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ORIGINAL ARTICLE

# Prevalence of Vancomycin Resistance among Clinical Isolates of MRSA from Different Governorates in Egypt

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***Key words:***
***MRSA, VRSA vancomycin resistance, vanA, vanB, virulence***

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## ABSTRACT

***Background****: The uncontrolled use of vancomycin led to an upsurge of vancomycin-resistant S. aureus (VRSA) throughout the world.* ***Objective****: The goal of this study is to screen vancomycin resistance among MRSA isolates, determine antimicrobial resistance pattern and evaluate the distribution of virulence genes among these isolates.* ***Methodology****: A total of 127 S. aureus clinical isolates were used, MRSA isolates were identified and antimicrobial sensitivity pattern for nine antimicrobial agents from different classes was assessed. In addition, vancomycin MIC was determined by standard agar dilution method and PCR identification of vancomycin resistance encoding genes vanA and vanB was performed. Moreover, the prevalence of eight different virulence genes was determined among different vancomycin resistance categories.* ***Results****: All isolates were identified phenotypically as MRSA. However, mecA gene was detected only in 95.28% of isolates. The highest and lowest percentage of resistance was recorded for clindamycin (82.68%) and trimethoprim (11.81%), respectively. Vancomycin resistance level was 23.62% of isolates, while vanA and vanB genes were detected only in 16.67% and 10% of VRSA isolates, respectively. The highest prevalence of virulence genes was found for icaA, followed by hld, hlb, icaD, hlg, hla, tsst and cna, respectively in the tested isolates. In addition, VRSA isolates showed higher mean virulence score (MVS) of 3.6 compared to VISA and VSSA isolates.* ***Conclusion****: This study highlights the alarming problem of the increasing incidence of VRSA infections in Egypt. Therefore, there is an urgent need to rationalize vancomycin consumption and to continuously monitor the prevalence of VRSA strains.*

## INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen of a great public health concern that can result in a wide range of clinical infections ranging from minor skin and soft tissue infections to serious and life-threatening systemic illnesses[1].

MRSA was first discovered in 1961 just two years after methicillin introduction to combat penicillin resistance of *S. aureus*. Methicillin resistance is mediated by *mecA* gene that encodes the low-affinity penicillin-binding protein PBP2[2].

Vancomycin was licensed as the first line glycopeptide antibiotic for the treatment of MRSA infections. Unlike the rapid appearance of methicillin resistance, reduced susceptibility or resistance to vancomycin took over three decades to evolve. Vancomycin resistance is mediated by *van* gene clusters that encode enzymes that replace the C terminal D-Ala-D-Ala residues of the peptidoglycan precursor with D-Ala-D-Lac[3]. However, vancomycin intermediate *S. aureus* (VISA) exhibits reduced susceptibility to vancomycin due to the synthesis of an unusually thickened cell wall containing dipeptides (D-Ala-D-Ala) capable of binding vancomycin and effectively sequestering them[4].

Vancomycin resistant *S. aureus* (VRSA) tends to be multi drug resistant (MDR) against a variety of currently available antibiotics making treatment of VRSA infections challenging and they continue to be a significant cause of mortality[5].

The ability of *S. aureus* to cause infections occurs through the expression of various virulence factors and liberation of different toxins that play a significant role in pathogenesis. One of these factors is collagen-binding protein that enables the bacteria to attach to collagen-rich tissues and inhibits the classical pathway of the complement system. Biofilm is another important factor that increases bacterial antibiotic resistance and provides a protected environment against the host's defenses. Hemolysins are one of exotoxins produced by *S. aureus* which have a cytolytic effect on a wide range of cells, primarily erythrocytes, platelets, monocytes, and neutrophils. Another significant toxin is toxic shock syndrome toxin, which can cause enormous T-cell activation and unregulated synthesis of proinflammatory mediators, causing capillary leak and shock syndrome[6].

The objective of this study is to detect vancomycin resistance in MRSA isolates, determine the

antimicrobial resistance pattern and detect different virulence genes among different vancomycin resistance categories.

## METHODOLOGY

### Isolation and identification of *S. aureus* isolates

Over the period between March 2019 to February 2021, a total of 268 clinical specimens were obtained from several hospitals located in three governorates: Damietta, Dakahlia and Cairo, Egypt. Identification of *S. aureus* isolates was performed using the biochemical standard methods[7].

### Phenotypic and genotypic identification of MRSA isolates

Methicillin resistance was determined using cefoxitin discs (30µg) (Oxoid, UK) by disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI)[8]. In addition, PCR amplification of *mecA* gene was also performed in all isolates using primers and conditions listed in Table 1.

