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ORIGINAL ARTICLE

Detection of Vancomycin Resistance among Hospital and Community-acquired Methicillin-resistant *Staphylococcus aureus* Isolates

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ABSTRACT

Background: *Methicillin-resistant Staphylococcus aureus (MRSA) is a significant pathogen, responsible for infections acquired in both nosocomial and community settings. The first line of defense against life-threatening MRSA infections is vancomycin. The development of vancomycin resistance and a possible failure of MRSA treatment is associated with elevated vancomycin MICs.* ***Objectives:*** *The aim of the study was to detect the antibiotic sensitivity pattern of methicillin-resistant S. aureus and the prevalence of vancomycin resistance among these isolates.* ***Methodology:*** *Fifty-one MRSA isolates were studied. Identification and antimicrobial susceptibility profiles of MRSA isolates were determined by Vitek 2.* ***Results:*** *Community-acquired MRSA (CA-MRSA) strains were isolated from abscesses in soft tissues and skin (76%), while Hospital-acquired MRSA (HA-MRSA) strains were isolated mainly from pus obtained from surgical site infections and diabetic foot (57.7%), We detected 4 VISA/VRSA isolates out of 51 MRSA strains with a prevalence rate of 7.8%. Generally, CA-MRSA isolates were more susceptible to antibiotics than HA-MRSA isolates, except for ciprofloxacin.* ***Conclusion:*** *The study revealed the emergence of VISA/VRSA strains in isolated MRSA that were equally distributed between non-hospitalized and hospitalized patients being more common in young patients suffering from soft tissue and skin infections. All VRSA/VISA isolates were susceptible to tigecycline and linezolid.*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant threat to health and is a major cause of infections acquired both in hospital and community settings[1]. Vancomycin is one of the primary antibiotics used to treat MRSA infections, However, *Staphylococcus aureus* (*S. aureus*) isolates with reduced susceptibility to vancomycin have emerged in the current years. The emergence of vancomycin-resistant *S. aureus* (VRSA) has been linked to the transfer of the vanA gene cluster from vancomycin-resistant enterococcus[2].

The first case of vancomycin-intermediate *S. aureus* (VISA) was reported in Japan in 1997, followed by increasing cases of VISA and VRSA globally, including in developed countries[3,4]. While the incidence of VRSA infections in the United States of America and Europe remains relatively low, higher rates have been discovered in other regions, particularly in developing countries[5]. Initially, VISA and VRSA strains were mostly hospital-acquired, but VRSA cases acquired from the community have also been reported[6]. The World Health Organization in 2017 listed VISA and VRSA as "antibiotic-resistant priority pathogens" in recognition of their significance[7].

The aim of the current study was to detect the antibiotic sensitivity pattern of methicillin-resistant *S. aureus* (MRSA) isolated from various clinical specimens and to detect the prevalence of vancomycin resistance among these isolates. Also, to compare the vancomycin resistance between hospital and community MRSA strains.

METHODOLOGY

Sample collection

Various clinical samples (pus, wound, blood, body fluids, ear, and nasal swabs…) were collected from outpatients and inpatients referred to the Central Microbiology Laboratories of Kasr Al-Ainy University Hospitals during the period from January to June 2023. The study proposal was approved by the Ethical Committee of the Faculty of Medicine, Cairo University (approval number: N-188-2023). Patients' demographic and clinical data were obtained from Kasr Al-Ainy University Hospitals database.

Cultivation and identification

Gram staining and inoculation on Blood Agar, Nutrient Agar, and MacConkey's agar (Oxoid, UK), were performed. Culture plates were incubated at 37° C aerobically for 24-48 hours. Blood samples and body fluids were inoculated into blood culture bottles and were directly incubated in BACT/ALERT 3D (Biomeriuex, France). Identification of *Staphylococcus aureus* isolates was done according to the standard microbiological methods by Gram staining, positive catalase, and coagulase tests and by mannitol fermentation on mannitol salt agar[8].

Antimicrobial susceptibility

Detection of MRSA and VRSA strains by VITEK® 2 AST- P592 cards:

VITEK® 2 AST- P592 cards (Biomerieux, France) were used to evaluate the antibiotic susceptibility of all identified *S. aureus* isolates by measuring the Minimal Inhibitory Concentration (MIC). Isolates that had a positive cefoxitin screen and an oxacillin MIC of ≥ 4 µg/ml were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), while isolates with a negative cefoxitin screen and an oxacillin MIC of ≤ 2 µg/ml were categorized as methicillin-sensitive *Staphylococcus aureus* (MSSA) and were excluded from the study[9]. Based on patients' history, MRSA isolates were further divided into Hospital-Acquired (HA) or Community-Acquired (CA) categories. Infections that occurred in outpatients or inpatients who had an MRSA isolate less than 48 hours after hospitalization were classified as CA-MRSA.

