Molecular Identification of Methicillin Resistant *Staphylococcus aureus* and Evaluation of Panton-Valentine Leukocidin Gene as a Sole Marker for CA-MRSA

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Abstract: Background: Infections caused by MRSA represent a growing problem and a challenge for healthcare institutions. **Aim of work**: To compare the efficacy of phenotypic methods for identification of MRSA with genotypic method and to evaluate if PVL gene could be used as sole marker for CA-MRSA. **Material and methods**: 88 isolates of MRSA were included in this study. Phenotypic identification was done by Oxacillin DD, PBP2a latex agglutination and Cefoxitin DD. Molecular detection of *mec-A* gene & PVL gene was done by real time PCR. **Results:** The PVL gene was detected among 46.8% of CA-MRSA, while only in 12.2 % HA-MRSA. 88 *mec-A* positive isolates, were identified as MRSA by oxacillin DD, PBP2a latex agglutination and cefoxitin DD methods with sensitivity of 92%, 98.8% and 100% respectively. Patients with PVL postive CA- MRSA were significantly younger males mostly of skin and soft tissues origin (p = 0.002). It showed distinctive antibiogram profile being significantly more sensitive to Levofloxacin, Moxifloxacin, Ciprofloxacin, Gentamicin, Clindamycin (p<0.001) and Tetracycline (p <0.05). **Conclusion:** The best phenotypic method for detection of MRSA is the combination of the Cefoxitin DD and the latex agglutination test. The presence of PVL gene cannot be used as a sole marker for CA-MRSA. [Sahar M Ali, Eman AM Bayoumi, Tarek A Alshazly. **Molecular Identification of Methicillin Resistant Staphylococcus aureus and Evaluation of Panton-Valentine Leukocidin Gene as a Sole Marker for CA-MRSA.** *J Am Sci* 2013;9(8):108-115]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 16

Keywords: CA- MRSA, PVL, *mec-A*, Realtime PCR.

Abbreviations: MRSA: Methicillin-resistant Staphylococcus aureus, CA-MRSA Community-associated MRSA, HA-MRSA: Hospital-associated MRSA, DD: disc diffusion, PBP2a: Penicillin-Binding Protein 2a, PCR: Polymerase chain reaction, PVL: Panton-Valentine leukocidin and LOS: Length of stay.

1.Introduction

Methicillin-resistant *Staph. aureus* (MRSA) strains were first described in England in 1961 shortly after methicillin became available for clinical use. They have subsequently spread throughout the world and are an important cause of nosocomial and community associated infections. Therefore, rapid and accurate detection of methicillin resistant strains in staphylococci is very essential in order to choose appropriate therapy, to prevent unnecessary use of glycopeptides antibiotics and to take necessary measures for infection control. The mechanism of resistance is due to acquisition of the *mec-A* gene, which encode for low-affinity penicillin-binding protein 2a (PBP2a). Therefore, presence and absence of *mec-A* gene indicates methicillin resistance and methicillin susceptibility in staphylococci respectively. Polymerase chain reaction (PCR) for the amplification of the *mec-A* is considered the gold standard for the detection of methicillin resistance in *S.aureus* **(Mohanasoudaram & Lalitha, 2008)**.

However, not all laboratories can include molecular biology techniques in their routine clinical practice. Therefore, it is desirable to identify an accurate, rapid and cost-effective phenotypic method for the detection of MRSA **(Datta *et al.*, 2011)**. The incidence of Community-associated MRSA (CA-MRSA) infection is rising in the developed world **(Chheng *et al.*, 2009)**. CA-MRSA has become increasingly important as a cause of skin and soft tissue infections (SSTIs), particularly in patients presenting to emergency departments **(Fridkin *et al.*, 2005; DeLeo *et al.*, 2010).**

Panton-Valentine leukocidin (PVL) is a cytotoxin produced by Staphylococcus aureus. PVL exhibits highly specific lytic activity against polymorphonuclear cells, monocytes, and macrophages in humans and rabbits **(Cribier *et al.*, 1992)**. PVL is a bicomponent toxin that consists of the polypeptides lukS-PV and lukF-PV. Genes for these PVL components have been found in the genomes of various temperate phages **(Narita *et al.*, 2001)**. The PVL gene is mainly associated with necrotic lesions of the skin and subcutaneous tissues, such as furuncles, and also with community-acquired, severe, necrotizing pneumonia **(Lina *et al.*, 1999)**.

