Egyptian Journal of Medical Microbiology Volume 29 / No.3 / July 2020 97-104 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Prevalence of *van*A Gene among Methicillin Resistant *S. aureus* Strains Isolated from Burn Wound Infections in Menoufia University Hospitals

[1]Amira H. Elkhyat*, [1]Amal F. Makled, [2]Ahmed M. Albeltagy, [3]Tarek F. Keshk, [1]Amal M. Dawoud

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt
[3]Department of Plastic Surgery, Faculty of Medicine, Menoufia University, Egypt

Key words:
Vancomycin resistant Staphylococcus aureus – MRSA - mecAgene - vanAgene

Corresponding Author:
Amira Hamed Abou El-Soud ELkhyat
MD of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University
Tala City, Menoufia Governorate, Egypt
Tel: 01111156488
amirahamed61@yahoo.com

ABSTRACT

Background: *Staphylococcus aureus is a leading cause of burn wound infection. Vancomycin resistance among methicillin-resistant Staphylococcus aureus (MRSA) is becoming a worldwide growing threat.* ***Objectives:*** *to detect the prevalence of MRSA in burn patients and its antibiotic susceptibility patterns. In addition, the resistance patterns of MRSA to vancomycin and the prevalence of vanA gene among MRSA isolates were investigated.* ***Methodology:*** *A total 250 clinical samples were obtained from patients admitted to Burn Unit in Menoufia University Hospitals. Identification and antimicrobial susceptibility testing of S. aureus isolates were performed. Cefoxitin disk diffusion method was used to identify MRSA strains. Vancomycin resistance was determined by agar dilution method. Detection of mecA and vanA genes by multiplex PCR was done.* ***Results:*** *Staphylococcus aureus represented 43.3% of all isolates. By cefoxitin disc diffusion method, 94% (79/84) of isolated S. aureus were MRSA that showed a high resistance to most antimicrobials used with rates ranged from 40.5 % to 100%. Phenotypically among MRSA isolates, vancomycin sensitive S. aureus (VSSA), vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA) were 59.5%, 15.2%, 25.3% respectively. Among MRSA isolates, 17 (21.5%) isolates had vanA gene by PCR (16 isolates were VRSA and one isolate was VSSA).* ***Conclusion:*** *This study is considered as an alarm demonstrating that implementation of proper infection control measures is mandatory to control spread of such resistant strains in our hospital.*

INTRODUCTION

The first barrier of defense against microbial invasion is skin, and once burns occur, it becomes more susceptible to infection, which is the main cause of morbidity and mortality in burn patients. Many microbes are accused of burn wound infections but S. *aureus* remains a leading cause of infections in burn centers leading to delayed wound healing and prolonged hospitalization [1].

Antibiotic resistance of *S. aureus* may be due to production of many enzymes, changes in its cell wall structure and the genetic mutations [2]. Methicillin resistance of *S.aureus* (MRSA) is due to alteration in low-affinity penicillin binding protein (PBP2a) that is encoded by *mec*A gene located in chromosomal mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec) leading to resistance to methicillin, and various broad-spectrum β-lactams like third-generation cephalosporins, cefamycins and carbapenems[3].

Vancomycin is the most reliable therapeutic agent against MRSA. The widespread use of vancomycin has contributed to the growing burden of both vancomycin-intermediate-resistant *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA)[4]. The less susceptibility to vancomycin in VISA is due to unusual increased thickness of cell wall that contains D-alanyl-D-alanine capable of binding vancomycin. However, VRSA is caused by *van* genes that encode for a ligase enzyme leading to production of D-Ala-D-lactate for building of peptidoglycan which has much less affinity to vancomycin than instead of D-Ala-D-Ala unites. Although eleven *van* genes are known for vancomycin resistance, *van* A gene is the commonest gene that causes high vancomycin resistance level [5].

Because of the global spread of vancomycin resistance among MRSA strains constituting one of the most serious growing challenges, the goal of this study came to detect the prevalence of MRSA in Burn Unit of Menoufia University Hospital and to investigate the resistance patterns of MRSA to vancomycin and the prevalence of *vanA* gene among MRSA isolates

DOI: https://doi.org/10.51429/EJMM29313

METHODOLOGY

Collection of samples and identification of *Staph aureus* isolates:

This study included 250 patients admitted to Burn Unit of Menoufia University Hospitals from January 2018 to December 2019. All the patients were subjected to full history taking and thorough clinical examination. Burn wound swabs were taken from all patients following cleaning of any remnant ointments. Written informed consents were taken from included patients and the study protocol was approved by the Ethical Committee of Faculty of Medicine.

