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ORIGINAL ARTICLE

Emergence of Vancomycin Resistant *Staphylococcus aureus* Isolated from Patients in ICUs of Zagazig University Hospitals

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ABSTRACT

Background*: Staphylococcus aureus infections treatment has become more complicated with emergence of a methicillin-resistant Staphylococcus aureus (MRSA) strain. Vancomycin has become the drug of choice for the treatment of serious MRSA infections Since the 1980s, however its efficacy is now being doubted due to the emergence of vancomycin resistant staph aureus (VRSA) that are always methicillin-resistant.* ***Objectives:*** *to investigate the emergence of vancomycin resistant Staphylococcus aureus among Staphylococcus aureus isolates from patients admitted to our hospital ICUs, and to determine the sensitivity of these isolates to different antimicrobial agents also to investigate for the presence of vanA gen in VRSA.* ***Methodology:*** *a total of 114 Staphylococcus aureus isolates were recovered from different clinical samples of patients admitted to ICUs over two years. All isolates were identified by Matrix-assisted laser desorption ionization–time of flight mass spectrometry and its antimicrobial susceptibility was investigated by VITEK 2 instrument, MRSA were identified, vancomycin MIC was confirmed by E test, mecA and vanA genes were investigated by PCR technique.* ***Results:*** *among Staphylococcus aureus isolates, there were 90/114 (78.9%) diagnosed as MRSA, the most active antimicrobial agent against these MRSA isolates was tigacyclin (100%) followed by linezolid (87.8%). we detected the emergence of VRSA that were 8.8% (10/114), all VRSA isolates were previously diagnosed as MRSA, percentage of VRSA among MRSA was 11% . The results of PCR showed that there were 88/90 MRSA isolates carrying mecA gene and all VRSA (10isolates) were positive for vanA and mecA genes.* ***Conclusion:*** *The results of this study can be considered as an advance warning about the emergence and dissemination of VRSA strains in our region.*

INTRODUCTION

Staphylococcus aureus (S. aureus) is an important cause of human disease, it can cause a wide range of infections from mild infections to life-threatening diseases, including skin and soft tissue infections, bacteremia, pneumonia, endocarditis, sepsis, and toxic shock syndrome[1]. The incidence of these bacterial infection had decreased due to discovery of penicillin in 1940, whoever *S. aureus* began producing β-lactamase, that destroys the penicillin β-lactam core ring and cause penicillin resistance[2]. The introduction of β-lactamase-resistant methicillin was significantly controlled the penicillin-resistant *S. aureus infection,* however, methicillin-resistant *S. aureus* (MRSA) strain was then emerged and spread globally**.** MRSA is characterized by the presence of *mec*A gene, that encodes an alternative penicillin binding protein 2a (PBP2a) with low-affinity to β-lactam antibiotics[3]**.**

Vancomycin has been used for treatment of penicillin-resistant and methicillin-resistant *S. aureus* since 1954. It has been used as the treatment of choice for serious infections caused by MRSA worldwide[4]. Glycopeptides as vancomycin inhibit cell wall synthesis in gram-positive bacteria by binding to the C-terminal D-Ala-D-Ala of the pentapeptide precursors of peptidoglycan and blocking the transglycosylation and transpeptidation reactions[5]. Vancomycin has proven remarkably reliable results, whoever its efficacy was questioned due to the emergence of *Staph. aureus* with reduced susceptibility to it[6]**.** The first *S. aureus* displaying vancomycin intermediate resistance (VISA) was isolated in Japan in 1996[7]. These VISA strains had thickened and poorly cross-linked cell wall layer that presents increased amounts of D-Ala-D-Ala building blocks of cell wall as a binding site for vancomycin which lead to diminished effects of vancomycin due to competition[8]. In 2002, the first Vancomycin-resistant *S. aureus* (VRSA) was reported in the United States that was due to acquisition of the *van*A resistance determinant from enterococci[9]. The *van*A gene product is a ligase that produces D-Ala-D-lactate (Lac), a substitution for D-Ala-D-Ala which is a building block for peptidoglycan synthesis that is much less affinity to

glycopeptides such as vancomycin[10], the resistance caused by *van*A-type resistance, was the first to be elucidated and it is the most common, that is characterized by high levels of resistance to glycopeptides as vancomycin[11]**.** There are many reports that record the detection of VRSA in Iran, India and other countries worldwide[6]**.**

The aim of this study was to investigate the emergence of vancomycin resistant S. aureus isolated from clinical samples of patients admitted to intensive care unit of Zagazig university hospital, and to determine the sensitivity of these isolates to different antimicrobial agents. Further investigation for the presence of Van A gen in VRSA strain was another aim of this study.