The purpose of the present study is to compare the efficacy of phenotypic methods for identification of MRSA with genotypic method using direct detection of *mec-A* gene by PCR and secondly to

assess the prevalence of the PVL toxin gene in the overall MRSA by real-time- PCR to evaluate if PVL toxin gene could be used as sole marker for CA-MRSA infections.

2.Material and Methods

Bacterial Isolates:

A total of 88 isolates of MRSA from different clinical samples during the period from August 2011 to July 2012 in Al-ansar hospital Madinah, KSA were studied. All the isolates were identified to the species level by using conventional techniques like colony morphology on 5% sheep blood agar, catalase test, slide and tube coagulase test

Case definition and source of data

MRSA isolates were further classified in to HA-MRSA and CA-MRSA according to CDC definition, which define the CA-MRSA as MRSA infection in an individual who has : MRSA identified within 48 hours of admission to a hospital with no history of hospitalization, surgery, dialysis, or residence in a long-term care facility within 1 year of the MRSA culture date and has no permanent indwelling catheter or percutaneous medical device (e.g., tracheostomy tube, gastrostomy tube, or Foley catheter) with no known prior positive culture for MRSA and HA-MRSA infections are those isolets which not fulfilling the previous criteria **(CDC, 2005).**

The secondary data of the patients were obtained from the laboratory investigation register and patients' medical record files. Data of the study subjects included basic demographic profiles, ward admitted, specimen type, length of hospital stay, clinical notes, and details of risk factors associated with HA-MRSA infections as mentioned above.

Phenotypic Detection of MRSA

- Disc diffusion methods: In all confirmed *S. aureus* isolates, Oxacillin and Cefoxitin disc diffusion (DD) methods were performed for the identification of MRSA. Four to five colonies from overnight growth was inoculated into 4 to 5 ml Mueller-Hinton broth (Hi-Media, Mumbai, India) and incubated at 37°C until turbid to 0.5 McFarland standard, and inoculated on two separate Mueller-Hinton agar (MHA) plates, then 1 µg/ml Oxacillin disc (Oxoid®) and 30 µg/ml Cefoxitin disc (Oxoid®) placed aseptically and incubated at 37 °C for 16-18 hrs in Cefoxitin and full 24 hrs for Oxacillin disc diffusion method. Oxacillin DD test was interpreted according to CLSI guidelines as follows: Resistance (≤10 mm), moderately sensitive (11-12 mm) and sensitive (≥13 mm), whereas Cefoxitin DD ≥ 22 mm as sensitive and ≤ 21 mm as resistant **(CLSI, 2008).**
- Penicillin Binding Protein 2a (PBP2a) latex test: MRSA isolates were also tested for production of (PBP2a) by using the Latex Agglutination Test (Oxoid®) based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, according to the manufacturer's instructions **(Louie *et al.*, 2000)**.
- Biochemical and antibiogram susceptibility testing were then performed using VITEK- 2 cassette (bioMérieux, Inc.) with identification card (GP card) and the antibiotic sensitivity test card (AST-580). Antibiotics included in the susceptibility testing were Amp/sulbactam Cefazolin, Rifampin, Gentamicin, Clindamycin, Tetracycline, Mupirocin , Linezolid, Vancomycin, Erythromycin, , Trimethoprim/sulpha(Co-trimoxazole), Moxifloxacin, Levofloxacin, Ciprofloxacine and Fusidic Acid.
- **Genotypic detection of *mec A* and PVL genes by real time PCR:** A real-time polymerase chain reaction was performed to determine the genes encoding *mec A* and PVL genes. Block cycler PCR for detection of the *mec A* and *luk F-PV/luk S-PV* genes fragment was performed according to the method described by **Lina *et al.*, 1999** and **Jonas *et al.*, 1999. Extraction of DNA**: 1 ml overnight culture of S. aureus was centrifuged at 16,000 xg for 30 seconds and the cell pellet re-suspended with 200 µl phosphate buffer solution (pH 7). Genomic DNA was extracted using the high pure PCR Template Preparation Kit (Roche Applied Science, Germany) with an additional step of incubation at 37ºc for 30 minutes with 0.5 µl of 10 mg/ml lysostaphin solution (Sigma, USA) prior to cell lysis.