All the specimens were cultured on different media (Oxoid, UK) and processed according to standardized microbiological methods. *S. aureus* was isolated after inoculation on 5% sheep blood and mannitol salt agars at 37C for 24 hours[6]. Any creamy or golden yellow colonies with or without hemolysis were identified using standard microbiological techniques(Gram stain, catalase test and coagulase test) and by MASTASTAPH (ATCC, USA) which is a rapid, latex agglutination test to detect coagulase and/or protein A that are associated with *S. aureus*. Then, *Staph aureus* isolate**s** were maintained on trypticase soy broth containing 20% glycerol at - 80 C [7].

Antimicrobial susceptibility testing:

Disk diffusion method:

Antimicrobial susceptibility testing for *Staph aureus* isolates was performed using Kirby-Bauer disk diffusion method against different antimicrobial agents (Oxoid) as recommended by CLSI, 2018[8].The tested antimicrobials included ampicillin (10 µg), penicillin (10µg), amoxicillin/ clavulanic acid (20/10 µg),linezolid (30 µg),ceftriaxone (30 µg), cefepime (30 µg),amikacin (30µg), gentamicin (10µg), tetracycline (30µg), tigecycline (30 µg),chloramphenicol (30µg), ceftazidime (30µg), ciprofloxacin (5µg),levofloxacin (5µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg) and cefoxitin (30 µg) for the detection of methicillin resistance and erythromycin (15 µg) and clindamycin (2 µg) disks at 15 mm apart were also used on same plate for the detection of inducible clindamycin resistance [8].

Detection of MRSA by cefoxitin disk diffusion method:

If zone size ≥22 mm, the strain is methicillin susceptible and if zone ≤21 mm, the strain is MRSA [8]

Detection of inducible clindamycin resistance using the D-test:

Isolates resistant to erythromycin and sensitive to clindamycin were tested for inducible clindamycin resistance by detection of a D-shaped zone around clindamycin [8].

Detection of vancomycin resistance:

Methicillin resistant *Staph aureus* isolates were tested for MIC of vancomycin by agar dilution method as recommended by CLSI. Bacterial isolates were classified into VRSA, VISA, and VSSA according to the following MIC ranges VSSA ≤2 µg/mL, VISA 4-8 µg/mL and VRSA MIC ≥16 µg/mL respectively [8]

Detection of *mec*A and *van*A genes by multiplex PCR:

DNA extraction:

Cellular DNA was obtained from *S.aureus* isolates grown overnight on blood agar plates using DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. The used primers were designed and synthesized by Qiagen(Germany) (Table 1). Amplification of the target genes was doneusing PCR master mix (Taq green PCR Master Mix, (Qiagen, Germany).The PCR program was performed in the Thermocycler Apparatus (Biometra, Germany) that consisted of an initial denaturation (5 min at 95°C), followed by 40 cycles: DNA denaturation (1 min at 94°C), primer annealing (1 min at 46°C), and primer extension (1 min at 72°C)], followed by final extension (10 min at 72°C). Synthesized DNA fragments were detected on 1.5% agarose gels by ethidium bromide staining. A DNA ladder (100–1000 bp) was used to estimate allele sizes in base pairs (bp) for the gel [9, 10]

Table 1: Primers used in PCR

Target genes	Primer sequence (5'-3')	Reference	Size (bp)
*mec*A	Forward: CCTAGTAAAGCTCCGGAA Reverse: CTAGTCCATTCGGTCCA	[9]	314
*van*A	Forward: ATGAATAGAATAAAAGTTGC Reverse: TCACCCCTTTAACGCTAATA	[10]	474

Statistical analysis

Computer SPSS program version 20 was used. The results were expressed as ranges and mean± SD. Chi-square test was done and p value <0.05 was considered as significant.

RESULTS

About 250 patients with burn were included in this study (56% males and 44% females with mean age 22.3 ± 16.2 years old). A total of 171/250 specimens (68.4%) showed positive cultures (148 with single growth and 23with mixed growth (2 isolates for each). During this study, 194 different pathogens were isolated. The most frequent isolate was *S. aureus* (43.3%) followed by *Pseudomonas spp.* (29.4%) as shown in table 2.