### Antimicrobial susceptibility testing

By disc diffusion method, the antimicrobial susceptibility of all isolates to nine antimicrobial agents belonging to different classes was performed[9] using discs of linezolid (30µg), clindamycin (2µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), doxycycline (30µg), ciprofloxacin (5µg), levofloxacin (5µg), gentamicin (10µg), nitrofurantoin (300µg) and azithromycin (15µg). All discs are (Oxoid, UK).

By standard agar dilution method, minimum inhibitory concentration (MIC) of vancomycin was calculated and the results were interpreted according to recommendations of CLSI[8].

Isolates that are resistant to at least one agent from three different antimicrobial categories were determined as MDR[10].

### Molecular detection of some resistance and virulence genes

#### *DNA extraction*

A rapid DNA extraction method (colony PCR) was performed. In PCR tubes, five fresh colonies were suspended in 100µl of sterile, nuclease-free water. The product was boiled in the thermocycler for 10 minutes at 95°C, after which it was stored at -20°C[11].

#### *Detection of vancomycin resistance encoding genes by PCR*

The vancomycin resistance encoding genes (*vanA* and *vanB*) were screened among VRSA isolates. Primer pairs and PCR conditions are showed in Table 1.

#### *Detection of some virulence genes*

The virulence genes of collagen-binding protein (*cna*), intercellular adhesion genes (*icaA, icaD*), hemolysins (*hla, hlb, hlg and hld*) and toxic shock syndrome toxin (*tsst*) genes were detected by PCR using primers and conditions in Table 1.

The following reaction was used for all resistance and virulence genes. For a total volume of 25µl: 12.5µl Dream Taq Green PCR Master Mix (2X) (Thermo Scientific™), 7.5µl nuclease-free water, 1µl of each primer, and 3µl of bacterial DNA. For negative control, the reaction was performed without a DNA template.

**Table 1: List of oligonucleotide primers, annealing temperature, expected amplicon size and PCR conditions used in this study**

| Gene name | Type | Nucleotide sequence (5` to 3`) | Amplicon size (bp) | Annealing temperature (°c) | PCR conditions | Ref. |
|---|---|---|---|---|---|---|
| *mecA* | Fw<br>Rv | TGCTATCCACCCTCAAACAGG<br>AACGTTGTAACCACCCCAAGA | 286 | 50 | one cycle at 95°C for 2 min, followed by 40 cycles of (95°C for 30 sec, the primer's specific temperature for 30 sec and 72°C for 40 sec). Finally, one cycle at 72°C for 5 min. | (12) |
| *vanA* | Fw<br>Rv | GCA ATA GAG ATA GCC GCT AAC A<br>TGA CTT GCC ATG CAA AGC TG | 236 | 55 | | This study |
| *vanB* | Fw<br>Rv | GATGGTGCGATACAGGGTCTG<br>GAT GTT GTC CAC TTC GCC GAC | 389 | 53 | | This study |
| *cna* | Fw<br>Rv | GTCAAGCAGTTATTAACACCAGAC<br>AATCAGTAATTGCACTTTGTCCACTG | 423 | 54 | | (12) |
| *icaA* | Fw<br>Rv | CTCAATCAAGGCATTAAACAGGC<br>ACATGGCAAGCGGTTCATACT | 393 | 53 | | (12) |
| *icaD* | Fw<br>Rv | TGGTCAAGCCCAGACAGAGG<br>TGATAATCGCGAAAATGCCC | 242 | 56 | | (12) |
| *hla* | Fw<br>Rv | GTAATAACTGTAGCGAAGTCTGGTGA<br>AAACACATATAGTCAGCTCAGTAACA | 700 | 50 | one cycle at 95°C for 5 min, followed by 40 cycles of (95°C for 30 sec, primer's specific temperature for 45 sec and 72°C for 1 min). Then, one cycle at 72°C for 7 min. | (13) |
| *hlb* | Fw<br>Rv | GCAATATAAACGCGCTGATTTAATCG<br>GAGTGCCTTTATTGACATTAAGGTCG | 517 | 50 | | (13) |
| *hlg* | Fw<br>Rv | GACATAGAGTCCATAATGCATTYGT<br>ATAGTCATTAGGATTAGGTTTCACAAAG | 390 | 55 | | (13) |
| *hld* | Fw<br>Rv | AAGAATTTTTATCTTAATTAAGGAAG8GAGTG<br>TTAGTGAATTTGTTCACTGTGTCGA | 111 | 58 | | (13) |
| *tsst* | Fw<br>Rv | CGTAAGCCCTTTGTTGCTTG<br>TGTCAGACCCACTACTATAC | 143 | 53 | | (13) |

Fw: Forward Rv: Reverse bp: base pair

After electrophoresis on a 1.5% agarose gel stained with ethidium bromide, PCR products were visualized by UV illumination and compared with a 100 bp DNA ladder (enzynomics).