Table (1): primers for *mecA*, *Luk-S (PV)* & *luk-F (PV)* genes

Primer	Target gene	Sequence (5.-3.)	Amplicon size	Ref.
MecA 1 MecA2	MecA	GTA GAA ATG ACT GAA CGT CCG ATA A GTA GAA ATG ACT GAA CGT CCG ATA A	310 bp	Jonas ***et al.***,1999
Luk-PV-1 Luk-PV-2	Luk-S(PV) luk-F(PV)	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A GCA TCA AGT GTA TTG GAT AGC AAA AGC	433bp	Lina ***et al.,*** 1999

PCR for *mecA* gene detection was performed using Real time (SYBR Green I) PCR:

Extracted DNA (2 µL) was added to a hot-start reaction mixture for each test. The final 20 µL real-time PCR reaction contained 1× LightCycler FastSart DNA Master SYBR Green I (Roche Applied Science, Indianapolis, IN), 2% DMSO (Sigma, St Louis, MO),

5 mmol/L of magnesium chloride, 0.5 µmol/L of each primer *mecA1* and *mecA2* (Table 1) yielding a 310-base-pair product Included in each run blank (water), negative (methicillin-susceptible *Staphylococcus epidermidis*), and positive (MRSA) controls. Real-time PCR was accomplished using the Light Cycler (Roche Applied Science), and the conditions consisted of an initial step FastStart DNA *Taq* polymerase activation phase at 95°C for 10 min; a 45-cycle amplification phase consisting of a 95°C segment for 10 s, a 65°C segment for 10 s, and a 72°C segment for 12 s; with fluorescence acquisition at the end of each extension. The amplification program was followed immediately by a melt program starting at 95°C then 15s at 65°C, and a gradual increase to 95°C at a rate of 0.1°C per second followed by cooling at 40ºc for 30s. The presence of amplified DNA was measured by detection of energy emitted at 640 nm.

PCR for *luk F-PV* and *luk S-PV* gene detection was performed using Real time (fluorescence probe) PCR:

Oligonucleotide primers and fluorescence-labeled hybridization probes, designed for amplification and sequence specific detection of both *luk F-PV* and *luk S-PV* were obtained from TIB Molbiol (Berlin, Germany). The primer sequences for the genes were as shown in table (1) Amplification mixtures contained 4 µl of light-cycler fast start DNA Master Plus Hybridization Probes Mix (Roche Diagnostics), 2 mM of $MgCl_2$ (total concentration), 0.5 µm of each primer oligonucleotide and 2 µl of template DNA in a final volume of 20 µl. Reactions were performed under the following thermal cycling conditions: 10 min at 95°C, followed by 45 cycles of 95°C segment for 10 s and 55°C segment for 10s and 72ºC segment for 20s followed by cooling at 40ºC for 30s .The presence of amplified DNA was measured by detection of energy emitted at 640 nm

Statistical analysis

Data were analyzed using using Statistical Package for Social Sciences (SSPS) software version 21. Chi square test and t-test were used to determine the statistical significance. The *P* value less than 0.05 was considered to be significant.

3.Results:

The study included 210 staphylococcal aureus isolated from different sources; 122 were methicillin sentsitive (58.1%) and 88 were methicillin resistant staph (41.9%). The performance of conventional method in the accurate identification of MRSA was evaluated by keeping the *mecA* gene as a genotypic marker for MRSA. Among 88 *mecA* positive isolates, 81, 87 and 88 isolates were identified as MRSA by oxacillin DD, PBP2a latex agglutination and cefoxitin DD methods with sensitivity of (92%, 98.8% and 100%) respectively (Table 2).

According to CDC criteria for definition of CA-MRSA and HA-MRSA 47 out of 88 MRSA strains were defined as CA-MRSA (46.6%) while the remaining 41 were defined as HA-MRSA (53.4%). The PVL gene was evaluated by Real time PCR in the 88 MRSA strain and the results revealed that 22 out of 47 (46.8%) CA-MRSA isolates harbored this gene, while only 5 of 41 (12.2 %) HA-MRSA strain harbored this gene. A statistical significant difference was found between HA-MRSA and CA-MRSA regarding the PVL gene carriage (p=0.001) meanwhile no significant difference was found within CA-MRSA isolates (Table 3, Fig 1).

