

ORIGINAL ARTICLE

Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Intermediate Susceptibility and Vancomycin Resistance Among *Staphylococcus aureus* Isolated From Tertiary Care Hospital in Egypt

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ABSTRACT

Key words:

Staphylococcus aureus,
MRSA, VISA, MIC, VRSA

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Background: Methicillin-resistant *Staphylococcus aureus* (*S.aureus*) (MRSA) is a major cause of hospital acquired infections. Vancomycin is the first-line of treatment for severe MRSA infections. Elevated vancomycin MICs in MRSA are associated with a risk of vancomycin resistance development and treatment failure. **Objectives:** This study aimed to detect MRSA, vancomycin-intermediated resistant *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) among *S. aureus* isolates from neonates admitted to neonatal intensive care unit (NICU) of Egypt Children's hospital for health insurance. **Methodology:** 91 *S. aureus* isolates were recovered from different clinical samples of neonates and were collected from June 2016 to February 2017 methicillin resistance detected by cefoxitin disc (30 µg) and the MIC of vancomycin was determined for MRSA isolates by using E-test. **Results:** (84.6%) were MRSA. The vancomycin MIC range was 0.38-4 µg /mL. Two MRSA isolates (2.6 %) were VISA. No vancomycin resistance was detected. **Conclusion:** the MIC of vancomycin was increased but without development of vancomycin resistance, so efficient infection control measures and antibiotic policy should be adopted in hospital to avoid development of new resistant strains.

INTRODUCTION

Staphylococcus aureus is one of the major causes of community and hospital acquired infections, leading to high morbidity and mortality¹. The antibiotic treatment of *S. aureus* infections is complicated by the increasing prevalence of methicillin-resistant *S.aureus* (MRSA)². Egypt has the highest rate of MRSA among *S. aureus* clinical isolates in comparison to southern and eastern Mediterranean countries and other African countries³. MRSA is mediated by alteration in protein called low-affinity penicillin binding protein (PBP2a). PBP2a is encoded by *mec A* gene which is present in chromosomal mobile genetic element called Staphylococcal cassette chromosome *mec* (SCC*mec*)⁴. Vancomycin remains one of the first-line treatment options for severe infections caused by MRSA⁵. The increased incidence of MRSA has led to more frequent use of vancomycin which leads to the emergence of new strains of *S. aureus* with decreased susceptibility to vancomycin and other glycopeptides⁶. The first vancomycin-intermediate *S. aureus* (VISA) isolate was detected in Japan In 1996⁷. It has reduced susceptibility to vancomycin not due to the presence of *vanA* or any of the other known vancomycin resistance

determinants but due to unusual increasing of cell wall thickness that containing D-alanyl-D-alanine targets capable of binding vancomycin^{7, 8}. vancomycin-resistant *S. aureus* (VRSA) was firstly reported in the USA in 2002⁹. According to the CLSI guidelines, MIC breakpoints for vancomycin were defined as follows: susceptible, ≤2 µg/ml; intermediate, 4–8 µg/ml; and resistant, ≥16 µg/ml¹⁰

Creeping phenomenon is the phenomenon of an increasing vancomycin MIC within the susceptible range for MRSA and it has been demonstrated by several studies^{11,12} so this study was aimed to detect the emergence of MRSA, VISA and VRSA among *Staphylococcus aureus* isolates from infected neonates admitted to the neonatal I.C.U.

METHODOLOGY

A total 91 isolates of *S. aureus* were selected from various clinical samples including blood (n=56 samples), wounds swabs (n=24 samples), and pus (n=11 samples) from neonates admitted to the NICUs of Egypt children's hospital for health insurance during period from June 2016 to February 2017.

Isolation and Identification of *Staphylococcus aureus*:

The specimens were inoculated on blood agar and mannitol salt agar (Hi Media, India) and incubated aerobically at 37°C for 24 hours at the laboratory of Medical Microbiology Department; Faculty of Medicine of Ain Shams University. The strains of *Staphylococcus aureus* were identified on the basis of colony morphology, Gram's stain, and positive catalase & coagulase tests¹³.

Antimicrobial susceptibility testing:

The antibiotic susceptibility was carried out by the Kirby-Bauer disc diffusion method¹⁷ and they included penicillin (10µg), gentamicin (10µg), erythromycin (15µg), ciprofloxacin (10µg), Levofloxacin (10µg), Clindamycin (2 µg), linezolid (30 µg), Rifampicin (5 µg), Amikacin (30 µg) azithromycin (30 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg).

Inoculum of 0.5 McFarland standards turbidity was prepared from *S.aureus* isolates then a sterile cotton swab was dipped into the inoculum suspension and streaked on surface of Mueller-Hinton agar plate. The antibiotic discs were placed on the surface of the media. Then the media plates were incubated at 37°C for 18-24hrs. The diameters of the zone of the inhibition were measured and interpreted as either susceptible, intermediate or resistant¹⁰. ATCC 25923 *S.aureus* strain was used as control strain

Detection of MRSA by Cefoxitin disc diffusion method:

All strains were tested with cefoxitin discs (30 mg) (Oxoid) on Mueller-Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 18 hours incubation at 35°C. Zone size was interpreted according to CLSI¹⁴ criteria; susceptible ≥ 22 mm; resistant ≤ 21 mm.