The current study collected a total of 51 MRSA isolates. Vancomycin resistance and other antibiotic sensitivity pattern were detected by VITEK® 2 AST-P592 cards (Biomerieux, France). The following antibiotics were tested: ciprofloxacin, gentamicin, clindamycin, erythromycin, teicoplanin, tigecycline, linezolid, tetracycline, rifampicin, fusidic acid, and sulphamethoxazole- trimethoprim). According to CLSI guidelines; isolates that had a vancomycin MIC of ≤ 2 µg/ml were categorized as vancomycin-sensitive *Staphylococcus aureus* (VSSA), while those with a MIC between 4-8 µg/ml were classified as vancomycin-intermediate *Staphylococcus aureus* (VISA). Isolates with a MIC of ≥ 16 µg/ml were classified as vancomycin-resistant *Staphylococcus aureus* (VRSA)[9].

Statistical analysis

SPSS Win statistical program version 28 was used to analyze the data. The mean and standard deviation (SD) of numerical data were used for expression. Frequency and percentage were used to express qualitative data. The suitable method for examining the relationship between qualitative variables was the chi-square (Fisher's exact) test. Shapiro-Wilk and Kolmogrov-Smirnov tests were used to check for normality. The difference between the two groups regarding numerical variables was tested using the Mann-Whitney U test. Statistical significance was defined as a *P-value* less than or equal to 0.05. It was two-tailed for each test.

RESULTS

The present study was performed on 51 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. Vancomycin resistance of all isolates was tested for by VITEK® 2 AST- P592 cards. Isolates having a MIC of ≥ 16 µg/ml were classified as VRSA.

The studied isolates included 51 MRSA, 25 CA-MRSA, and 26 HA-MRSA. 33 (64.7%) isolates were obtained from males and 18 (35.3) from females. The most common age group was adults from 21-30 years old (29.4%), and MRSA strains were least isolated from 41-50 years old (9.8%). HA-MRSA was mainly obtained from patients over 40 years; however, CA-MRSA was common in younger age groups **(table 1)**.

The highest percentage of MRSA isolates were obtained from skin and soft tissue infections (n=34/51, 66.7%), followed by blood and synovial fluid (5.9%). Two MRSA isolates were collected from each of the following samples: sputum, CSF, nasal swabs, and ear swabs (3.9%). One MRSA isolate was obtained from bone biopsy, throat swabs, and facial follicles (2%) **(figure 1)**.

Generally, the CA-MRSA strains were found in soft tissues and skin abscesses (n=19/25, 76%), while HA-MRSA strains were recovered mainly from pus obtained from surgical site infections and diabetic foot (n=15/26, 57.7%), followed by blood and synovial fluid (11.5%), then sputum and CSF (7.7%) **(table 1)**.

Table 1): Characteristics of patients and source of specimens:

	CA-MRSA (n= 25) No. (%)	HA-MRSA (n= 26) No. (%)	*P-value*
Age			
Mean ± SD	**34.16 ± 17.2**	**40.15 ± 19.4**	**0.197**
18-40 years	**18 (72%)**	**13 (50%)**	
41-70 years	**7 (28 %)**	**13 (50%)**	**0.108**
Sex			**0.202**
Male (n= 33)	**14 (56%)**	**19 (73.8%)**	
Female (n= 18)	**11 (44%)**	**7 (26.9%)**	
Source of specimen			**0.166**
Pus (34)			
Abscess in skin, soft tissues	**19 (76%)**	**0**	
Diabetic foot	**0**	**4 (15.4%)**	
Surgical site infection	**0**	**11 (42.3%)**	
Others (17)			
Blood	**0**	**3 (11.5%)**	
Sputum	**0**	**2 (7.7%)**	
CSF	**0**	**2 (7.7%)**	
Synovial fluid	**0**	**3 (11.5%)**	
Bone biopsy	**0**	**1 (3.8%)**	
Ear swab	**2 (8%)**	**0**	
Nasal swab	**2 (8%)**	**0**	
Throat swab	**1 (4%)**	**0**	
Facial follicles	**1 (4%)**	**0**	

