAAATGACTGAACGTCCGATGA-3′	310	21
mecA- Rev	5′-CCAATTCCACATTGTTTCGGTCTAA-3′		
luk-PV-For	5′- ATCATTAGGTAAAATGTCTGGACATGATCCA-3′	433	22
luk-PV-Rev	5′- GCATCAAGTGTATTGGATAGCAAAAGC-3′		
msrA- For	5′- GAAGCACTTGAGCGTTCT-3′	287	
msrA-Rev	5′- CCTTGTATCGTGTGATGT-3′		

Table 2. PCR amplification conditions of *mecA* gene (21), *luk-PV* and *msrA* genes (20).

Reaction	Cycling condition				
	Initial incubation	Denaturation	Annealing	Extension	Final extension
***mecA* gene**	94°C 5 min	94°C 60 sec	62°C 30 sec	72°C 35 sec	72°C 10 min
Number of cycles 30					
***luk-PV* gene**	95°C 5 min	94 °C 30 sec	55°C 30 sec	72°C 1 min	72°C 5 min
Number of cycles 30					
***msrA* gene**	95°C 5 min	94°C 30 sec	50°C 30 sec	72°C 30 sec	72°C 4 min
Number of cycles 30					

RESULTS

During the study period, 423 patients with burn injury were included. The number, gender, age, burn degree and sample obtained for culture in relation to the study group is shown in Table 3.

Among hospitalized patients, positive culture was detected in 170 (77.9%) of the 218 samples, while in the community patients, positive culture was detected from 183 (89.26%) of the 205 samples, Table 4.

The identification of growth results showed that the predominant isolates were Gram-negative bacilli (71.76%) among hospitalized patients followed by *S. aureus* (22.35%) indicating burn wound infection. From the community samples, *S. epidermidis*, a normal skin flora was the predominating (86.88%) and few species of other Gram-positive organisms but no Gram-negative bacilli were isolated from the community-burn samples, Table 5.

All isolated *S. aureus* (41) were subjected to antimicrobial susceptibility, Table 6. All *S. aureus* were resistant to methicillin; the hospital isolates showed resistance to ceftazidime (100%), and to erythromycin, penicillin G and amoxicillin-clavulanate. Resistance to the newer agents such as meropenem and imipenem was observed in 57.89% of the isolates, while all isolates were founded to be susceptible to vancomycin. The three community *S. aureus* isolate*s* were found to be all susceptible to oxacillin, imipenem, ciprofloxacin and doxycycline.

Comparison of *S. aureus* susceptibility to methicillin, oxacillin and cefoxitin and their possession of *mecA* were used to detect MRSA. All *S. aureus* isolates showed resistance to methicillin, while 65.85% showed resistance to cefoxitin and 58.53% to oxacillin. PCR detection of *mecA* gene in *S. aureus* has shown that 36 (87.8%) isolates were positive for this gene, Table 7 and Figure 1.

PCR detection of *mecA*, *pvl* and *msrA* genes in *S. aureus* isolated showed that *mecA* was detected in 36 isolates (33 hospitals and 3 community), *mrsA* was detected in 17 isolates (14 hospital and 3 community), while *pvl* was detected in 3 isolates (1 hospital and 2 community), Figures 1 and 2. The results of sequencing were subjected to BLAST tools and the query revealed to be firstly *Staphylococcus xylosus* macrolide resistance plasmid pJW231 *mph* (*C*), *msrA* and *erm* (48) genes, strain JW231 followed by *Staphylococcus aureus* pEP2104 *msr* (*A*) gene for ABC-F type ribosomal protection protein Msr (A) identity of 98%.

Table 3. The number, gender, age, burn degree and sample obtained for culture in relation to the study group (*n*=423).