Table 2: Number and percentage of growth from burn swabs and the isolated organisms from positive cultures

Burn swabs			No.	%
No growth (sterile burn)			79	31.6
Growth		**Single growth**	148	59.2
		Mixed growth	23	9.2
		Total	171	68.4
Total			250	100
Isolates	**Gram-positive**	***S. aureus***	84	43.3
	Gram-negative	***Pseudomonas spp.***	57	29.4
		Klebseilla spp.	23	11.9
		Enterobacter spp.	22	11.3
		E.coli	7	3.6
	Fungi	***Candida* spp.**	1	0.5
Total			194	100

About 94% (79/84) of isolated *S.aureus* were MRSA by cefoxitin disc diffusion method, and the inducible clindamycin resistance (D zone) was observed in 3 isolates only (3.8%) among MRSA.

All MRSA showed (100%) resistance to penicillin and ampicillin. On the other hand, all isolated MRSA strains (100%) were sensitive to linezolid and tigecycline. Other MRSA antibiograms were illustrated in Fig 1.

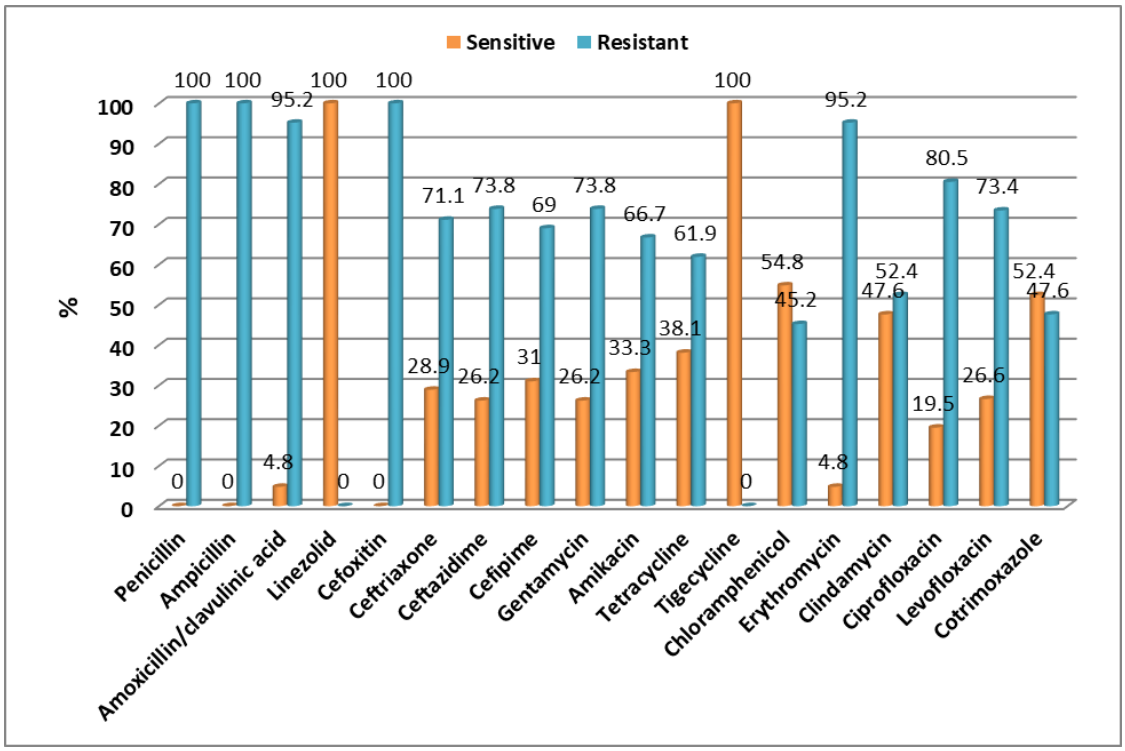

Fig. 1: Antibiotic susceptibility pattern of MRSA isolates

Among 79 MRSA isolates, agar dilution method showed that 47 (59.5%) as VSSA, 12(15.2%) as VISA, and 20 (25.3%) as VRSA as shown in Fig 2,3.