METHODOLOGY

This study was done in the microbiology unit and ICUs of Zagazig University Hospitals. A total of 114 *Staph aureus* isolates were obtained randomly from different clinical samples of patients admitted to the ICUs over 24 months period (November 2014 to October 2016). Approval for this study was obtained from Research Administration and Research Ethics Committee of Faculty of Medicine, Zagazig University. The collected samples included (blood culture, sputum, endotracheal aspirate, urine, body fluid, wound swab samples) that were transported to the microbiology laboratory and inoculated on blood agar, MacConkey agar and chocolate agar plates that incubated in aerobic, anaerobic and CO_2 condition at 37°C for 24-48 hours.

Identification

Staph. aureus isolates were identified by Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the **VITEK MS** system (Biomerieux. Inc, Durham, USA), also Gram staining, coagulase test (oxoid LTd; UK) and catalase test were performed. Chromogenic media was used for MRSA identification (Chrom ID ^{TM}MRSA agar. Biomerieux, France)

Antibiotic susceptibility testing:

Antibiotic susceptibility testing was carried out using Vitek 2 System (AST-GP67 card) for Gram-positive cocci (Biomerieux. Inc, Durham, USA), the following antibiotics were included: cefoxitin, bezylpenicillin, ampicillin, oxacillin, gentamycin, streptomycin, levofloxacin, ciprofloxacin, moxifloxacin. erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, vancomycin, tetracycline, tigecycline, nitrofurantion, rifampicin and trimethoprim/ sulphamethazol. Susceptibility of MRSA isolates to vancomycin were confirmed by E test strips (Biomerieux, France) that were performed according to the manufacturer's instructions and in accordance with the guidelines of the Clinical and Laboratory Standards Institute[12].

PCR

All *Staph. aureus* isolates were analyzed by PCR to detect *mec*A and *van*A genes as follow:

DNA was extracted from isolated *Staph. aureus* colonies by using QIAamp® DNA Mini kit (Qiagen GmbH, Germany), for DNA amplification TIANGE genomic DNA kit was used as described by the manufacture. PCR amplification conditions were as follows using thermal cycler (Gene Amp, PCR system 9700)

-PCR amplification of *van*A gene:

PCR amplification conditions were initial denaturation at 98°C for 2 min followed by 30 cycles of denaturation at 98°C for 40 s; annealing at 52°C for 45 s; extention at 72°C for 30 s and final extension at 72°C for 5 min.

- PCR amplification of *mec*A.

PCR amplification conditions were initial denaturation at 98°C for 2 min for, 35 cycles of denaturation at 98°C for 10 s, primer annealing at 50°C for 1 min, and extension at 72°C for 1 min. and final extension at 72°C for 5 min.

The amplified PCR products were visualized on 2% agarose gel stained with ethidium bromide and examined under ultraviolet light. A single DNA band at 310bp was recorded as positive for *mec*A gene and at 1032 bp was recorded as positive for *van*A gene. Primers used in this study are shown on Table 1.

Table 1: Primer used for detection of genes encoding *van*A and *mec*A genes in *staph aureus* isolates.

Primer	*Nucleotide Sequence (5′—3′)*	*Amplicon size*	*Reference*
*van*A F	ATGAATAGAATAAAAGTTGC	1032	**Saha et al.[13]**
*van*A R	TCACCCCTTTAACGCTAATA		
*mec*A F	TGGCTATCGTGTCACAATCG	310	**Dias et al.[14]**
*mec*A R	CTGGAACTGTTGAGCAGAG.		