Parameter	Hospital group	Community group	P-value
No. of patients	218	205	
Sex of patients			
Female	168 (77.1%)	106 (51.7%)	< 0.1
Male	50 (22.9%)	99 (48.3%)	
Age range (y)*			
Female	1-78	1-75	
Male	1-67	1-67	
Mean Age in year (SD)			
Female	24.5 (16.4)	19.2 (18.8)	
Male	18.8 (18.1)	17.7 (19.4)	
Degree of Burn			
Female			
First degree	0 (0%)	20 (18.9%)	
Second degree	40 (23.8%)	76 (71.7%)	< 0.1
Third degree	128 (76.2%)	10 (9.4%)	
Male			
First degree	0 (0%)	15 (15.2%)	
Second degree	37 (74%)	80 (80.8%)	< 0.1
Third degree	13 (26%)	4 (4%)	
Specimen obtained			
Swab	169 (77.5%)	205 (100%)	
Tissue	49 (22.5%)	(0%)	

***Age below one year was regarded as year**

Table 4. The results of growth among female and male samples in both the hospital and community group patients.

Sex of patients and culture results	Hospital group No. (%)	Community group No. (%)	P-value
Female patients	168	106	
Positive culture	132 (60.5)	94 (45.85)	0.0321
Negative culture	36 (16.5)	12 (5.85)	
Males patients	50	99	
Positive culture	38 (17.4)	89 (43.41)	0.0239
Negative culture	12 (5.5)	10 (4.87)	
Total	218 (100)	205 (100)	

Table 5. The bacterial species isolated from both the hospitalized and community group specimens.

Isolated microorganism	Hospitalized group No. (%)	Community group No. (%)
Staphylococcus aureus	38 (22.35)	3 (1.6)
Staphylococcus xylosus	3 (1.76)	5 (2.7)
Staphylococcus lentus	2 (1.1)	3 (1.6)
Staphylococcus hemolyticus	1 (0.5)	2 (1.1)
Staphylococcus capitis	1 (0.5)	1 (0.5)
Staphylococcus hominis	1 (0.5)	3 (1.6)
Staphylococcus simulans	1 (0.5)	0 (0)
Staphylococcus caprae	1 (0.5)	2 (1.1)
Staphylococcus epidermidis	0 (0)	159 (86.88)
Gram-positive bacilli	0 (0)	5 (2.7)
Gram-negative bacilli	122 (71.76)	0 (0)
Total	170 (100)	183 (100)

Table 6. Antimicrobial susceptibility test of *Staphylococcus aureus* isolates from the hospital and community group samples using disk diffusion test.

Antibacterial agent		Hospital *S. aureus* isolates (*n=38*)			Community *S. aureus* isolates (*n=3*)		
		R	S	I	R	S	I
Methicillin	No.	38	0	0	3	0	0
	%	100	0	0	100	0	0
Oxacillin	No.	24	14	0	0	3	0
	%	63.1	36.8	0	0	100	0
Ceftazidime	No.	38	0	0	3	0	0
	%	100	0	0	100	0	0
Cefoxitin	No.	25	13	0	2	1	0
	%	65.8	34.2	0	66.7	33.3	0
Ciprofloxacin	No.	26	11	1	0	3	0
	%	68.4	28.9	2.63	0	100	0
Gentamicin	No.	19	17	2	1	2	0
	%	50	44.7	5.26	33.3	66.7	0
Amoxicillin-Clavulanate	No.	30	8	0	2	1	0
	%	78.9	21.1	0	66.7	33.3	0
Imipenem	No.	22	16	0	0	3	0
	%	57.9	42.1	0	0	100	0
Meropenem	No.	22	16	0	1	2	0
	%	57.9	42.1	0	33.3	66.7	0
Vancomycin	No.	0	38	0	0	3	0
	%	0	100	0	0	100	0
Doxycycline	No.	22	9	7	0	3	0
	%	57.9	23.7	18.4	0	100	0
Erythromycin	No.	35	3	0	3	0	0
	%	92.1	7.9	0	100	0	0
Penicillin G	No.	30	8	0	3	0	0
	%	78.9	21.0	0	100	0	0
Piperacillin-Tazobactam	No.	31	7	0	2	1	0
	%	81.8	18.4	0	66.7	33.4	0

*** R: resistant, S: susceptible, I: intermediate response.**

Table 7. Susceptibility of *Staphylococcus aureus* isolates to methicillin, cefoxitin and oxacillin to determine MRSA phenotypically and PCR detection of *mecA*.