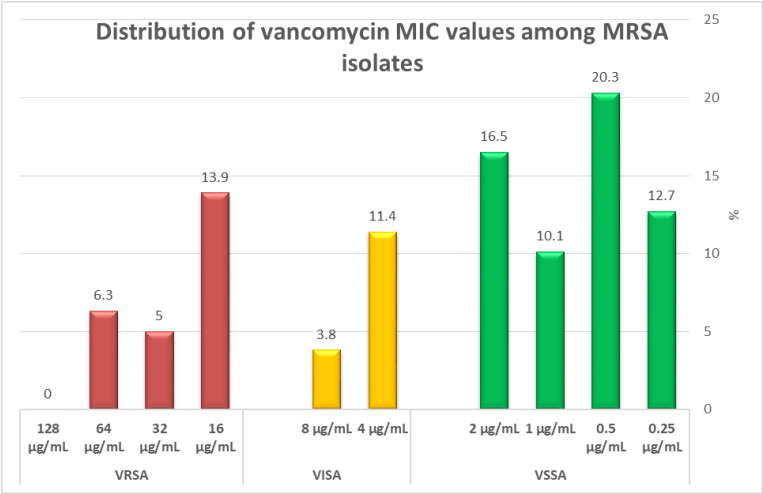

Fig. 2: Vancomycin MIC values among MRSA isolates

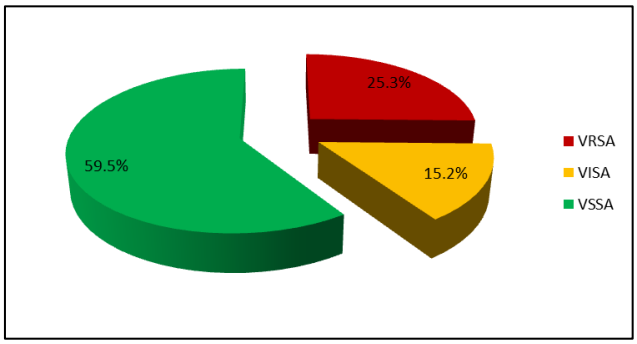

Fig. 3: Distribution of VSSA, VISA and VRSA among 79 isolates of MRSA

Considering PCR as the gold standard, *mec*A gene was detected in 78 isolates out of 79 isolates which were phenotypically identified by cefoxitin disk diffusion test so; sensitivity, specificity and diagnostic accuracy of cefoxitin disk diffusion test were 100%, 83.33%and 98.81% % respectively as shown in table 3.

Table 3: diagnostic value of cefoxitin disc diffusion method for the prediction of MRSA as diagnosed by PCR for detection of *mec*A gene

cefoxitin disc diffusion method	**PCR for detection of *mec* A gene**			
		+ve for gene	**-ve for gene**	**Total**
	Resistant	78	1	79
		92.9%	1.2%	94.1%
	Sensitive	0	5	5
		0%	5.9%	5.9%
	Total	78	6	84
		92.9%	7.1%	100%
Sensitivity	100%		95% CI (95.38% --- 100%)	
Specificity	83.33%		95% CI (35.88% --- 99.58%)	
Positive Predictive Value (PPV)	98.73%		95% CI (92.87% --- 99.79)	
Negative Predictive Value (NPV)	100%			
Accuracy	98.81%		95% CI (93.54% --- 99.97%)	

Among 79 MRSA isolates, 17 (21.5%) isolates had *van*A gene by PCR (16 isolates were VRSA and one isolate was VSSA. There was highly significant difference between vancomycin MIC for MRSA & presence of *van*A gene. About 80% of VRSA strains had *van*A gene compared to 97.9% of VSSA strains that didn`t have the gene as shown in table 4.

Table 4: Relation between presence of *van* A gene by PCR and vancomycin susceptibility by agar dilution method among MRSA isolates

	***Van* Agene**		***Test of sig***	***P value***
	Positive (n=17) No.(%)	**Negative (n=62) No.(%)**		
Vancomycin MIC for MRSA: VSSA (n=47)	1 (2.1)	46 (97.9)	54.25	<0.001
VISA (n=12)	0(0.0)	12 (100.0)		
VRSA (n=20)	16 (80.0)	4 (20.0)		
Total (MRSA= 79)	17(21.5)	62(78.5)		

DISCUSSION

Infection is the main cause of morbidity and mortality in burn patients. Burn wounds provide ideal environment for multiplication of bacteria due to plentiful supplies of nutrients and moisture. The immunosuppressive status of the patients leads to free multiplication of microorganisms [11].This fact was achieved in this research as 68.4% of our patients had burn wound infections, which was nearer to El Sebaey's[12] result who reported that 63.9% of burn patients had wound infections.