**Statistical analysis**

Correlation between the results was evaluated using chi-square test, except for the correlation between degree of virulence among different vancomycin resistance categories, One Way ANOVA test was used in addition to chi-square test. Statistical significance was considered at P-value ≤ 0.05. Data were analyzed using the SPSS software (version 20.0; SPSS, Chicago, IL, USA).

**Ethical consideration**

The Research Ethics Committee of Faculty of Pharmacy, Mansoura University certified that the research work conducted on clinical isolates from human was approved (2022 – 125).

## RESULTS

**Isolation and identification of isolates**

In the present study, a number of 127 isolates out of 268 specimens were identified as *S. aureus* by Gram staining, D-mannitol fermentation, catalase and coagulase production. The identified *S. aureus* isolates were collected from different clinical sources including wound (n=39), urine (n=34), blood (n=23), throat swab (n=10), sputum (n=8), nose swab (n=6), vaginal swab (n=4), pus (n=2) and nail swab (n=1).

**Identification of MRSA isolates**

All the 127 isolates were identified phenotypically as MRSA. However, molecular identification of MRSA revealed that only 121 isolates harbored *mecA* gene (Fig. 2A).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing revealed that high resistance levels were detected to clindamycin (82.68%) and linezolid (75.59%), followed by azithromycin and doxycycline (37.80%). Lower resistance levels were observed for gentamicin, levofloxacin, ciprofloxacin, and nitrofurantoin (22.05%, 21.26%, 20.47% and 19.96%, respectively). The lowest level of resistance was observed for trimethoprim (11.81%).

Concerning vancomycin resistance, 30 isolates (23.62%) exhibited MICs ≥16µg/ml and they were considered as VRSA. In addition, eight isolates (6.30%) showed MICs values ranging from 4 to 8µg/ml and were classified as VISA. The remaining 89 isolates (70.08%) exhibited MICs ≤ 2 µg/ml and were identified as VSSA. A total of 114 isolates (89.76%) were determined to be MDR (Fig. 1).

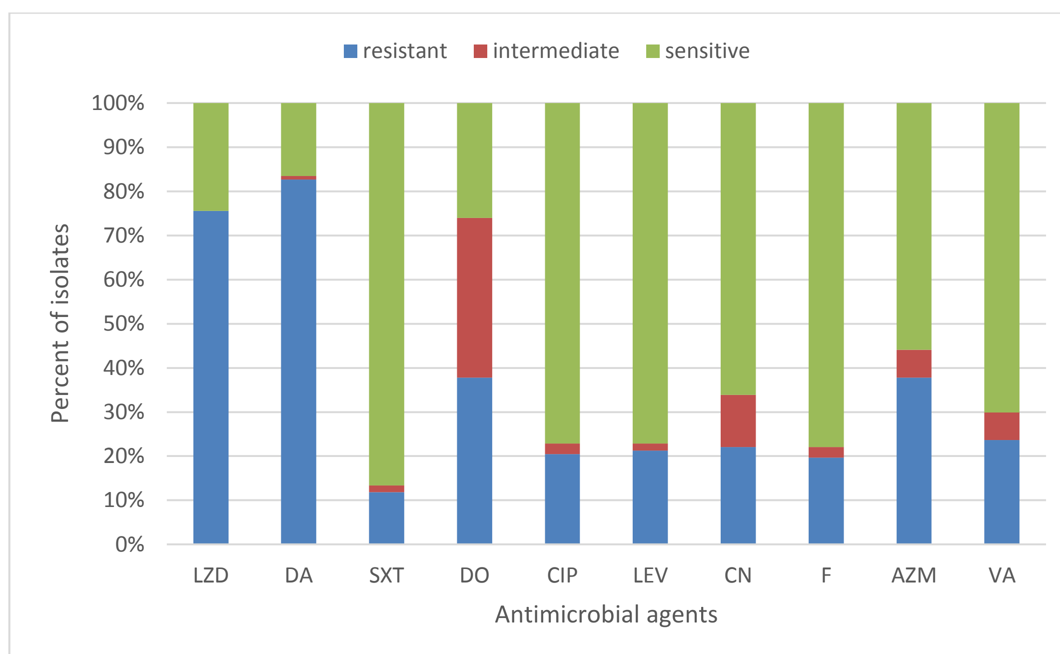


**Fig. 1:** Percentage of antibiotic resistance among the 127 MRSA isolates
**LZD**: linezolid; **DA**: Clindamycin; **SXT**: Trimethoprim-Sulfamethoxazole; **DO**: Doxycycline; **CIP**: Ciprofloxacin; **LEV**: Levofloxacin; **CN**: Gentamycin; **F**: Nitrofurantoin; **AZM**: Azithromycin

A comparison of antimicrobial resistance between VRSA, VISA and VSSA isolates is shown in Table 2. Resistance to clindamycin and linezolid was significantly recorded among VRSA isolates (P=0.001). In contrast, the highest percentage of resistance among VISA isolates was significantly reported for azithromycin (P=0.003). Different antimicrobial susceptibility patterns (67 patterns) were observed among VRSA, VISA and VSSA isolates (

Table 3), where 17 distinct patterns were related with VRSA. Pattern P6 was common among the three categories, while patterns P16, P17, P29, P33 and P55 were common among both VRSA and VSSA. The most prevalent pattern among VSSA isolates was P17.