Statistical analysis:

Data were analyzed using SPSS 20. Chi Square was used to compare categorical variables. P value of 0.05 was considered statistically significant

RESULTS

There were 114 *Staph. aureus* isolates included in the study, its antimicrobial sensitivity was done using Vitek2 instrument that diagnosed, 68/114vancomycin susceptible *Staph. aureus* (VSSA), 10 / 114 VISA and 12/114 VRSA strains. There were 90 /114 (78.9%) MRSA strains.

We confirmed the sensitivity results of MRSA isolates to vancomycin by E test to detect its minimum inhibitory concentration (MIC) (Fig. 1). The isolates were reported as (VSSA) when ≤ 2 µg/ml, vancomycin intermediate *Staph. aureus* (VISA) when MIC4-8µg/ml while these strains were defined as vancomycin resistant *Staph. aureus* (VRSA) with MIC ≥16 µg/ml according to **CLSI**[(12)]. The results reveled that among MRSA isolates, there were 10/90 VRSA (11.1%) in addition to 12/90 VISA (13.3%), and 68/90 VSSA (75.5%). The percentage of confirmed VRSA in comparison to the total number of *Staph. aureus* isolates were 8.8% (10/114).

All MRSA showed 100% resistant to pencillin, oxacillin and ampicillin. The most active antibiotic against isolated MRSA was tigecylclin (100%) followed by linezolid (87.8%) and quinupristin/dalfopristin (83.3%). There were 81/90 multidrug resistant (MDR) MRSA isolates (90%). Antimicrobial sensitivity test results for MRSA isolates are illustrated in (Fig.2).

All VRSA were resistant to most of the antibiotics; the most active antibiotic against isolated VRSA was tigecylclin (100%) followed by linezolid (60%) and quinupristin/dalfopristin 40%. (Fig. 3)

Prior use of vancomycin, hospitalization for more than 28 days, were identified as significant risk factors in patient who were suffering from infection due to MRSA resistant to vancomycin (X^2 =7.813, 33.277 respectively and P value was significant <0.05* for both) while age and sex of these patients were not significant risk factors.

As regard to PCR results there were 88 MRSA isolates carrying *mec*A gene and there were 2 isolates diagnosed as MRSA and were negative for *mec A* gene. All VRSA (10 isolates) were carrying *van*A gene and *mec*A gene. There were no VSSA or VISA carrying *van A* gene among MRSA isolates (Fig. 4).

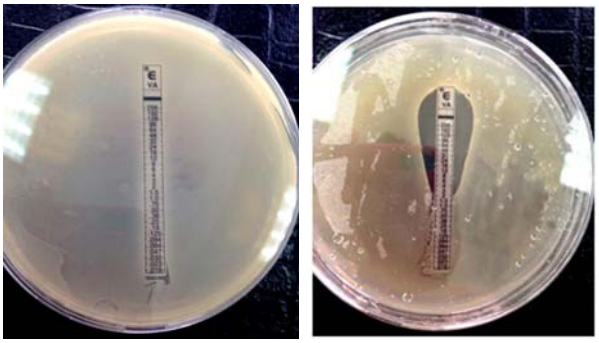

Fig. 1: E test results show VRSA and VSSA.