In this study, *S. aureus* was the most common isolated bacteria (43.3%), followed by *Pseudomonas* spp. (29.4%), *Klebseilla* spp.(11.9%), *Enterobacter* spp.(11.3%), *E.coli* (3.6%) and *Candida* spp. (0.5%). Similar results were reported by AL-Aali et al. [13] in KSA and Chen et al. [1] in China. On the other hand, El Sebaey[12] reported that the most common isolate of burn wound infection was *Klebsiella* spp. (47.5%). However, Ikram et al.[14] and Jasem et al. [15] found that *P.aeruginosae*, *K.pneumoniae* and *S. aureus* were the most frequent isolates.

One of the worldwide health problems is methicillin Resistant *Staphylococcus aureus* (MRSA) especially in burn centers as it leads to poor outcomes like prolonged hospitalization, sepsis and death[16]. Detection of MRSA is essential for proper infection control measures. Cefoxitin is a strong inducer for *mec*A regulatory system so it can be used as a marker for *mec*A gene detection[17].

In this study, about 94% of *S.aureus* were MRSA by cefoxitin disc diffusion method. Previous studies done in Egypt by Fakhr and Fathy[18],Mashaly et al., [19] Zaki and Hager[4], Amer and Gamal[20]and Abdel-Maksoud et al. [21]detected the prevalence of MRSA 100%, 92%, 84.6%, 78.9% and 76.6% respectively. Similarly, high rates of MRSA were reported in other parts of the world: 84.32% in India [22] and 72% in Bangladesh[23]. However, lower rates of MRSA were reported in Iran (42.73%) by Asadpour and Ghazanfari[5] and in Egypt (43.8%) by ElSayed et al. [24] The lowest rate (19 %) reported in Ghanaian Burn Unit by Amissah et al. [25] This significant variability in different regions, which may be due to differences of local antibiotic policy and the infection control practices in different health care facilities, needs to a periodic evaluation of MRSA [4].

Considering PCR as the gold standard, we found that sensitivity, specificity and diagnostic accuracy of cefoxitin disk diffusion test were 100%, 83.33% and 98.81% respectively. *mec*A gene was detected in 78 isolates out of 79 isolates phenotypically detected by cefoxitin disk diffusion test. These results came in a line with the data published by Siyahkali et al. [26] who found that sensitivity and specificity of disk diffusion were 100% and 85% respectively. Also, Islam and Shamsuzzaman[27] found that both sensitivity and specificity of cefoxitin disc diffusion method were 100%. The isolate that was MRSA positive but negative for *mec*A gene might carry another gene like *mec* C gene [28]

The MRSA isolated strains in the current study showed a high resistance not only to beta-lactams but also to most antimicrobials used with rates ranged from 40.5.% to 100%. This result was completely identical to numerous researches published in Egypt by ElFekyet al. [29], Mashaly et al. [19] and Zaki and Hager[4]. Also, the high resistance rate of MRSA was published in India by Otta et al. [16] and in Sudan by Khederet al. [30]. Resistance to methicillin and other beta-lactam antibiotics is mediated by *mec* A gene which is a part of staphylococcal chromosome cassette *mec* (SCCmec), a mobile genetic element that may contain genetic structures that encode

non-β-lactam antibiotics resistance. Also, the response of MRSA in hospitals to the antibiotics selection pressure may explain the high resistance rate of MRSA[21].

Detection of erythromycin induced clindamycin resistance by D test is important to avoid treatment failure with clindamycin for MRSA isolates. When this test is positive, it means it is resistant to clindamycin [8]. In this study, inducible clindamycin resistance was diagnosed in three isolates (3.8%) among MRSA. This result was nearer to the result of Zaki and Hager[4] (3.9%) and Abdel-Maksoud et al. [21] (5.3%) but lower than Adhikari et al.[3] (10%). This means that reporting MRSA as clindamycin sensitive without D-test may lead to prescribing inappropriate clindamycin therapy. On the other hand, negative D-test confirms susceptibility to clindamycin[31].

All MRSA isolates were sensitive to linezolid and tigecyclin. This means that these drugs could be suitable options for treatment [27].