**Table 2: Prevalence of antimicrobial resistance among vancomycin-resistant, intermediate and susceptible isolates**

| Antimicrobial agent | Number of resistant isolates (%) | | | P value |
|---|---|---|---|---|
| | VRSA N=30 | VISA N=8 | VSSA N=89 | |
| DA | **30** (100%) | **3** (37.5%) | **72** (80.9%) | 0.001* |
| LZD | **26** (86.67%) | **2** (25%) | **68** (76.4%) | 0.001* |
| DO | **10** (33.33%) | **3** (37.5%) | **35** (39.33%) | 0.842 |
| AZM | **7** (23.33%) | **7** (87.5%) | **34** (38.2%) | 0.003* |
| CN | **5** (16.67%) | **1** (12.5%) | **22** (24.72%) | 0.52 |
| LEV | **6** (20%) | **1** (12.5%) | **20** (22.47%) | 0.789 |
| CIP | **5** (16.67%) | **1** (12.5%) | **20** (22.47%) | 0.671 |
| F | **8** (26.67%) | **0** (0%) | **17** (19.1%) | 0.234 |
| SXT | **3** (10%) | **0** (0%) | **12** (13.48%) | 0.495 |

**LZD**: linezolid; **DA**: Clindamycin; **SXT**: Trimethoprim-Sulfamethoxazole; **DO**: Doxycycline; **CIP**: Ciprofloxacin; **LEV**: Levofloxacin; **CN**: Gentamycin; **F**: Nitrofurantoin; **AZM**: Azithromycin; *: significant difference

**Table 3: Distribution of antimicrobial resistance patterns among VRSA, VISA and VSSA isolates**

| Pattern number | Resistance pattern | No. of isolates (%) | | |
|---|---|---|---|---|
| | | VRSA N=30 | VISA N=8 | VSSA N=89 |
| P1 | - | 0 (0%) | 0 (0%) | 3 (3.37%) |
| P2 | AZM | 0 (0%) | 1 (12.5%) | 2 (2.25%) |
| P3 | DA | 2 (6.67%) | 0 (0%) | 0 (0%) |
| P4 | LZD | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P5 | DO | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P6 | LZD, DA | 4 (13.33%) | 1 (12.5%) | 4 (4.49%) |
| P7 | LZD, DO(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P8 | DA, DO | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P9 | DA, DO(i) | 1 (3.33%) | 0 (0%) | 0 (0%) |
| P10 | DO, F(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P11 | DO, AZM | 0 (0%) | 3 (37.5%) | 0 (0%) |
| P12 | DO(i), CN(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P13 | DO(i), AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P14 | CN(i), AZM | 0 (0%) | 1 (12.5%) | 0 (0%) |
| P15 | CN, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P16 | LZD, DA, DO | 4 (13.33%) | 0 (0%) | 7 (7.87%) |
| P17 | LZD, DA, DO(i) | 4 (13.33%) | 0 (0%) | 16 (17.98%) |
| P18 | LZD, DA, SXT | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P19 | LZD, DA, AZM | 0 (0%) | 0 (0%) | 2 (2.25%) |
| P20 | LZD, DA, AZM(i) | 1 (3.33%) | 0 (0%) | 0 (0%) |
| P21 | LZD, DA, CN(i) | 3 (10%) | 0 (0%) | 0 (0%) |
| P22 | LZD, DO, AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| P23 | DA, DO(i), AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |

| | | | | |
|---|---|---|---|---|
| **P24** | DA, CN, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P25** | DO(i), CIP(i), CN(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P26** | DA, DO(i), AZM(i) | 1 (3.33%) | 0 (0%) | 0 (0%) |
| **P27** | DO, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P28** | LZD, DA, SXT, DO | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P29** | LZD, DA, SXT, DO(i) | 1 (3.33%) | 0 (0%) | 2 (2.25%) |
| **P30** | LZD, DA, DO, AZM | 0 (0%) | 0 (0%) | 2 (2.25%) |
| **P31** | LZD, DA, DO, AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P32** | LZD, DA, DO(i), AZM | 0 (0%) | 1 (12.5%) | 1 (1.12%) |
| **P33** | LZD, DA, DO(i), CN(i) | 1 (3.33%) | 0 (0%) | 1 (1.12%) |
| **P34** | LZD, DA, DO, CN(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P35** | LZD, DA, DO(i), F | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P36** | DA, CIP, LEV, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P37** | CIP, LEV, CN, AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P38** | LZD, DA, DO, CN, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P39** | LZD, DA, SXT, DO, LEV(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P40** | LZD, DA, SXT, DO, CN(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P41** | LZD, DA, SXT, DO(i), CN | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P42** | LZD, DA, SXT, DO(i), CN(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P43** | LZD, DA, DO(i), CN(i), AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P44** | LZD, DA, SXT, F(i), AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P45** | LZD, DA, DO, CN, F | 1 (3.33%) | 0 (0%) | 0 (0%) |
| **P46** | DA, DO, CIP(i), CN, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P47** | DA, DO(i), CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P48** | DA, CIP, LEV, CN, AZM | 0 (0%) | 1 (12.5%) | 0 (0%) |
| **P49** | LZD, DA, SXT, DO, F, AZM | 1 (3.33%) | 0 (0%) | 0 (0%) |
| **P50** | LZD, DA, DO, CIP, LEV, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P51** | LZD, DA, DO(i), CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P52** | LZD, DA, DO, CIP, LEV, CN | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P53** | DA, SXT, CIP, LEV, CN, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P54** | DA(i), DO, CIP, LEV, CN(i), AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P55** | LZD, DA, DO, CIP, LEV, CN, F, AZM | 1 (3.33%) | 0 (0%) | 7 (7.87%) |
| **P56** | LZD, DA, SXT(i), DO, CIP, LEV, CN, F, AZM | 1 (3.33%) | 0 (0%) | 0 (0%) |
| **P57** | LZD, DA, SXT, DO, CIP, LEV, CN, F(i), AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P58** | LZD, DA, SXT, DO, CIP, LEV, CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P59** | LZD, DA, DO(i), CIP, LEV, CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P60** | LZD, DA, DO(i), LEV(i), CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P61** | LZD, DA, SXT(i), DO(i), CIP, LEV, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P62** | LZD, DA, DO(i), CIP, LEV, CN, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P63** | LZD, DA, DO, CIP, LEV, F, AZM | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P64** | LZD, DA, DO, CIP, LEV, CN(i), AZM(i) | 0 (0%) | 0 (0%) | 1 (1.12%) |
| **P65** | LZD, DA, DO(i), CIP, LEV, CN, F, AZM | 2 (6.67%) | 0 (0%) | 0 (0%) |
| **P66** | LZD, DA, DO, CIP(i), LEV, F, AZM | 1 (3.33%) | 0 (0%) | 0 (0%) |
| **P67** | LZD, DA, SXT, DO, CIP, LEV, F, AZM | 1 (3.33%) | 0 (0%) | 0 (0%) |

**LZD**: linezolid; **DA**: Clindamycin; **SXT**: Trimethoprim-Sulfamethoxazole; **DO**: Doxycycline; **CIP**: Ciprofloxacin; **LEV**: Levofloxacin; **CN**: Gentamycin; **F**: Nitrofurantoin; **AZM**: Azithromycin; **i**: intermediate

Correlation between clinical source and vancomycin resistance revealed that the highest percent of VRSA isolates (38.46%) were significantly recovered from wound (P=0.02), while no isolates was obtained from throat. Regarding VISA, urine was the major source for isolation (

Table 4).

**Table 4: Distribution of different vancomycin resistance categories among clinical sources**

| Clinical source | Wound N=39 (30.71%) | Urine N=34 (26.77%) | Blood N=23 (18.11%) | throat swab N=10 (7.87%) | Sputum N=8 (6.3%) | nose swab N=6 (4.72%) | vaginal swab N=4 (3.15%) | Other sources N=3 (2.36%) | Total N=127 (100%) |
|---|---|---|---|---|---|---|---|---|---|
| **VRSA** | **15** (38.46%) | **7** (20.59%) | **3** (13.04%) | **0** (0%) | **2** (25%) | **1** (16.67%) | **1** (25%) | **1** (33.33%) | **30** (23.62%) |
| **VISA** | **1** (2.56%) | **5** (14.71%) | **1** (4.35%) | **0** (0%) | **1** (12.5%) | **0** (0%) | **0** (0%) | **0** (0%) | **8** (6.3%) |
| **VSSA** | **23** (58.97%) | **22** (64.71%) | **19** (82.61%) | **10** (100%) | **5** (62.5%) | **5** (83.33%) | **3** (75%) | **2** (66.67%) | **89** (70.08%) |
| **P value** | 0.02* | 0.06 | 0.34 | 0.09 | 0.742 | 0.710 | 0.870 | 0.852 | |

**Detection of vancomycin resistance encoding genes by PCR**

Detection of *vanA* and *vanB* genes among 30 VRSA isolates showed that they were detected in 16.67% and 10% of isolates, respectively. *VanA* and *vanB* genes showed amplicons of 236bp and 389bp, respectively (Fig. 2B).