Comparing the demographic data of patients infected with CA -MRSA and HA-MRSA a significant difference was found as regard sources of specimen (CA-MRSA more common in skin and soft tissue infection than HA-MRSA (61.7% Vs 36.0%) while HA-MRSA more common in sputum and blood specimen (48.0% Vs 29.8% and 4% Vs 2.1% respectively , overall p=0.005) , the outcome of incision and drainage was more in CA-MRSA than HA-MRSA (53.2% Vs 28.0% , p <0.001), while HA-MRSA patients had higher Length Of Stay (LOS) and death rates than CA-MRSA patients (18.44±6.15 Vs 13.15±5.77, p =0.001 and 22% Vs 4.3 % , p =0.01, respectively) without any significant difference regarding the other parameters (male sex, Patient risk factors, and presentation).

Patients infected with PVL positive CA-MRSA were also compared to those infected with CA-MRSA lacking PVL gene and revealed that the PVL positive CA-MRSA patients were significantly younger (27.4±15.11 Vs 65.12±15.11 , p <0.001) with predominance of male sex (63.6% Vs 20% , p =0.002), the skin and soft tissue infection was the major source (90.9% Vs 36.0%) and the outcome of incision and drainage was greater (81.8% Vs 28.0%, p <0.001) compared to patients infected with CA-MRSA lacking PVL gene but without any significant difference as regard the other parameters (Patient risk factor, presentation , LOS and death rate). (Table 4).

The CA-MRSA strains were sensitive to Gentamicin (97.87%), Mupirocin (97.8%) Rifampicin (97.8%), Clindamycin (95.74%), Fusidic Acid (91.4%), Tetracycline (89.3%), Ciprofloxacin (80.85%), Moxifloxacin (78.72%), Levofloxacin (57.45%), and Erythromycin (10.64%). The HA-MRSA strains were sensitive to, Mupirocin (97.5%) and Rifampicin (97.5%), Fusidic Acid (87.8%), Tetracycline (68.3%), Gentamicin (65.85), Clindamycin (56.1%), Ciprofloxacin (19.51%), Moxifloxacin (12.20%) and Erythromycin (2.44%). All strains were susceptible to

Vancomycin, Trimethoprime-sulfamethoxazole (Cotrimoxazole) and Linezolid.

The CA-MRSA isolates were significantly more sensitive to Levofloxacin, Moxifloxacin, Ciprofloxacin, Gentamicin, Clindamycin (p <0.001) and Tetracycline (p <0.05) compared to HA-MRSA isolates (Table 5 & Fig 2).

Table (2): Comparison of different methods for Identification of MRSA

No. of isolates tested	Oxacillin (1 µg) DD		PBP2a latex agglutination		Cefoxitin (30 µg) DD	
	Positive	Negative	Positive	Negative	Positive	Negative
88	81	7	87	1	88	0
Sensitivity	92%		98.8%		100%	

Table (3): Prevalence of Pantone valentine leukocidin gene in CA -MRSA and HA-MRSA isolates

	CA-MRSA (n=47)		HA-MRSA (n=41)		*P* value
	No.	%	No.	%	
PVL positive	22	46.8	5	12.2	0.001*
PVL negative	25	53.2	36	87.8	
P value	0.7705		<0.001*		