Susceptibility	Methicillin No. (%)	Oxacillin No. (%)	Cefoxitin No. (%)	*mecA* No. (%)
Resistant	41 (100)	24 (58.53)	27 (65.85)	36 (87.8)
Susceptible	0	17 (41.46)	14 (34.14)	5 (12.19)
Total	41 (100)	41 (100)	41 (100)	41 (100)

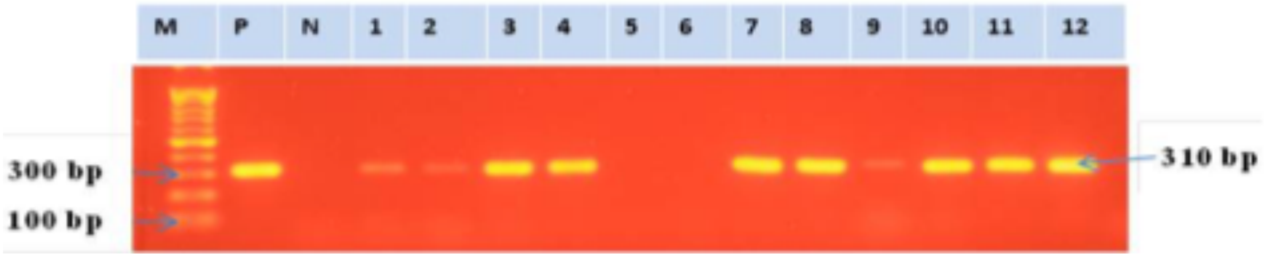

Figure 1 Agarose gel electrophoresis (1.5%) of PCR products of DNA extracted from *S. aureus* showing amplification of 310-bp fragment of *mecA* gene. M, 100 bp DNA marker (ladder); P: positive control; N: negative control; lanes 1, 2, 3, 4, 7, 8, 9, 10, 11 and 12 positive results; lanes 5 and 6 negative results.

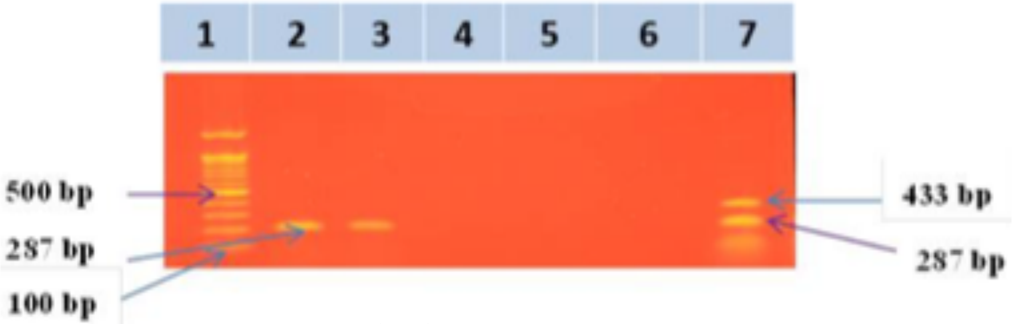

Figure 2. Agarose gel electrophoresis (1.5%) of PCR products of DNA extracted from S. *aureus* showing amplification of *msrA* and *pvl* gene. Lane 1: 100 bp DNA ladder; lane 2: positive control; lane 3: positive result *msrA* genes; lanes 4 and 5 negative results; lane 6: negative control; lane 7: positive result of multiplex PCR of *pvl* and *msrA*.