**Molecular detection of some virulence genes**

Investigation of biofilm encoding genes (*icaA* and *icaD*) revealed that they were found in 66.14% and 45.67% of isolates, respectively. Concerning hemolysin genes, *hld* gene was the most predominant (60.63%) followed by *hlb* (51.18%), *hlg* (41.73%) and *hla* genes (29.92%). Furthermore, *cna* gene was found in 10.24% of isolates while, *tsst* gene was detected in 13.39% of isolates (Fig. 2C).

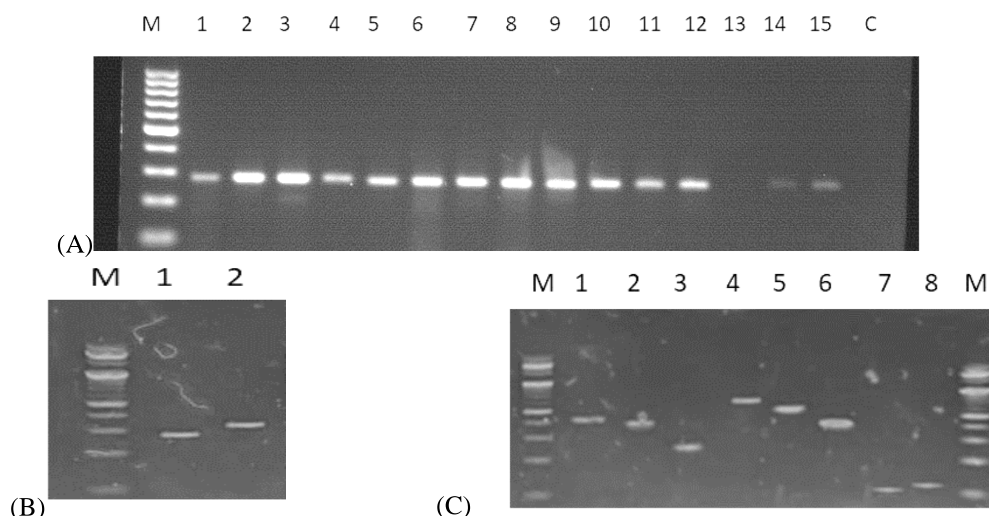


**Fig. 2:** Gel electrophoresis (1.5%) for detection of some resistance and virulence genes

(A) Detection of *mecA* gene (286bp), where lane M: 100bp DNA ladder; lane C: negative control; lane 13: negative sample; lanes 1-12,14 and 15: positive samples.

(B) Detection of vancomycin encoding genes, where lane M: 100bp DNA ladder; lane 1: *vanA* gene (236 bp); lane 2: *vanB* gene (389bp).

(C) Detection of some virulence genes, where lane M: 100bp DNA ladder; lanes 1-8: *cna* (423bp), *icaA* (393bp), *icaD* (242bp), *hla* (700bp), *hlb* (517bp), *hlg* (390bp), *hld* (111bp) and *tsst* (143bp) genes, respectively.

The distribution of virulence genes among vancomycin resistance categories revealed that VRSA isolates harbored the highest percent of *icaD*, *hla* and *hlb* genes. In addition, VISA isolates had the highest percent of *cna*, *hlg, hld* and *tsst* genes, where *hlg* gene was significantly more prevalent among VISA isolates (P=0.012). Moreover, *icaA* was the most prevalent gene (67.42%) in VSSA, and nearly identical level (66.67%) in VRSA isolates. Regarding other virulence genes, no significant difference was revealed between the three categories (Fig. 3).