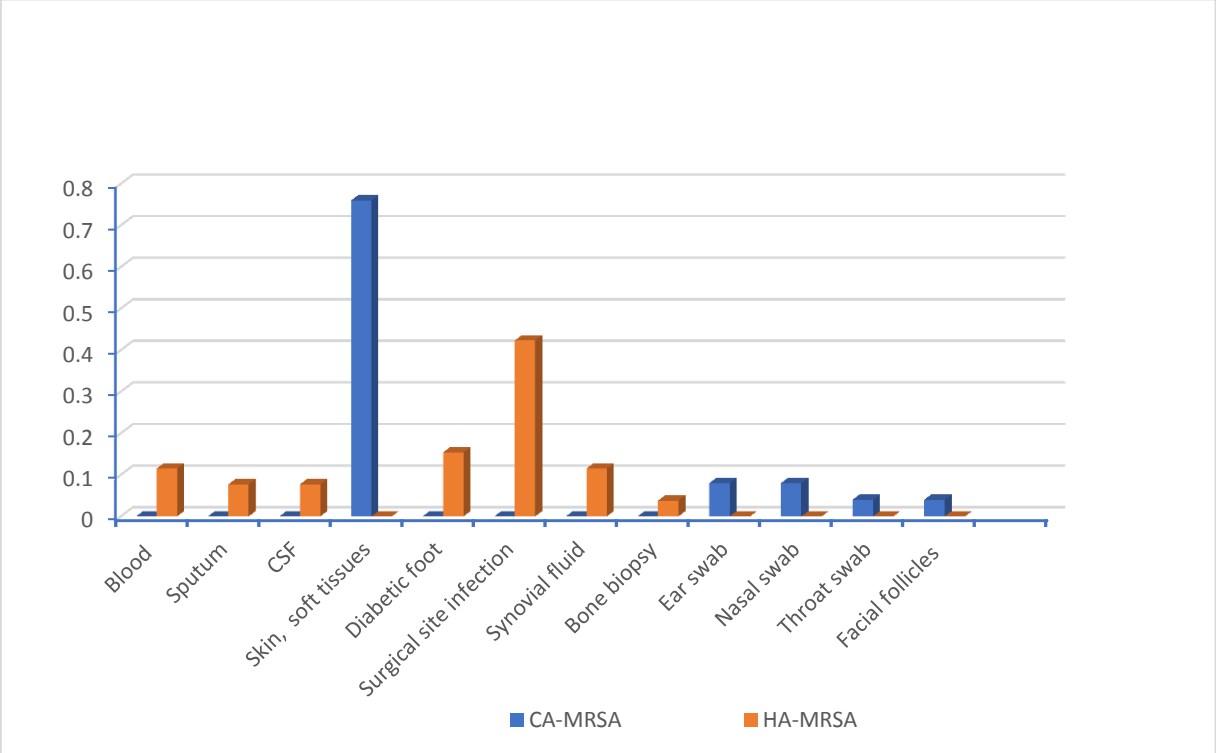

Fig. 1: Distribution of CA-MRSA and HA-MRSA isolates among different clinical specimens

Antimicrobial susceptibility

Generally, CA-MRSA isolates showed higher susceptibility to antibiotics than HA-MRSA isolates, except for ciprofloxacin; (16% *vs.* 7.7%, *P-value*=0.297). All MRSA strains were susceptible to linezolid and tigecycline.

HA-MRSA isolates showed higher resistance than CA-MRSA to gentamicin (23.1% *vs.* 16%, *P-value*=0.814), erythromycin (34.6 and 32%), and clindamycin (42.3 and 32 %), tetracycline (46.2 and 32% respectively), fusidic acid (76.9% *vs* 68%), sulfamethoxazole-trimethoprim (19.2% and 8%). All CA-MRSA strains were susceptible to teicoplanin and rifampicin. Inducible clindamycin resistance was present in 23.1% of HA-MRSA isolates compared to 12% of CA-MRSA isolates with no significance statistically (**table 2, figure 2)**.