The growing prevalence of MRSA has increased the use of vancomycin over the past 3 decades leading to selective pressure that resulted in the emergence *S. aureus* strains with decreased susceptibility to vancomycin [32].

As recommended by CLSI 2018, the agar dilution method is the ideal for vancomycin MIC to determine VISA and VRSA strains. Unfortunately, most microbiology laboratories in Egypt depend on the disk diffusion method to determine *S. aureus* susceptibility to vancomycin, which does not give reliable results. It can leave many VISA/VRSA isolates undetected and they will give inhibition zones with sizes similar to those of the vancomycin-susceptible ones [8].In this study, about 21.5% of MRSA was VRSA that was coincided with Mashaly et al.[19] who reported that 21.7% of MRSA was VRSA. However, Amr and Al Gammal [20] in Zagazig University Hospitals reported a lower result (11%).

In this study, about 15.2% of MRSA was VISA that was higher than data reported by Zaki and Hager[4] (2.6%) and Abdel-Maksoud et al [21] (1.2%). On the contrary, Osman et al. [33] and Ghoniem et al. [34] found a higher prevalence of VISA that were 22% and 20.68% respectively.

There are many studies conducted in this regard and the results were different as follows; ElFeky et al. [29] in Egypt found that 15% of MRSA isolates were VISA and no VRSA was detected. Asadpour and Ghazanfari [5] in Iran recognized 2.73% and 7.27% of MRSA as VRSA and VISA respectively. Park et al. [35] in South Korea found that 14 isolates (21.2%) were VISA and no VRSA was detected. Such variation in incidence of VRSA and VISA may be due to regional differences in antibiotic policies and infection control measures [5].

In this study, 6.3 %of MRSA exhibited vancomycin MIC higher than 32µg/mL. This may point to emerging vancomycin resistance of MRSA at a high-level. For this, it was necessary and inevitable to determine the *van*A gene that provides a high level vancomycin resistance. This gene can be transferred from enterococci to MRSA via plasmid leading to development of VRSA [36].In this study, 17 out of 79 MRSA isolates (21.5%) had *van*A gene by multiplex PCR. This result was higher than that detected by ElFeky et al. [29] who detected *van*A in 12%of MRSA isolates.

In the current study, 80% of VRSA and 2.1% of VSSA were *van*A gene positive respectively. Variable results were detected by Thati et al. [37] and Mahmood and Flayyih[38] who reported that *van*A gene presented in 86% and 4.5% respectively in their phenotypically detected VRSA.

The MRSA may be resistant to vancomycin, but *van*A gene does not exist because of other *van* genes such as *van*B, *van*C, *van*D, *van*E, and *van*G, which may be present in these *van*A-negative VRSA[19]This hypothesis was confirmed in this research, as 20% of VRSA were *van*A gene negative

In a study done by Asadpour and Ghazanfari[5] all VISA strains were free of *van*A gene. This was in consistent with the finding reported in this study, as all phenotypically VISA isolates were negative for *van*A gene. This intermediate resistance may be due to increased cell wall thickness leading to sequestration of vancomycin molecules in peptidoglycan layer, causing decreased susceptibility of *S. aureus* to vancomycin[30]

Detection of vancomycin resistance among MRSA isolates is a serious alarm demonstrating the need for new effective therapeutic agents [29].

Conclusion and Recommendations:

Continuous surveillance to monitor the changing patterns of vancomycin MICs levels among MRSA isolates is mandatory. Detection of high percentage of MRSA, VISA, and VRSA isolates in burn unit necessitates the implementation of infection control measures and proper use of effective antibiotics to control such multi-resistant strains.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Fund: No fund was received