DISCUSSION

In this study, burn infection rate was 77.9% in hospitalized patients, mostly Gram-negative bacteria followed by Gram-positive bacteria, while in the community patients, positive culture was 89.26%, of which 180 isolates were normal flora and 3 were pathogenic Gram-positive bacteria. This high rate of infection was observed in a local study where infection rate was 78% from 50 swabs from patients admitted to hospitals in Baghdad (23).

From community-burn swabs, *S. epidermidis* was the main isolate, these are regarded as colonization by skin flora rather than true infection, while among hospital group, the majority of the samples were from infected wounds and resulted in isolation of Gram-negative bacteria (71.76%) followed by *S. aureus*, a well-known nosocomial pathogen, then other coagulase-negative staphylococci. This pattern of isolation was also showed by others where Gram-negative bacilli were more isolated than Gram positive cocci (24, 25). Different organisms may be blamed for infection such *Pseudomonas* spp. followed by *S. aureus* and *Klebsiella pneumoniae* (26). It is suggested that high frequency of Gram-negative bacteria rather than Gram-positive bacteria may be due to prolonged hospital stay and intensive use of antibiotics (27). Other reasons for this pattern may be related to bacterial virulence factors, prevalence in hospital environment, overcrowding in the hospital wards, frequent visitors to the burn patients, and contamination of wound at the time of injury, and even at the time of dressing by health workers.

In this study, the susceptibility of *S. aureus* isolates to commonly used antimicrobials in burn wounds was tested; *S. aureus* were resistant to methicillin and ceftazidime, while the majority were resistant to erythromycin, piperacillin-tazobactam but susceptible to vancomycin. These finding were in agreement with Kareem *et al.* revealing high resistance toward ceftazidime (95.2%) (28), and another study reporting 94% resistance to ceftazidime among 54 *S. aureus* isolates (29). For *S. aureus*, vancomycin is the most effective antibiotic against MRSA, such susceptibility makes it used for all resistant *S. aureus* as there is no chance for other drugs to control the infection effectively (30). Because we isolated only three *S. aureus* from community burns, we could not compare their suceptibility to the hospital isolates, although more sensitive response was detected among them. All three community-acquired *S. aureus* isolates were sensitive for oxacillin, ciprofloxacin, doxycyclin, imipeneme and vancomycin.

Resistance of to methicillin, oxacillin and cefoxitin in hospital *S. aureus* isolates were 100%, 63.1% and 65.78%, respectively. Oxacillin and cefoxitin disk diffusion susceptibility are used for screening MRSA. Accordingly, in this study and among all *S. aureus* isolates, 65.85% were resistant to cefoxitin and considered as MRSA. This was compatible with previous studies such as Degaim *et al.* study on 126 *S. aureus* isolates from burned patients where 85 (67.46%) isolate were considered to be MRSA (31). Another study reported that among 65 *S. aureus* isolates, 40 (61.53%) were confirmed to be MRSA using cefoxitin (32). A similar report by Kolhe *et al.* showed that among 70 isolates of *S. aureus* from burned patients, 43 (61.42%) were MRSA as determined by cefoxitin resistance (33).

Cefoxitin resistance is better than oxacillin and methicillin resistance to predict and screen MRSA. The accurate determination of methicillin resistance in staphylococci by the oxacillin disk diffusion method may be affected by various components of medium, temperature and the duration of incubation (34). Several reports indicate that oxacillin often failed to detect low-level heterogeneous MRSA populations (35). The current gold standard for MRSA detection is by detection of *mecA* gene since there is no optimal phenotypic method for detecting methicillin resistance in *S. aureus*. Cefoxitin results are easier to interpret and are thus more sensitive for the detection of *mecA*-mediated resistance than oxacillin results (36), whereas genotypic methods may have better accuracy and precision (37). In this study, 36 isolate (87.8%) showed PCR amplification of *mecA* gene and this is compatible with the studies for detection of *mecA* (38, 39).