Table (4): The demographic data of patients infected with CA -MRSA and HA-MRSA

Parameter		HA- MRSA strains (n=41)		CA-MRSA strains						*P* value	
				PVL^- (n=25)		PVL^+ (n=22)		Total		HA-MRSA & CA-MRSA strains	PVL- & PVL+
		N	%	N	%	N	%	N	%		
Male sex		20	48.8	5	20.0	14	63.6	19	40.4	0.520	0.002*
Age Range (Mean±SD)		(27-78) 56.2±12.5		(26-90) 65.1±15.1		(18-41) 27.4±15.1		(18-90) 46.5±20.1		0.009*t	<0.001*t
Source	Skin & soft tissue infections	14	34.1	9	36.0	20	90.9	29	61.7	0.005*	0.002*
	Sputum	13	31.7	12	48.0	2	9.1	14	29.8		
	Blood	11	26.8	1	4.0	0	0.0	1	2.1		
	Urine	2	4.9	2	8.0	0	0.0	2	4.3		
	Other	1	2.4	1	4.0	0	0.0	1	2.1		
Patient risk factors	Diabetes	14	34.1	7	28.0	8	36.4	15	31.9	0.824	0.540
	Dialysis	4	9.8	5	20.0	1	4.5	6	12.8	0.656	0.098
	Malignancy	4	9.8	1	4.0	1	4.5	2	4.3	0.305	1.000
Presentation	WBC at diagnosis Range (Mean±SD)	(10-30) 18.1±6.8		(6-22) 17.2±5.8		(7-27) 17.58±2.4		(6-27) 17.5±3.4		0.6123 t	0.747 t
	Fever >38° C	13	31.7	8	32.0	7	31.8	15	31.9	0.983	1.000
Treatment and outcome	Incision & drainage	10	24.4	7	28.0	18	81.8	25	53.2	0.005*	<0.001*
	Death	9	22.0	1	4.0	1	4.5	2	4.3	0.010*	0.924
	Length of stay (LOS)	(10-30) 18.4±6.2		(1-22) 12.55±4.55		(0-15) 13.8±6.25		(0-22) 13.2±5.8		0.0001*t	0.4228 t

t:- t-test and chi-square for other parameters

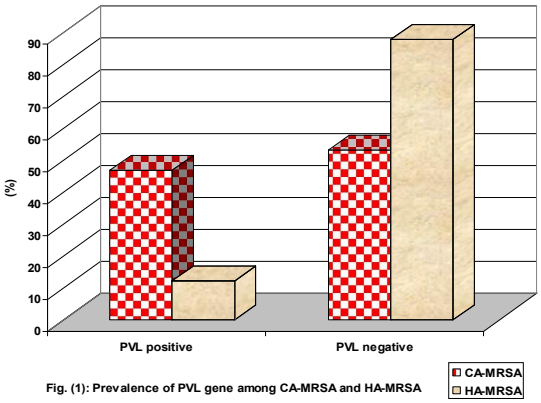

Fig. (1): Prevalence of PVL gene among CA-MRSA and HA-MRSA

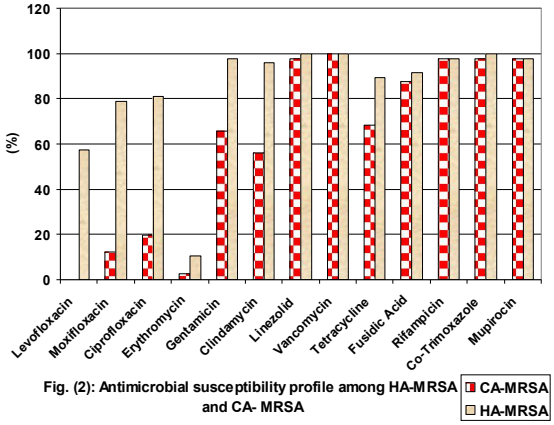

Fig. (2): Antimicrobial susceptibility profile among HA-MRSA and CA- MRSA

Table (5) Antimicrobial susceptibility profile among HA-MRSA and CA- MRSA

	HA-MRSA		CA-MRSA		Chi-square	
	N	%	N	%	X^2	*P*-value
Benzylpenicillin	0	0.00	0	0.00	.	
Amp/sulbactam	0	0.00	0	0.00	.	
Oxacillin	0	0.00	0	0.00	.	
Cefoxitin	0	0.00	0	0.00	.	
Cefazoline	0	0.00	0	0.00	.	
Levofloxacin	0	0.00	27	57.45	33.97	<0.001*
Moxifloxacin	5	12.20	37	78.72	38.84	<0.001*
Ciprofloxacin	8	19.51	38	80.85	33.02	<0.001*
Erythromycin	1	2.44	5	10.64	2.31	0.128
Gentamicin	27	65.85	46	97.87	15.87	<0.001*
Clindamycin	23	56.10	45	95.74	19.60	<0.001*
Linezolid	40	97.56	47	100.00	0.00	1.00
Vancomycin	41	100.00	47	100.00	0.00	1.00
Tetracycline	28	68.29	42	89.36	4.74	0.029
Fusidic Acid	36	87.80	43	91.49	0.04	0.828
Rifampicin	40	97.56	46	97.87	0.38	0.535
Trimethoprim/ Sulfamethoxazole	40	97.56	47	100.00	0.005	0.945
Mupirocin	40	97.56	46	97.87	0.38	0.535

4.Discussion

Staphylococcus aureus is one of the most significant human pathogens responsible for nosocomial and community acquired infections. It can cause a range of infectious diseases from mild conditions to severe life-threatening debilitation. Infections caused by MRSA represent a growing problem and a challenge for healthcare institutions **(Morellion *et al.*, 2005)**.