REFERENCES

1. Chen K, Lin S, Li P, Song Q, Luo D, Liu T and Zhang W. Characterization of *Staphylococcus aureus* isolated from patients with burns in a regional burn center, Southeastern China. BMC infectious diseases 2018; 18(1): 51.
2. Akhtar RW, Hannan A, Saleem S, Qaisar A and Jahan S (2018): Frequency of vancomycin Resistant *Staphylococcus Aureus* Among Clinical Isolates Of MRSA collected From Tertiary Care Hospital Of Lahore, Pakistan. Pakistan Armed Forces Medical Journal 2018, 68(3): 580-84.
3. Adhikari R, Dutt Pant N, Neupane S, Neupane M, Bhattarai R, Sabita Bhatta S, Chaudhary R, and Lekhak B. Detection of Methicillin Resistant *Staphylococcus aureus* and Determination of Minimum Inhibitory Concentration of Vancomycin for *Staphylococcus aureus* Isolated from Pus/Wound Swab Samples of the Patients Attending a Tertiary Care Hospital in Kathmandu, Nepal .Canadian Journal of Infectious Diseases and Medical Microbiology 2017, Article ID 2191532, 6 pages.
4. Zaki W and Hager R Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate Susceptibility and Vancomycin Resistance among *Staphylococcus aureus* Isolated from Tertiary Care Hospital in Egypt. Egyptian Journal of Medical Microbiology 2018; 27(3): 53-58
5. Asadpour L and Ghazanfari N (2019): Detection of vancomycin non-susceptible strains in clinical isolates of *Staphylococcus aureus* in northern Iran. International Microbiology 2019; 22(4): 411-417.
6. Cheesbrough M. Microbiological tests in district laboratory practice in tropical countries, part II. Great Britain: Cambridge University Press; 2000. 1–266.
7. Isenberg HD. Clinical microbiology procedures handbook. 2nd ed. Washington D.C.: ASM press; 2004
8. Clinical and Laboratory Standards Institute 2018 Performance standards for antimicrobial disk susceptibility tests; 28th edition, CLSI supplement M100. Wayne, PA
9. Choi SM, Kim SH, Kim HJ, et al. Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among staphylococcus species. J Korean Med Sci 2003; 18:631–636.
10. Saha B, Singh AK, Ghosh A, Bal M. Identification and characterization of a vancomycin-resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). J Med Micro- boil 2008; 57:72–79.
11. Agnihotri N, Gupta V, Joshi M Aerobic bacterial isolate from burn wound infections and their antibiotics a five-year study. J Burns 2004; 30: 241-3.
12. El Sebaey A. Epidemiologic Features of Hospital-acquired Burn Wound Infections (HABWI) and Infection Prevention Precautions at a University Hospital in Egypt. American Journal of Infection Control 2019; 47(6): S23.
13. AL-Aali K. Microbial Profile of Burn Wound Infections in Burn Patients, Taif, Saudi Arabia. Arch Clin Microbiol. 2016; 7:2.
14. Ikram S, Asher N, Farooq B, and Rehman A. Spectrum of Bacterial Pathogens Isolated from Burn Wound Patients. Annals of PIMS-Shaheed Zulfiqar Ali Bhutto Medical University 2018; 14(3): 218-221.
15. JASEM, Meroj A. et al. The most frequent bacterial infections in burn injuries at burn units of two hospitals in Baghdad. Iraqi Journal of Public Health 2018; 2 (1): 12-15. ISSN 2521-7267.
16. Otta S, Dash JK and Swain B (2015): Aerobic bacteriology of burn wound infections. CHRISMED J Health Res 2015; 2:337-41.
17. Anand KB, Agrawal P, Kumar S, and Kapila K: Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene detection of MRSA. Indian Journal of Medical Microbiology 2009; 27(1): 27-29.
18. Fakhr A and Fathy F. Bacterial Pattern and Risk Factors of Hospital Acquired Infections in a Tertiary Care Hospital, Egypt. Egyptian Journal of Medical Microbiology 2018; 27 (1): 9-16
19. Mashaly M, El-Mashad N and El-deeb H (2019): Detection of VanA type vancomycin resistance among MRSA isolates from an emergency hospital in Egypt. Comparative Clinical Pathology 2019; 28(4): 971-976.
20. Amr G and Al Gammal S. Emergence of Vancomycin Resistant *Staphylococcus aureus* Isolated from Patients in ICUs of Zagazig University Hospitals. Egyptian Journal of Medical Microbiology.2017; 26 (2):53-59
21. Abdel-Maksoud M, El-Shokry M, Ismail G, Hafez S, El-Kholy A, Attia E and Talaat M. Methicillin-Resistant *Staphylococcus aureus* Recovered from Healthcare- and Community- Associated Infections in Egypt. *Int J Bacteriol*.2016:5751785.
22. Shende S and Wadhai V. Detection of MRSA and VRSA *Staphylococcus aureus* from Tertiary Care Center, Chandrapur, Maharashtra. ISSN (PRINT): 2393-8374, (ONLINE): 2394-0697, volume-6,

Issue-1, International Journal of Current Engineering and Scientific Research (IJCESR) 2019.