Staphylococcus aureus expresses a variety of virulence factors, including Panton-Valentine-leucocidin. This cytotoxin produced by fewer than 5% of the *S. aureus* strains (40). PVL expression enhances MRSA pathogenicity and is a critical determinant in the choice of suitable antibiotics. Therefore, investigating the prevalence of the PVL marker among MRSA strains, which are a major health issue, is of high importance (41). In this study only 3 (7.31%) of *S. aureus* isolates showed PVL-encoding gene. The low prevalence was similar to what reported by Khosravi *et al.* from burn wounds where 46.8% out of total tested isolates were identified as *S. aureus* and the prevalence of *pvl* gene in MRSA strains were 7.23% (38), while much higher prevalence of *pvl* gene were reported in 24 MRSA reaching (79%) (42). This reflects that the prevalence of *pvl* varies between geographical locations and population and like other virulence factor may be transmissible and also affect the outcome of infection with PVL expressing MRSA.

Resistant to macrolides in staphylococci is carried out by an ATP-dependent efflux pump, encoded by *mrsA* gene (16), so expression of *mrsA* product indicate macrolides resistance which has become a problem in Gram-positive bacteria. Seventeen (41.46%) of our *S. aureus* isolates harboured the *msrA* gene indicating also resistance to macrolides. The prevalence of *msrA* gene has been reported differently in different regions, lower prevalence (10.2 and 10.5%) were reported by Zmantar *et al.* and Fasihi *et al.* respectively (43, 44). In comparison to our result, they are lower and again this variation in a certain gene can be explained to be due to the dynamic changes among bacteria and their ability to share virulence and resistance gene.

In conclusion, all *S. aureus* isolates were resistant to methicillin, ceftazidime while they were all susceptible to vancomycin and most of the species were showed to be MRSA. The prevalence of *msrA* gene among nosocomial and community-burn wound isolates of *S. aureus* was high, while few isolates were found to carry *pvl* gene.

REFERENCES

1. Plichta JK, Droho S, Curtis BJ, Patel P, Gamelli RL, Radek KA. Local burn injury impairs epithelial permeability and antimicrobial peptide barrier function in distal unburned skin. Critical Care Medicine. 2014;42(6):e420-31.2. Rafla K, Tredget EE. Infection control in the burn unit. Burns. 2011;37(1):5-15.

3. Bayram Y, Parlak M, Aypak C, Bayram I. Three-year review of bacteriological profile and antibiogram of burn wound isolates in Van, Turkey. Int J Med Sci. 2013;10(1):19-23.

4. Coban YK. Infection control in severely burned patients. World J Crit Care Med. 2012;1(4):94-101.

5. D'Avignon LC, Saffle JR, Chung KK, Cancio LC. Prevention and management of infections associated with burns in the combat casualty. The Journal of trauma. 2008;64(3 Suppl):S277-86.

6. Dai T, Huang YY, Sharma SK, Hashmi JT, Kurup DB, Hamblin MR. Topical antimicrobials for burn wound infections. Recent Pat Antiinfect Drug Discov. 2010;5(2):124-51.

7. Uwaezuoke JC, Aririatu LE. A Survey of Antibiotic Resistant Staphylococcus Aureus Strains from Clinical Sources in Owerri. Journal of Applied Sciences and Environmental Management. 2004;8:67-9.

8. Cui F, Li G, Huang J, Zhang J, Lu M, Lu W, et al. Development of chitosan-collagen hydrogel incorporated with lysostaphin (CCHL) burn dressing with anti-methicillin-resistant Staphylococcus aureus and promotion wound healing properties. Drug Deliv. 2011;18(3):173-80.

9. CLSI. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Second Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA, USA, ISBN: 1-56238-877-0 CLSI document VET01-S2. 2013.

10. Lindsay JA. For CA-MRSA, How much PVL is too much? Microbiology. 2009;155(Pt 11):3473-4.

11. Hu Q, Cheng H, Yuan W, Zeng F, Shang W, Tang D, et al. Panton-Valentine leukocidin (PVL)-positive health care-associated methicillin-resistant Staphylococcus aureus isolates are associated with skin and soft tissue infections and colonized mainly by infective PVL-encoding bacteriophages. Journal of Clinical Microbiology. 2015;53(1):67-72.