Table 2: Antimicrobial susceptibility pattern of CA-MRSA and HA-MRSA by VITEK 2:

Antibiotic	CA-MRSA (n= 25) No. (%)			HA-MRSA (n= 26) No. (%)			Total MRSA (n=51)	*P-value*
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	Total resistance	
Ciprofloxacin	20 (80%)	1 (4%)	4 (16%)	24 (92.3%)	0	2 (7.7%)	6 (11.8%)	0.297
Gentamicin	16 (64%)	5 (20%)	4 (16%)	15 (57.7%)	5 (19.2%)	6 (23.1%)	10(19.6%)	0.814
Erythromycin	17 (68%)	0	8 (32%)	16 (61.5%)	1 (3.8%)	9 (34.6%)	17(33.3%)	0.586
Clindamycin	17 (68%)	0	8 (32%)	15 (57.7%)	0	11 (42.3%)	19(37.3%)	0.447
Inducible clindamycin resistance	Positive	Negative		Positive	Negative		0.465	
	3 (12%)	22 (88%)		6 (23.1%)	20 (76.9)			
Vancomycin	23 (92%)	1 (4%)	1 (4%)	24 (92.3%)	0	2 (7.7%)	3 (5.9%)	1
Linezolid	25(100%)	0	0	26 (100%)	0	0	0	NA
Teicoplanin	25(100%)	0	0	25 (96.2%)	0	1 (3.8%)	1 (2%)	1
Tigecycline	25(100%)	0	0	26 (100%)	0	0	0	NA
Tetracycline	17 (68%)	0	8 (32%)	14 (53.8%)	0	12 (46.2%)	20(39.2%)	0.301
Fusidic acid	8 (32%)	0	17 (68%)	6 (23.1%)	0	20 (76.9%)	37(72.5%)	0.384
Rifampicin	25(100%)	0	0	25 (96.2%)	0	1 (3.8%)	1 (2%)	1

NA: not applicable, *P-value* cannot be calculated because the variables are constant

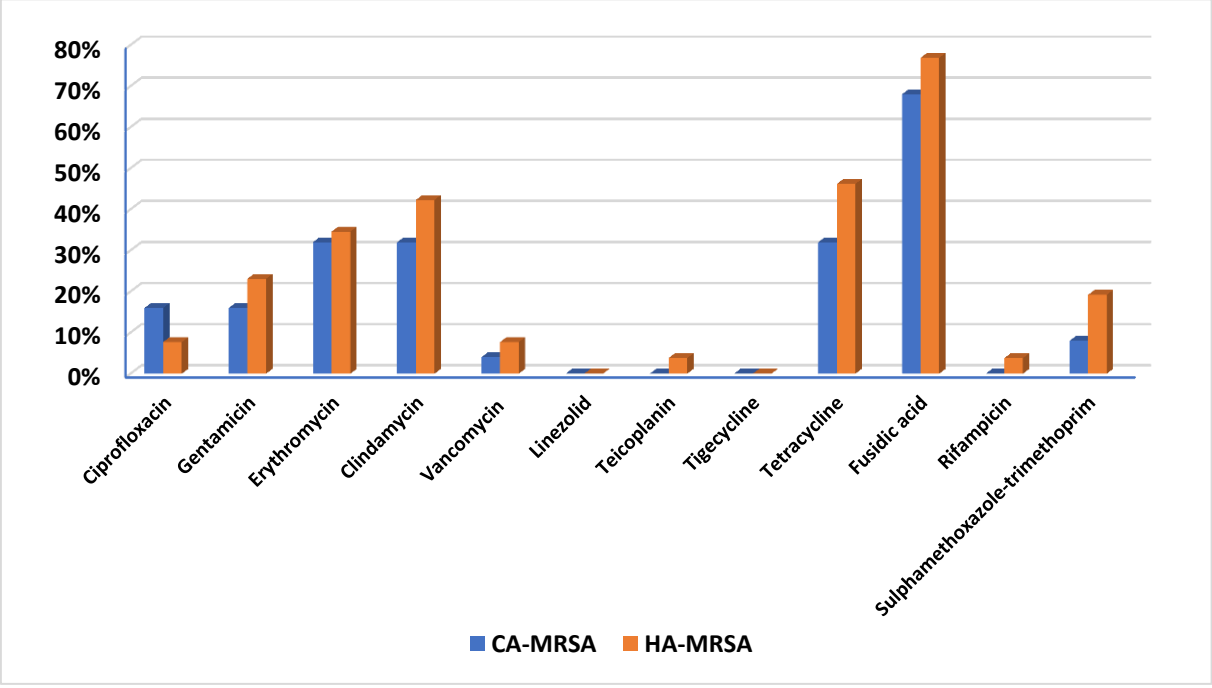

Fig. 2: Percentage of antimicrobial resistance among CA-MRSA and HA-MRSA isolates