Accurate and early diagnosis of methicillin resistance is vital in the management of patients with infections caused by *S. aureus*. Although many phenotypic methods have been developed to achieve this objective, the lacunae in the sensitivity of these tests in isolation may not ensure appropriate and timely treatment of all MRSA-infected patients. The current gold standard for MRSA detection is identification of the *mecA* gene. However, not all laboratories can include molecular biology techniques in their routine clinical practice. Therefore, it is desirable to identify an accurate, rapid and cost-effective phenotypic method for the detection of MRSA **(Datta *et al.*, 2011).** Methods based on disc diffusion, as well as microdilution with oxacillin, are often not entirely reliable at detecting some strains that harbor the *mecA* gene **(Velasco *et al.*, 2005)**.

In the present study, Among 88 *mec-A* positive isolates, 81, 87 and 88 isolates were identified as MRSA by oxacillin DD, PBP2a latex agglutination and cefoxitin DD methods Showing sensitivity of 92%, 98.8% and 100%, respectively. Various workers have shown that the cefoxitin disc method has better sensitivity than the oxacillin disc method for MRSA detection **(Skov *et al.*, 2003; Boutiba-Ben Boubaker *et al.*, 2004; Velasco *et al.*, 2005)**. This higher sensitivity to cefoxitin can be explained by the increased expression of the *mecA*-encoded protein PBP2a, cefoxitin being an inducer of the *mecA* gene **(Velasco *et al.*, 2005)**. Our study strengthens the point that cefoxitin is superior to oxacillin as an indicator of MRSA for the detection of methicillin resistance.

In our study, the latex agglutination test for detection of (PBP2a) had 98.8% sensitivity. Many studies have reported the sensitivity and specificity of the latex agglutination test to be ≥ 97% **(Louie *et al.*, 2000; Datta *et al.*, 2011)**. Latex agglutination has the advantages of being rapid, giving results on the same day, and easy to perform with very good sensitivity. This method could detect even low levels of PBP2a that are usually missed in routine disc diffusion methods. The only disadvantage is the cost factor. A study by **Rohrer *et al.* (2001)** proved that the sensitivity of the latex agglutination test can be improved (93.5 to 100 %) by induction with cefoxitin using growth from the edge of the inhibition zone of cefoxitin to perform the test. **(Rohrer *et al.*, 2001)**

According to our results, the best combination is the cefoxitin disc diffusion method and the latex agglutination test. Since the latex agglutination test is expensive, it cannot be applied to all tests. Therefore, isolates that give a zone diameter of less than 20 mm can be easily reported as MRSA and only those with zone diameters of 20–22 mm need to be confirmed by latex agglutination.

The Panton Valentine leukocidin genes code for the production of cytotoxins that cause tissue necrosis and leukocyte destruction by forming pores in cellular membranes. The screening of PVL gene among MRSA has gained importance in recent years due to high involvement of PVL toxin in CA-MRSA infections **(Kunsang *et al.*, 2012)**. Our study revealed that the prevalence of PVL gene was 46.8 % and 12.8% among CA- MRSA and HA-MRSA isolates, respectively. These findings were consistent with those

found in previous studies such as **Monecke *et al.* (2012)** and **Sobhy *et al.* (2012)** who found that the prevalence of PVL gene in CA-MRSA was 54.21% and 33.33% in Saudi and Egyptian studies, respectively. Meanwhile, in Tunisian study by **Ben Jomàa-Jemili *et al.* (2013)** the prevalence of PVL gene was 79% of CA-MRSA and 51% HA-MRSA, which is much higher than our results **(Monecke *et al.*, 2012, Sobhy *et al.*, 2012 and Ben Jomàa-Jemili *et al.*, 2013)**. This diversity of PVL gene carriage prevalence among various MRSA strains around the world might be explained by the strong association between PVL gene carriage with certain *mec A* gene subtype (Types IV & V) and the distinct geographical distribution of *mec A* subtypes as proposed by various recent studies **(David & Daum, 2010)**.