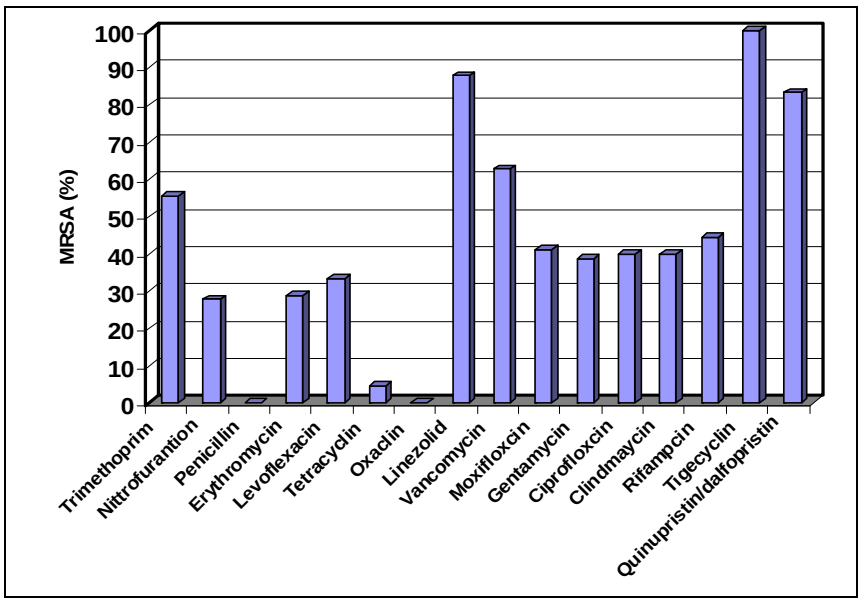

Fig. 2: Antimicrobial sensitivity pattern in MRSA isolates.

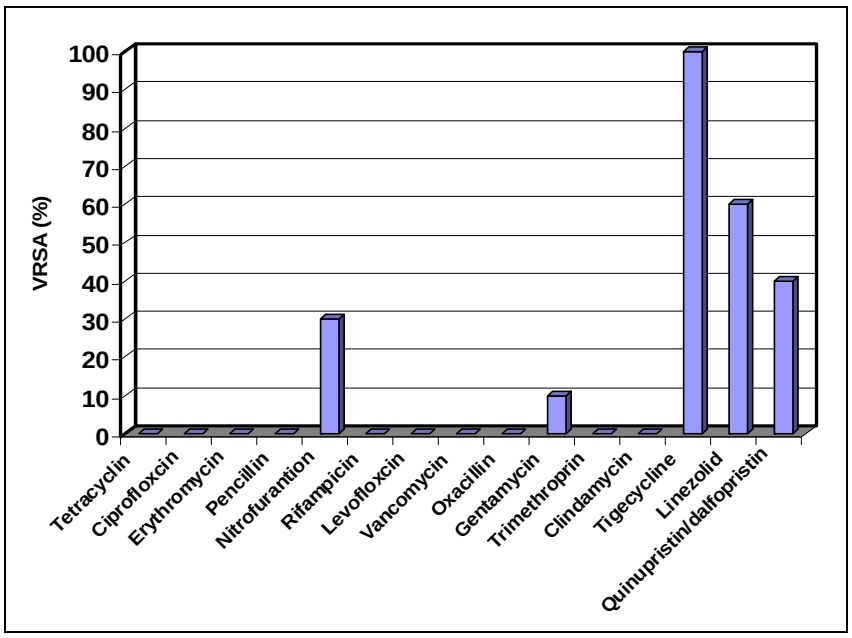

Fig. 3: Antimicrobial sensitivity pattern in VRSA isolates.

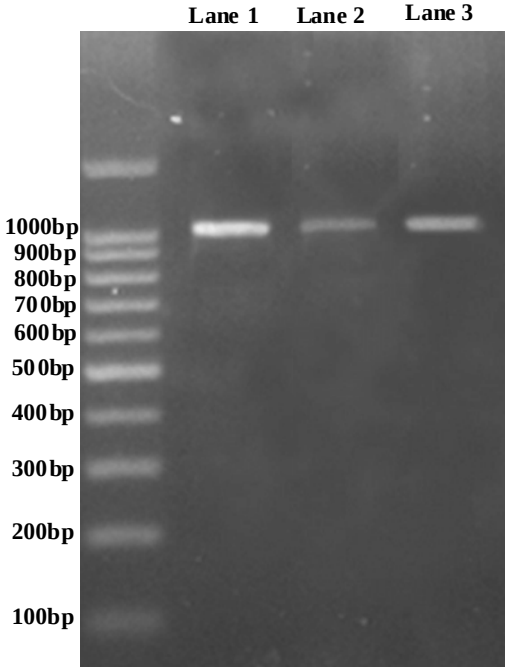

Fig. (4): PCR results of some VRSA isolates show *van*A gene (lane 1,2,3) at 1032 bp.