Regarding vancomycin; we detected 4 VISA/VRSA isolates out of 51 MRSA strains with a prevalence rate of 7.8 %. 3 VRSA and one VISA isolates, The first VRSA isolate was recovered from the blood of a 29-year-old hospitalized male patient with bacteremia, and the second one was isolated from a wound exudate of 48-year-old hospitalized patient suffering from pelvis fracture, this strain was also resistant to teicoplanin which is a glycopeptide antibiotic similar to vancomycin, so classified as glycopeptide-resistant *Staphylococcus aureus* (GRSA). The third one was a community-acquired strain obtained from a 21-year-old female patient with a soft tissue infection. One vancomycin-intermediate *Staphylococcus aureus* (VISA) isolate was detected in a male (25 years) who presented in the outpatient clinic with a wound infection in his foot.

We observed that VISA/VRSA strains were common in young patients and were obtained mainly from pus related to soft tissue and skin infections (75%), then blood (25%). No data was available about treatment options and outcomes. VRSA and VISA strains were sensitive to linezolid, tigecycline, ciprofloxacin, rifampicin, and sulfamethoxazole-trimethoprim. Characteristics of VRSA and VISA isolates were illustrated in **table (3)**.

Table 3: Characteristics of VRSA and VISA isolates:

		HA-VRSA (1)	**HA-VRSA (2)**
HA-VRSA	**Age**	29	48
	Sex	male	male
	Source of specimen	blood	Pus (wound, fracture pelvis)
	MIC of vancomycin (µg/ml)	≥ 32	≥ 32
	Antibiotic resistance	Erythromycin/Clindamycin/Tetracycline/Fusidic acid	Clindamycin/Teicoplanin/Tetracycline/Fusidic acid
CA-VRSA /VISA		**CA-VRSA**	**CA-VISA**
	Age	21	25
	Sex	Female	Male
	Source of specimen	Pus	Pus (wound infection)
	MIC of vancomycin µg/ml	≥ 32	4
	Antibiotic resistance	Clindamycin/Tetracycline/Fusidic acid	Benzylpenicillin/Oxacillin

DISCUSSION

Over the last forty years, Methicillin-resistant *S. aureus* (MRSA) has become a significant pathogen causing both nosocomial and community-acquired infections. It is crucial to detect MRSA rapidly and accurately so that proper antimicrobial therapy can be administered and the spread of these strains is controlled[10]. Our work was designed to detect the antibiotic sensitivity pattern of MRSA and to compare the prevalence of vancomycin resistance between hospital and community MRSA strains.

In the current work, Males exhibited higher MRSA isolation rates (64.7%) compared to females (35.3%) in most of the clinical samples. This finding was similar to those of Primo *et al.*[11], Ghoneim *et al.*[12], and Hadyeh *et al.*[1] who found that 57%, 56.55%, and 53.6% of patients respectively were males. This finding was in disagreement with Terry Alli *et al.*[13] and Ibrahim *et al.*[14] who found that MRSA isolates were prevalent among female patients.

In our work, MRSA isolates were prevalent among adults from 21-30 years old (29.4%). CA-MRSA strains were more prevalent in younger age groups, HA-MRSA strains have primarily been isolated from patients over the age of 40. Our findings were in agreement with Hadyeh *et al.*[1] who observed that MRSA isolates were most commonly isolated from adults (60.7%). Xie *et al.*[15] observed that patients over 60 years old were the primary source of HA-MRSA strains, whereas younger ones were the source of CA-MRSA strains.

The highest percentage of MRSA strains have been obtained from pus specimens (66.7%) in the present study. Generally, CA-MRSA strains were found in abscesses in the skin, and soft tissues (n=19/25, 76%), while HA-MRSA strains were found mainly in pus obtained from surgical site infections and diabetic foot (n=15/26, 57.7%), followed by blood and synovial fluid (11.5%), then sputum and CSF (7.7%).

Vysakh and Jeya[16] reported that exudates displayed the highest percentage of MRSA isolates (91%), blood (5%), respiratory specimens, and urine (2% each). A higher prevalence of MRSA in pus specimens, particularly in patients with burns, surgical wounds, and diabetic foot infections has been previously reported by another study[17]. Hadyeh *et al.*[1] also isolated 35.7% of

MRSA from wound infections. On the other hand; Xie *et al.*[15] reported that sputum was the primary source of HA-MRSA strain isolation (56.4%).

A correlation has been shown between the excessive use of antibiotics and the rise in antibiotic resistance in developing nations. Without a prescription, it is simple to obtain antibiotics, which encourages their unrestrained use in both hospital and outpatient settings[18].