Detection of Inducible Clindamycin Resistance:

Erythromycin resistant isolates were tested for inducible clindamycin resistance by D-test as per CLSI guidelines¹⁵.

Detection of Vancomycin MIC:

Minimum Inhibitory Concentrations of the MRSA isolates to vancomycin were also determined by E. test strips (Hi media). Muller Hinton plates were inoculated by direct colony suspension method of 0.5 McFarland equivalent inoculums prepared in sterile normal saline.

Plates were incubated for 24 hours at 37°C. MIC was interpreted as the zone of inhibition corresponding to a concentration gradient on the E test strips, according to the manufacturer's guidelines. Results were interpreted according to CLSI guidelines¹⁶.

RESULTS

Ninety one *S. aureus* isolates were tested for methicillin sensitivity, 14 (15.4%) were MSSA and 77 (84.6%) MRSA by the cefoxitin disc (figure 1).

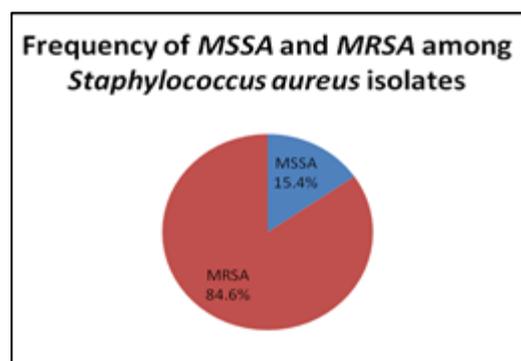


Fig. 1: Frequency of MSSA and MRSA among *Staphylococcus aureus* isolates

Most of MRSA isolates were isolated from blood samples (91.1%) followed by wound swabs (79.2%). The prevalence of MSSA and MRSA among clinical isolates was listed in table 1.

Table 1: Frequency of MSSA and MRSA among clinical isolates

Clinical Samples	MSSA N(%)	MRSA N(%)
Blood (56)	5 (8.9%)	51 (91.1%)
Wound (24)	5 (20.8%)	19 (79.2%)
Pus (11)	4 (36.4%)	7 (63.6%)

MRSA showed 100% resistance to penicillin. The most sensitive antibiotic against isolated MRSA was linezolid (93.5%) followed by Rifampin and Amikacin, (58.44%) for each. Antimicrobial sensitivity test results for MRSA isolates are illustrated in (table 2).

Table 2: Antibiotic susceptibility patterns of MSSA and MRSA isolates.

Isolates	MRSA (n:77)			MSSA (n:14)	
	Sensitive N(%)	Resistant N (%)	Intermediate resistant	Sensitive N(%)	Resistant N (%)
Gentamycin	22 (28.6%)	55 (71.4%)	-	9 (64.3%)	5(35.7%)
Amikacin	45 (58.44%)	30 (38.96%)	2 (2.6%)	10 (71.4%)	4 (28.6%)
Clindamycin	35 (45.5%)	42 (54.5%)	-	10 (71.4%)	4 (28.6%)
Azithromycin	26 (33.8%)	51 (66.2%)	-	8 (57.1%)	6 (42.9%)
Levofloxacin	35 (45.5%)	37 (48%)	5 (6.5%)	9(64.3%)	5(35.7%)
Ciprofloxacin	30 (39%)	44 (57.1%)	3 (3.9%)	8(57.1%)	6(42.9%)
Erythromycin	24 (31.2%)	53 (68.8%)	-	7 (50%)	7(50%)
Rifampin	45 (58.44%)	27 (35.06%)	-	14 (100%)	-
Linezolid	72 (93.5%)	5 (6.5%)	-	14 (100%)	-
Cotrimoxazole	37 (48.1%)	40 (51.9%)	-	13 (92.9%)	1(7.1%)

The inducible clindamycin resistance (D zone) was observed in 3 isolates (3.9%) among MRSA as shown in figure (2).



Fig. 2: Erythromycin induced clindamycin resistance in MRSA isolate.

The MIC of vancomycin ranged from 0.38-4µg/mL and its mean± SD was 1.233 ± 0.793. The vancomycin MIC of MRSA isolates and its percentage was listed in table 3.

Table 3: Frequency of Vancomycin MIC of MRSA isolates and its percentage

Vancomycin MIC		
	N	%
0.38 µg/mL	4	5.2
0.5 µg/mL	13	16.9
0.75 µg/mL	14	18.2
0.94 µg/mL	3	3.9
1 µg/mL	15	19.5
1.5 µg/mL	15	19.5
2 µg/mL	7	9.1
2.5 µg/mL	2	2.6
3.5 µg/mL	2	2.6
4 µg/mL	2	2.6

Seventy five isolates (97.4%) were sensitive to vancomycin (VSSA) and 2 isolates (2.6%) were intermediately susceptible to vancomycin (VISA) with MIC 4 µg/mL as shown in figure 3.