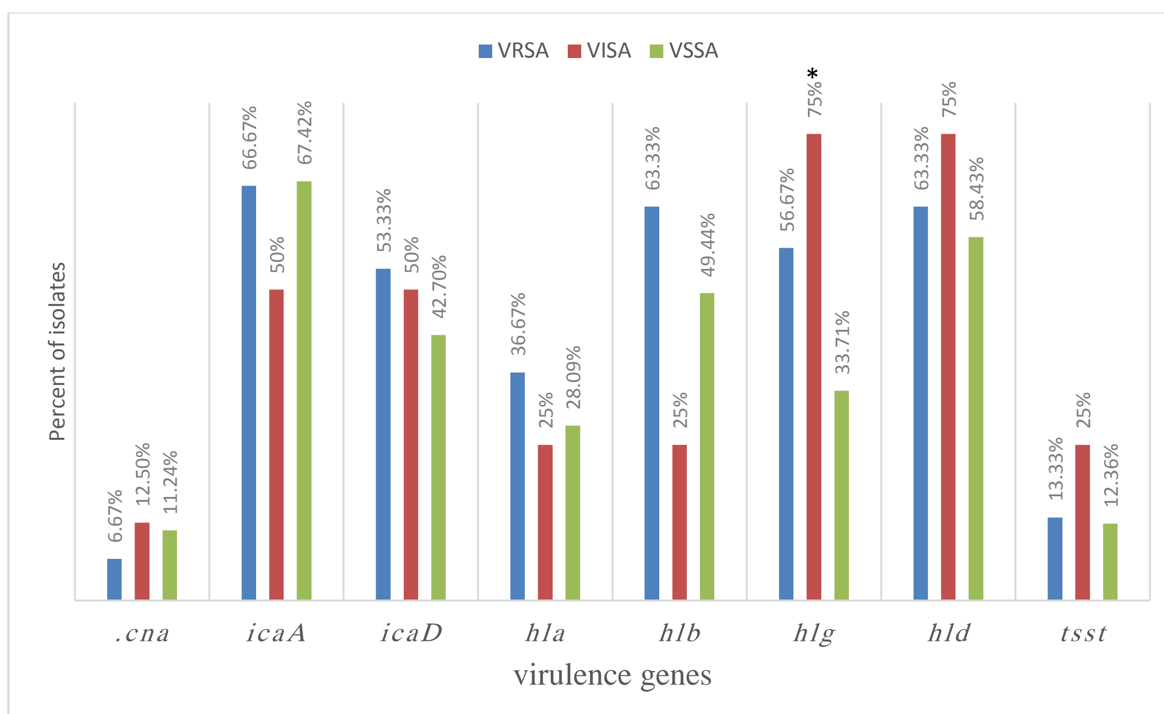


**Fig. 3:** Distribution of virulence genes among VRSA, VISA and VSSA isolates
***cna*:** collagen binding protein; ***icaA***: intercellular adhesion gene A; ***icaD***: intercellular adhesion gene D; ***hla***: α-hemolysin; ***hlb***: β-hemolysin; ***hlg***: γ-hemolysin; ***hld***: δ-hemolysin; **tsst**: toxic shock syndrome toxin; *: significant difference

The mean virulence score, which is the sum of all the VS of the isolates divided by the number of isolates, was determined for different categories. The results revealed that VRSA isolates had the highest MVS compared to VSSA and VISA isolates with no significant difference between the three categories ( Table 5).

**Table 5: Correlation between the degree of virulence among different vancomycin resistance categories**

| Percent of isolates | Score of virulence genes | | | | | | | | MVS |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | |
| **VRSA** | 0.00% | 3.33% | 20.00% | 30.00% | 20.00% | 13.33% | 13.33% | 0.00% | 3.60 |
| **VISA** | 0.00% | 0.00% | 50.00% | 0.00% | 12.50% | 37.50% | 0.00% | 0.00% | 3.38 |
| **VSSA** | 7.87% | 10.11% | 19.10% | 21.35% | 24.72% | 11.24% | 3.37% | 2.25% | 3.03 |
| **P value** | 0.205 | 0.275 | 0.128 | 0.261 | 0.672 | 0.628 | 0.202 | 0.648 | 0.214 |

**MVS**: mean virulence score

## DISCUSSION

MRSA is associated with both community-acquired and nosocomial infections and is considered a significant public health concern[14]. The rising incidence of MRSA has led to an overuse of vancomycin as a first-line glycopeptide, resulting in decreasing sensitivity to vancomycin and the emergence of VRSA strains. Therefore, VISA and VRSA have become more common in various regions of the world[15]. Several studies performed in Egypt during 2016-2019 have reported rapid increasing levels of MRSA emergence (73-92%)[12, 16, 17]. Surprisingly, we found in our study that all the 127 *S. aureus* isolates were MRSA. This high prevalence of MRSA was recorded earlier in Iran , where a study conducted in 2007 reported that 98% of the isolated *S. aureus* was MRSA[18].

Interestingly, six isolates (4.72%) out of the 127 MRSA isolates were negative for *mecA* gene in our study. Similarly, Rania *et al.* (2018) reported absence of *mecA* gene in 5.5% of their MRSA isolates[19]. These findings provides a clear evidence that there might be other possible mechanisms implicated in methicillin resistance such as *mecB* gene discovered by Becker *et al.* and *mecC* gene which is a unique homologue of *mecA* gene[20, 21].