Regarding the antimicrobial resistance in our study; a high degree of resistance among MRSA isolates was encountered for fusidic acid (72.5%) followed by tetracycline (39.2%), clindamycin (37.3%), erythromycin (33.3%), and gentamicin (19.6%) respectively.

Our results were similar to Mundhada *et al.*[19] who observed a high percentage of erythromycin resistance. AbdEl-Mongy *et al.*[20] revealed increasing resistance to many antibiotics among *S. aureus* isolates, oxacillin (84%), and erythromycin (78%). Other studies reported (47.4%) and (60.4%) resistance percentage to tetracycline among *S. aureus* isolates respectively[21, 22].

In both tertiary and primary care settings, vancomycin-resistant *S. aureus* appears to be a significant concern. The extensive use of vancomycin may be associated with the emergence of VISA/VRSA. Dealing with VRSA is difficult, as these strains exhibit resistance to several antibiotics. As a result, the available treatment options are limited, which may result in insufficient antibiotic therapy, leading to higher rates of mortality and morbidity in affected patients[23].

We detected 4 VISA/VRSA isolates out of 51 MRSA strains with a prevalence rate of 7.8%. In a study conducted in Pakistan, the resistance pattern of clinical *S. aureus* isolates was investigated, and it was found that only one isolate was resistant to vancomycin[23]. A similar isolation rate was obtained by another study conducted in Minoufiya, Egypt at the Neonatal ICU, which detected 2 VISA/VRSA strains (4%) out of 50 MRSA strains isolated from the blood culture of neonates[20].

In Egypt, four studies reported higher isolation rates of VISA/VRSA; Ghoniem *et al.*[12] conducted a study at the National Liver Institute and revealed that the VRSA prevalence rate was 20.68%. El-Sayed *et al.*[24] detected 11 (13.8%) VRSA isolates out of 200 MRSA and one strain of VISA from an outpatient clinic in the rural area of Kafr-Eldawar in Egypt. Othman *et al.*[25] detected 30 VISA strains out of 100 MRSA (30%) by broth microdilution method. In a study conducted by Ibrahiem *et al.*[26], it was found that out of 127 *S. aureus* isolates tested using the standard agar dilution method, 30 isolates (23.62%) showed MICs ≥16µg/ml and they were classified as VRSA. Higher results were also reported in Pakistan (37.9, and 57.9% respectively)[27,28]. On the other hand; out of 112 MRSA isolates no VRSA strains were reported by Hadyeh *et al.*[1].

The incidence rates of VRSA strains vary across the globe. Before 2006, its total prevalence was 2%, which increased to 5% between 2006-2014 and further to 7% between 2015-2020. The occurrence of VRSA was found to be highest in Africa at 16%, followed by Asia (5%), North America (4%), South America (3%), and finally Europe (1%)[10]. It seems that VRSA is more common in developing than in developed countries. This could be attributed to higher public hygiene, rational use of antibiotics in treatments, and improved hospital-associated infection surveillance in the majority of developed nations[29,30]. However, the limited resources available for testing may lead to an inaccurate representation of VRSA prevalence in developing countries, as the total number of tests conducted may not accurately reflect the actual number of infections[10].

We observed that VISA/VRSA strains were equally distributed between non-hospitalized and hospitalized patients being more common in young patients with soft tissue and skin infections (75%). In Egypt, Abdel Gawad *et al.*[31] revealed in their study that the incidence of VRSA isolates in specimens obtained from hospitalized patients was equal to specimens from non-hospitalized patients.

An urgent need to control the rapid emergence of vancomycin resistance is evident, and this can be achieved through various measures such as educating healthcare professionals about nosocomial infections, implementing policies for infection control, and promoting the responsible use of antibiotics[32].

CONCLUSION AND RECOMMENDATIONS

The current work revealed the emergence of VISA/VRSA strains in isolated MRSA that were equally distributed between non-hospitalized and hospitalized patients being more prevalent in young patients with soft tissue and skin infections. All VRSA and VISA isolates were susceptible to linezolid and tigecycline. The decreased susceptibility of MRSA to vancomycin highlights the need for alternative treatment methods. Staff and hospitalized patients can significantly reduce MRSA spread by maintaining proper hygiene, and the implementation of well-defined antibiotic stewardship programs at both regional and national levels can further contribute to this effort.

The current work has many limitations, including the small sample size and the absence of molecular confirmation of the *van A* gene which should be addressed in future research.

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