DISCUSSION

Diseases caused by methicillin-resistant *S. aureus* have been associated with high morbidity and mortality rates, since the late 1970s it has been associated with several hospital outbreaks worldwide[15]. Vancomycin was considered to be the best antimicrobial agent for the treatment of these serious infections caused by multidrug resistant MRSA but unfortunately the emergence of decrease in vancomycin susceptibility of MRSA and isolation of vancomycin intermediate and resistant *S. aureus* have been reported from many countries[16].

In this study the percentage of MRSA in the studied *Staph. aureus* isolates was 78.9% (90/114) this results is in agreement with previous studies done in Egypt by Abdel-Maksoud et al.[17], Ghoniem et al.[18] and El Kholy et al.[19]. Also this result was in agreement with other studies done in different part of the world as those of Hasan et al.[20], Ghenghesh et al.[21], Thati et al.[22] and

Baddour et al.[23] that were done in Iran, Lebanon, India and Saudi Arabia respectively. However this results was higher than the result of Osman et al.[24] that was done in Sudan and concluded that MRSA was only 41%.

Most MRSA isolates (90%) were multidrug resistant, this in agreement with previous study of Abdel-Maksoud et al.[17] that reported that MDR of MRSA isolate were (85%) and also in agreement with the results of Kheder et al.[25] and Saderi et al.[26].

Tigecycline was the most effective antibiotic against MRSA isolates (100%) this finding is in agreement with Brandon et al.[27].

As regards to vancomycin MIC results by Etest among MRSA, there were13.3% VISA (12/90) this was near to the results of a studies of Osman et al.[24] and Muneeri et al.[28] that were12%, 14.43% respectively. On the other hand, these results were lower than the results of Ghoniem et al.[18] who reported that VISA percentage was 20.68%, and higher than that of Abdel-Maksoud et al.[17], Kheder et al.[25], Abdollahi et al.[29]. These results were reported from Egypt and other developing countries as Iran and Sudan, however VISA isolates have been also recorded from many other countries around the world, including United States, France, Australia, Scotland, Brazil, Japan, South Korea, China[4].

In the current study we detected the emergence of VRSA among the studied *staph aureus*, MIC by E test revealed that VRSA percentage was (8.8%) this result was lower than the results recorded in Iran by Hasan et al.[20] that was 28% and recorded in Egypt by Ghoniem et al.[18] that was 20.68% and by El-Banna et al.[30] that was 20.13%. The percentage of VRSA among MRSA isolates in this study was 11%, which was also lower than the result of Dubey et al.[31] that was 16.8%.

This result was higher than the results reported in Nigeria by Moses et al.[32] that concluded VRSA was (5.4%), also lower than the results of Thati et al.[22] that was (1.9%)

As regards PCR result, *mec A* gene was detected in 88/90 of diagnosed MRSA isolates, 2 isolates were not carrying this gene, this result was in agreement with the result of Hawraa et al.[33].

We detected *van A* gene in all VRSA isolates diagnosed by E test, these results is in agreement with previous studies done in Egypt by El-Banna et al.[30], El-Daker et al.[34] and both concluded that all VRSA isolates in each study were *van A* gene positive. On the other hand some author recorded the presence of some VRSA isolates that were negative for *van A* gene as Thati et al.[22], Tiwari and Sen[35].

One of the limitations of this study was the small number of isolates that were only collected from patients admitted to our ICUs, the other is that, our results is of single center study and this findings may not be applied to other hospitals in Egypt.

The result of this study can be considered as an advance warning about the emergence and dissemination of VRSA in our region. The elevation of vancomycin resistance rates is mostly due to excessive use of this antimicrobial agent for treatment of MRSA, which represents an impending threat to patient health. VRSA is a local and global health threat that requires proper prescription of antimicrobial agents, adherence to the infection control principles and continuous epidemiologic surveillance.

Further multicenter wider studies are recommended to investigate the prevalence, potential sources, and mode of transmission of VRSA.

CONCLUSION

This study highlights the emergence of VRSA among the studied *s. aureus* that were isolated from patients admitted to ICUs. Tiegacyclin, linezolid and quinupristin/dalfopristin were the most active antimicrobial agents that can be used to replace vancomycin for MRSA treatment. The implementation of antibiotic stewardship programs and development of regulations for the antibiotic use is essential.

Conflict of interest: The authors declare no conflict of interest

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