Resistance of *S. aureus* to different antimicrobial agents is rapidly increasing worldwide due to the overuse and misuse of antibiotics, lack of culture susceptibility testing and selling of drugs without prescriptions in the developing countries[22]. Antimicrobial susceptibility testing revealed that highest resistance levels were detected to clindamycin (82.68%) and linezolid (75.59%) which were consistent with the results reported by Lupinacci *et al.* (89%) and Armin *et al.* (77.3%), respectively[23, 24]. In contrast, lower levels were reported by Shebi *et al.*[25]. The levels of resistance to azithromycin and doxycycline were found to be 37.80%, while resistance to gentamicin was 22.05%. These levels were higher compared to Mohanty *et al.*[26]. However, in our study we reported a lower resistance level to levofloxacin and ciprofloxacin compared to Mohanty *et al.* (2019) who reported a higher resistance to ciprofloxacin (52.1%)[26]. For nitrofurantoin, the level of resistance was 19.96% which is comparable to 20% resistance reported by Hasan *et al.*[27]. In addition, the lowest level of resistance was observed for trimethoprim-sulfamethoxazole (11.81%) which is similar to that reported by ElSayed *et al.*[28]. In this study, 114 (89.76%) isolates were considered MDR which was close to results (92.9%) obtained by Kot *et al.*[29].

The prevalence of VRSA isolates in this study was 23.62%. A Previous study conducted in Egypt by Mashaly *et al.*(2018) reported a similar result (21.7%)[17]. However, this rate shows an increase than that reported in 2017 by El-Kady *et al.* in Egypt (15.7%)[30]. This variation in VRSA prevalence may be geographically based. It may also reflect changes through time due to the overuse of vancomycin for the treatment of infections caused by MRSA. In addition, inadequate monitoring of defined antibiotic policies, and insufficient surveillance for vancomycin resistance may be a contributor[31].

Comparison of antimicrobial resistance pattern between VRSA, VISA and VSSA revealed that there was a significant difference regarding resistance to clindamycin and linezolid (P=0.001). A previous study reported highly significant differences in resistance rates to all antibiotics between VRSA and VSSA ($P< 0.001$)[32].

Correlation between the clinical source and vancomycin resistance revealed that the highest percent of VRSA isolates was significantly recovered from wound, which is in consistence with Cong *et al.* who stated that wound is the most common source of VRSA isolates[33].

In this study, *vanA* and *vanB* genes were detected in 16.67% and 10% of isolates, respectively. A previous study carried out by Aubaid *et al.* showed that 6.9% and 12.5% of isolates contained *vanA* and *vanB* genes, respectively[34]. Higher percentages (34% and 37%) were reported by Solhjoo for *vanA* and *vanB* genes, respectively[35]. Absence of *van* genes in some VRSA isolates supports the possible presence of other mechanisms of resistance. Cell wall thickening is assumed to be the responsible to the development of vancomycin resistance in these isolates[36].

Investigation of virulence genes revealed that the highest prevalence of virulence genes was found for *icaA*, followed by *hld, hlb, icaD, hlg, hla, tsst* and *cna* as they were harbored by 66.14%, 60.63%, 51.18%, 45.67%, 41.73%, 29.92%, 13.39% and 10.245% of isolates, respectively. Higher frequencies of the *hld*, *hlg, hlb*, *hla* and *tsst* genes were recorded by El-baz *et al.*[13]. A previous study by Hassan *et al.* revealed higher prevalence of *icaA*, *icaD* and *cna* genes[12]. In addition, VRSA isolates, in this study, harbored the highest percent of *icaD*, *hla* and *hlb* genes, while VISA isolates had the highest percent of *cna*, *hlg, hld* and *tsst* genes. A previous study by El-sayed *et al.* estimated similar result that VRSA isolates harbored the highest percent of *icaA* and *icaD* genes[28]. However, Jin *et al.* reported that VISA isolates had higher biofilm formation capacity encoded by biofilm encoding genes[37].

Several studies have suggested three different correlations between virulence and resistance: an increase in resistance followed by a decrease in virulence; an increase in resistance followed by an increase in virulence; and an increase of resistance has no effect on virulence as reviewed in Cepas & Soto, 2020[38]. Our results revealed that VRSA isolates showed higher, however, non-significant MVS compared to VISA and VSSA, which is consistent with that estimated by El-sayed *et al.*[28]. Thus, our results do not support the correlation between vancomycin resistance and virulence.

## CONCLUSION

The present study highlights the unexpectedly high prevalence of MRSA as well as the alarming rising rate of VRSA infections in Egypt. These urge a strong need for the government implementing antimicrobial stewardship programmes in order to effectively control these isolates in all health care settings. In addition, absence of *vanA* and *vanB* genes in some VRSA isolates necessitates the identification and study of new resistant determinants for surveillance of vancomycin-resistance. Furthermore, virulence and resistance seem to be independent, as no correlation between vancomycin resistance and virulence was detected in our study.

VRSA and VSSA did not differ significantly in the context of the virulence determinants studied.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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