Fig. 3: MIC of Vancomycin at 4µg/mL.

DISCUSSION

Methicillin-resistant *S. aureus* (MRSA) are well established in both the healthcare setting and in the community. They are among the most common causes of nosocomial infections as intravenous catheter associated infections, ventilator associated pneumonias and surgical wound infections in some industrialized countries¹⁷. Precise recognition of MRSA and VRSA confines could be valuable for deciding the fitting treatment¹⁸.

In the present study, 84.6% of *S. aureus* infections are caused by MRSA. Previous studies done in Egypt by Amer and Gamal¹⁹, Abdel-Maksoud et al.²⁰ and Ghoniem et al.²¹ and detected the prevalence of MRSA 78.9%, 76.6% and 71.7% respectively .

However, lower prevalence of MRSA in comparison to our study was reported by Kshetry et al²². (37.6) and Dibah et al²³. (46.3%). Also our results were higher than

the result of many studies done in different regions such as Sudan, India and Pakistan²⁴⁻²⁶. The cause of variable prevalence of the MRSA infections between regions is due to differences of local antibiotic policy and the infection control practices in different health care facilities²².

Fifty Four (91.1%) of blood samples were MRSA. Lower detection rate of MRSA detection were observed by Wyllie *et al*²⁷. (50%) and Mustaq *et al*²⁸. (32.6%).

Also in the present study MRSA were isolated by 63.6% from pus and this results was higher than Dilnessa and Bitew²⁹ (20.3%), Abbas *et al*³⁰ (43.8%) and Mustaq *et al*²⁸ (32.5%).

Susceptibility test profiles of MRSA isolates revealed resistance to commonly used antimicrobial agents Erythromycin, Azithromycin and aminoglycosides and this results is in agreement with other studies conducted by Khadri & Alzohairy³¹ and Islam & Shamsuzzaman¹⁸.

Most of isolates were sensitive to Vancomycin (97.4%) and linezolid (93.5%). These results were similar to Hafeez *et al*²⁶ and Kaleem *et al*³² and this suggests that these drugs could be suitable treatment options. Regarding the sensitivity of ciprofloxacin and levofloxacin it was 61% and 54.5% respectively and these results is comparable to the results of Bhatt *et al*³³ who found approximately 75-80% of isolates were resistant to ciprofloxacin and levofloxacin, a finding which must be considered.

In the present study, inducible clindamycin resistance was found in 3.9% of MRSA isolates. This result was lower than Adhikari³⁴ (10%). Detection of erythromycin induced clindamycin resistance is important to avoid treatment failure with clindamycin for these isolates as the strains that showed positive *D*-test should reported resistant to clindamycin¹⁶

Most of MRSA isolates were sensitive to vancomycin 97.4% with MIC of vancomycin ranged from 0.38-4 µg/ml. This results was in agree with Kshetry *et al*²² and Amatya *et al*³⁵. However Vancomycin resistant strains have been isolated in Japan, The USA, France, Korea, South Africa, Brazil and Scotland, hence, the problem of glycopeptides resistance is global³⁶. Increasing of vancomycin MICs within the susceptible range of MRSA is called creep phenomenon. Several studies have demonstrated this phenomenon past years^{11,37}. In the present study we found this phenomenon in MRSA isolates, we have 15 isolates (19.5%) with vancomycin MIC 1 µg/mL, another 15 isolates with MIC 1.5 µg/mL. 13 isolates were founded to have higher MICs 2 µg/mL (table 2). This MIC creeping is associated with treatment failure as reported by Soriano *et al*¹² and Kullar *et al*³⁸.

There were only two isolates (2.6%) with intermediate resistant to vancomycin (VISA) with MIC was 4 µg/ml. Osman *et al*²⁴ and Ghoniem *et al*²¹ Founded higher prevalence of VISA that were 12% and

20.68% respectively. VISA is caused by mutations accumulation. It has several genetic mechanisms of resistance which differ from vancomycin resistance mediated by the *van* gene in *Enterococci* and *Staphylococci*³⁹.

The mechanisms behind the resistance of *S. aureus* to vancomycin may be the thickening of cell wall caused by differentially regulated cell wall biosynthesis and stimulatory pathways, reduced cross-linking of peptidoglycan, decreased autolytic activity of the enzymes responsible to cell-wall turnover, altered surface protein profile, dysfunction of the *agr* system and changes to growth characteristics⁴⁰.

Conclusion and Recommendation:

This study highlights the high prevalence of MRSA among the studied *S. aureus* and the detection of VISA in neonatal infections. linezolid and Rifampin were the most sensitive antibiotics for MRSA. Efficient infection control measures and antibiotic policy should be adopted in hospital to prevent the transmission of these strains between patients and also to prevent development of new resistant strains.

Limitation of this study was the inability to perform Polymerase Chain Reaction (PCR) for *Van A* gene detection in vancomycin decreased sensitivity isolates in addition the performed MIC.

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