12. Adler A, Temper V, Block CS, Abramson N, Moses AE. Panton-Valentine leukocidin-producing Staphylococcus aureus. Emerging Infectious Diseases. 2006;12(11):1789-90.

13. Tappe D, Schulze MH, Oesterlein A, Turnwald D, Muller A, Vogel U, et al. Panton-Valentine leukocidin-positive Staphylococcus aureus infections in returning travelers. American Journal of Tropical Medicine and Hygiene. 2010;83(4):748-50.

14. Monecke S, Muller E, Buechler J, Rejman J, Stieber B, Akpaka PE, et al. Rapid detection of Panton-Valentine leukocidin in Staphylococcus aureus cultures by use of a lateral flow assay based on monoclonal antibodies. Journal of Clinical Microbiology. 2013;51(2):487-95.

15. Lim JC, You Z, Kim G, Levine RL. Methionine sulfoxide reductase A is a stereospecific methionine oxidase. Proc Natl Acad Sci U S A. 2011;108(26):10472-7.

16. Juda M, Chudzik-Rzad B, Malm A. The prevalence of genotypes that determine resistance to macrolides, lincosamides, and streptogramins B compared with spiramycin susceptibility among erythromycin-resistant Staphylococcus epidermidis. Memorias do Instituto Oswaldo Cruz. 2016;111(3):155-60.

17. Connie RM, donald CL, George M. Textbook of Diagnostic Microbiology 3rd ed: Newark Saunders Elsevier; 2007. p. 303-15.

18. Sabzghabaee AM, Abedi D, Fazeli H, Javadi A, Jalali M, Maracy MR, et al. Antimicrobial resistance pattern of bacterial isolates from burn wounds in an Iranian University Hospital. J Res Pharm Pract. 2012;1(1):30-3.

19. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966;45(4):493-6.

20. Anyanwu NCJ, Abdullahi IO, Ameh JB, Ella EE. Molecular detection of PVL, msrA genes and antibiotic susceptibility pattern of staphylococcus aureus from skin and soft tissue infections in Zaria, Nigeria. Scientific Journal of Microbiology. 2013;2(2):43-52.

21. Elhassan MM, Ozbak HA, Hemeg HA, Elmekki MA, Ahmed LM. Absence of the mecA Gene in Methicillin Resistant Staphylococcus aureus Isolated from Different Clinical Specimens in Shendi City, Sudan. Biomed Res Int. 2015;2015:895860.

22. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. J Vis Exp. 2012(62).

23. Jebur M. Therapeutic efficacy of Lactobacillus acidophilus against bacterial isolates from burn wounds. N Am J Med Sci. 2010;2(12):586-91.

24. Hussien IA, Habib KA, Jassim KA. Bacterial Colonization of Burn Wounds. Baghdad Science Journal. 2012;9(4).

25. Shrivastava G, Bhatambare GS, Lunawat A, Patel KB, Maheshwari T. Pattern of microorganism from burn wounds and their trends of susceptibility to antibiotics in tertiary care center. Int J Health Syst Disaster Manage. 2016;4(2):53-7.

26. Mohamed H. One year prevalence of critically ill burn wound bacterial infections in surgical ICU in Egypt: Retrospective study. Egyptian Journal of Anaesthesia. 2016;32(3):431–4.

27. Irfan M, Ahmed I, Shafee M, Tareen AM, Rehman MFU, Khan SA. Microbiological Investigation of Burn Patients in Burn Intensive Units, in Quetta, Pakistan. Journal of Infection and Molecular Biology. 2014;2(4):74 – 6.

28. Kareem SM, Al- Jubori SS, Ali MR. Prevalence of PVL Gene among Methicillin Resistance *S. aureus* Isolates in Baghdad City. World Journal of Pharmaceutical Research. 2015;4(5):455-71.