23. Hasan R, Acharjee M, Noor R. Prevalence of vancomycin resistant *Staphylococcus aureus* (VRSA) in methicillin resistant *S. aureus* (MRSA) strains isolated from burn wound infections. Tzu Chi Medical Journal 2016; 28(2):49–53
24. ElSayed N, Ashour M and Amine AEK. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt. GERMS 2018; 8(3):134-139.
25. Amissah NA, Buultjens AH, Ablordey A, van Dam L, Opoku-Ware A, Baines SL, Bulach D, Tetteh CS, Prah I, van der Werf TS, Friedrich AW, Seemann T, van Dijl JM, Stienstra Y, Stinear TP and Rossen JW. Methicillin Resistant *Staphylococcus aureus* Transmission in a Ghanaian Burn Unit: The Importance of Active Surveillance in Resource-Limited Settings. Front. Microbiol 2017; 8:1906.
26. Siyahkali M, Dadras O, Zohar I, Seyedalinaghi S and Hejazi N. Evaluation of Sensitivity and Specificity of the Method Cefoxitin-disk Diffusion in Detection of Methicillin-resistant *Staphylococcus Aureus* in Clinical Samples of Two Hospitals, Tehran, Iran. Journal of International Translational Medicine 2018; 6(3): 141-143.
27. Islam B and Shamsuzzaman M. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant, vancomycin-resistant, and Panton-Valentine leukocidin positive *Staphylococcus aureus* in a tertiary care hospital Dhaka, Bangladesh. Tzu Chi Medical Journal 2015; 27(1): 10-14.
28. Paterson GK, Harrison EM, and Holmes MA. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. Trends in microbiology 2014; *22*(1): 42–47.
29. ElFeky DS, Awad AR, Elshobaky MA, &Elawady BA. Effect of Ceftaroline, Vancomycin, Gentamicin, Macrolides, and Ciprofloxacin against Methicillin-Resistant *Staphylococcus aureus* Isolates: An *in Vitro* Study. Surgical Infections 2019; 20(10):1-9.
30. Kheder S, Ali N, and Fathelrahman A. "Prevalence and antimicrobial susceptibility pattern of methicillin resistant staphylococcus in a sudanese surgical ward," Pharmacology &Pharmacy 2012: 3, 1: 103–108.
31. Patel M, Waites K, Moser S, Cloud G and Hoesley C, "Prevalence of inducible clindamycin resistance among community- and hospital-associated *Staphylococcus aureus* isolates," Journal of Clinical Microbiology 2006; 44 (7): 2481–2484.
32. Tenover FC, Biddle JW and Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. Emerg Infect Dis 2001 Mar; 7(2):327-32.
33. Osman HB, Abdel Halim RM, Gomaa FAM, Amer MZ. Vancomycin MIC Distribution among Methicillin-Resistant *Staphylococcus Aureus*. Is Reduced Vancomycin Susceptibility Related To MIC Creep? Open Access Maced J Med Sci. 2019; 7(1):12-18.
34. Ghoniem E, El Hendawy G, Moteleb T, Hassan H, & Khalil H. Characterization of vancomycin-resistant *Staphylococcus aureus* in the National Liver Institute. Menoufia Medical Journal 2014; 27(4): 825.
35. Park JW, Lee H, Kim JW and Kim B. Characterization of Infections with Vancomycin-Intermediate *Staphylococcus aureus* (VISA) and Staphylococcus aureus with Reduced Vancomycin Susceptibility in South Korea. Scientific reports 2019; 9(1):1-9.
36. Khanam, S, Haq JA, Shamsuzzaman S, Rahman M and Mamun Z: Emergence of Vancomycin Resistant *Staphylococcus aureus* during Hospital Admission at a Tertiary Care Hospital in Bangladesh. Bangladesh J.Infec. Dis. 2016; 3: 11–16.
37. Thati V, Shivannavar CT and Gaddad M. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. The Indian journal of medical research 2011; 134(5): 704–708.
38. Mahmood HA and Flayyih MT. Detection of *van*A gene of vanco- mycin resistant *Staphylococcus aureus* by PCR technique. Int J 2014; 7: 209–216.