In the present study, PVL gene was included to evaluate the hypothesis that "PVL toxin could be used as sole marker for CA-MRSA infections, In contrast to many researchers who considered the PVL gene a reliable marker for CA-MRSA infections and reported the presence of PVL toxin only in CA-MRSA and none from HA-MRSA isolates. **(Soo Ko, 2005; D'Souza *et al.*, 2010)**. Our results were in agreement with other studies which reported that the presence of PVL toxin cannot be used as a sole marker for CA-MRSA **(Monecke *et al.*, 2012; Shallcross *et al.*, 2013)**.

The present study demonstrated that a high proportion of patients identified in our Institution had CA-MRSA (53.4%) infections. Our findings confirm our clinical impression that CA-MRSA had emerged in our community. Patients with CA- MRSA were significantly younger 46.54±20.11, p 0.009) and had different distribution of clinical infections compared with HA- MRSA patients. Most of CA-MRSA infections were of skin and soft tissues types (61.7%), which responded quickly to wound care (incision & drainage) when indicated (53.3%). Bloodstream infections and respiratory tract infections were more common among HA- MRSA cases (4 % and 48%, respectively) (P= 0.005 for all comparisons). This distribution for CA-MRSA infections was consistent with those of previous studies **(Roosney *et al.*, 2007; Mesrati *et al.*, 2010; Shallcross *et al.*, 2013; Neha *et al.*, 2013)** . Surgical drainage is crucial for the cure of skin and soft tissues infections and, therefore, is recommended for the treatment of these conditions in all patients **(Stevens *et al.*, 2005)**.

Notably, PVL-positive CA-MRSA demographics didn`t differ from those of the overall CA-MRSA isolates which was similar to those reported in other studies **(Boyle-Vavra & Daum, 2007; Daskalaki *et al.*, 2010)**. However, it is important to emphasize that the association between PVL genes and particular clinical manifestations needs further work to demonstrate the role of other exotoxin genes or combinations of genes as an important factors in the pathogenesis.

Extensive use of antibiotics might exert selective pressures on bacteria, and only those strains that carry or acquire resistance genes are able to adapt and survive **(Ma *et al.*, 2006)**.

In our study, Both CA-MRSA and HA-MRSA were resistant to traditional β-lactam antibiotics. However, CA-MRSA isolates were susceptible to most non-β-lactam antimicrobial drugs, including several orally available agents**.** The CA-MRSA isolates were significantly more sensitive to Levofloxacin, Moxifloxacin, Ciprofloxacin, Gentamicin, Clindamycin (p<0.001) and Tetracycline (p<0.05) compared to HA-MRSA isolates. Comparable results were obtained by various authors **(Kaplan, 2005, Anbumani *et al.*, 2006)**. The narrow spectrum of resistance in CA-MRSA might be due to determinants harbored on genetic elements present on the SCC **(Donnio *et al.*, 2004)**. The enhanced sensitivity of CA-MRSA isolates to several oral antibiotics enables clinicians to have a number of options when selecting empiric treatments of putative CA-MRSA infections. Also, this distinct pattern of susceptibility to more than two non-β-lactam antimicrobials can be used as one of the characteristic defining criteria to identify CA-MRSA **(Munckhof *et al.*, 2008)**.

Conclusion

- The best phenotypic method for detection of MRSA is the combination of the cefoxitin disc diffusion method and the latex agglutination test.
- The presence of PVL gene cannot be used as a sole marker for CA-MRSA and further studies are required to find a reliable marker or combination of markers to facilitate the recognition of CA-MRSA strains.
- PVL positive CA-MRSA is more prevalent in younger males with skin and soft tissue infections which have distinct pattern of susceptibility to certain non-β-lactam antimicrobial drugs, and can be effectively cured by incision and drainage, if indicated. Further studies needed to assess the validity of this distinct susceptibility pattern as one of the characteristic defining criteria for identification of CA-MRSA

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