29. Ahmad B, Khan F, Ahmed J, Cha SB, Shin M, Bashir S, et al. Antibiotic Resistance Pattern and Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus Colonization in Burns Unit of a Tertiary Care Hospital in Peshawar, Pakistan. Tropical Journal of Pharmaceutical Research. 2014;13(12):2091-9.

30. Ragbetli C, Parlak M, Bayram Y, Guducuoglu H, Ceylan N. Evaluation of Antimicrobial Resistance in Staphylococcus aureus Isolates by Years. Interdiscip Perspect Infect Dis. 2016;2016:9171395.

31. Degaim ZD, Shani WS, Hamim SS. Virulence factors of Methicillin Resistant Staphylococcus aureus (MRSA) isolated from burn patients. IntJCurrMicrobiol. 2015;4(7):898-906.

32. Ohadian Moghadam S, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin-resistant Staphylococcus aureus isolated from burn patients, Iran. J Infect Dev Ctries. 2014;8(12):1511-7.

33. Kolhe VS, R. M, M. MS. Incidence of Antibiotic Resistance in Methicillin Resistant Staphylococcus aureus In Burn Patients in a Tertiary Care Hospital, India. International Journal of Pharma and Bio Sciences. 2014;5(4):537 - 41.

34. Pramodhini S, Thenmozhivalli PR, R. S, Dillirani V, Vasumathi A, Agatha D. Comparison of Various Phenotypic Methods and mecA Based PCR for the Detection of MRSA. Journal of Clinical and Diagnostic Research. 2011;5(7):1359 - 62.

35. Demir T, Coplu N, Esen B. Comparative analysis of phenotypic and genotypic detection of methicillin resistance among *Staphylococcus aureus*. Indian Journal of Pathology and Microbiology. 2016;59(3):314-7.

36. Broekema NM, Van TT, Monson TA, Marshall SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in Staphylococcus aureus in a large-scale study. Journal of Clinical Microbiology. 2009;47(1):217-9.

37. Farahani A, Mohajeri P, Gholamine B, Rezaei M, Abbasi H. Comparison of different phenotypic and genotypic methods for the detection of methicillin-resistant Staphylococcus aureus. N Am J Med Sci. 2013;5(11):637-40.

38. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in Staphylococcus aureus strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. Burns. 2012;38(2):247-51.

39. Qureshi A, Ingle R, Musaddiq M, Ali Y, Khan Z. Status and Distribution of *mecA* Gene in Hospitalized Patient's MRSA Isolates. Bioscience Discovery. 2012;3:52-7.

40. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant Staphylococcus aureus in the genomics era. Journal of Clinical Investigation. 2009;119(9):2464-74.

41. Shariati L, Validi M, Hasheminia AM, Ghasemikhah R, Kianpour F, Karimi A, et al. Staphylococcus aureus Isolates Carrying Panton-Valentine Leucocidin Genes: Their Frequency, Antimicrobial Patterns, and Association With Infectious Disease in Shahrekord City, Southwest Iran. Jundishapur J Microbiol. 2016;9(1):e28291.

42. AL-Charrakh AH, AL-Hassnawi HH, AL-Khafaji JK. Molecular Characteristics of Community-Associated Methicillin-resistant Staphylococcus aureus (CAMRSA) Isolates from Clinical Specimens in Iraq. British Microbiology Research Journal. 2015;5(3):227-36.

43. Zmantar T, Kouidhi B, Miladi H, Bakhrouf A. Detection of macrolide and disinfectant resistance genes in clinical Staphylococcus aureus and coagulase-negative staphylococci. BMC Res Notes. 2011;4:453.

44. Fasihi Y, Saffari F, Ghahraman MRK, Neyestanaki DK. Molecular Detection of Macrolide and Lincosamide-Resistance Genes in Clinical Methicillin-Resistant *Staphylococcus aureus* Isolates from Kerman, Iran. Arch Pediatr Infect Dis. 2017;5(1):1-5.