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In Vitro Susceptibility to Glycopeptides, Linezolid, Tigecycline, and Ceftaroline against Methicillin Resistant *Staphylococcusaureus* Isolates in a Tunisian University Hospital

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ABSTRACT

Background: *Vancomycin remains the drug of choice for treatment of serious methicillin-resistant Staphylococcus aureus (MRSA) infections. However, Strains with reduced susceptibility to vancomycin have been reported in several countries around the world.Therefore, new antibiotics are introduced into the treatment.*

Objectives: *The present study aimed to determine the vancomycin, teicoplanin, linezolid, tigecycline and ceftaroline susceptibility patternand to investigate the presence and the frequency of heterogeneous vancomycin intermediate S. aureus (hVISA) among clinical isolates of MRSA in Tunisia.*

Methods: *A total of 162 non duplicate MRSA strains isolated between 2017 and 2018 were investigated. Vancomycin, teicoplanin, tigecycline, linezolid and ceftaroline minimum inhibitory concentrations (MIC) values were detected by broth microdilution method and interpreted according to the European Committee on Antimicrobial Susceptibility testing criteria. Etest GRD, Etestmacromethod, Mueller-Hinton screen agar, and population analysis profile-area under the curve (PAP-AUC) methods were used to detect hVISA.*

Results: *The MIC_{50}, MIC_{90}, and MIC ranges were respectively 1, 1, and 0.5-2 µg/ml for vancomycin; 1, 2, and 0.125-4 µg/ml for teicoplanin; 2, 2, and 0.5-4 µg/ml for linezolid; and 0.25, 0.5, and 0.064-0.5 µg/ml for tigecycline. Twelve strains were suspected as hVISA by EtestGRD, 28 by screen agar, and one by Etestmacromethod, but only one strain was confirmed hVISA by PAP-AUC. No vancomycin, tigecycline and linezolid resistance was found among MRSA isolates. Four strains were teicoplanin resistant, and four were intermediate to ceftaroline.*

Conclusion: *The prevalence of hVISA, teicoplanin and ceftaroline resistance are low. Linezolid and tigecycline were found to be highly active against MRSA isolates. Therefore, they could be considered as alternative agents for the treatment of serious infections. Continuous and regular monitoring of MICs at local and regional level is necessary to guide clinician in their empiric antibiotic selection.*

Keywords: *Methicillin resistant Staphylococcus aureus, glycopeptide, linezolid, tigecycline, ceftaroline, MIC.*

INTRODUCTION

Glycopeptides, vancomycin and teicoplanin are considered the treatment of choice for severe methicillin-resistant *Staphylococcus aureus* (MRSA) infections for decades. However, strains with reduced susceptibility to vancomycin, including vancomycin intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) and even vancomycin resistant *S. aureus* (VRSA) have been reported since 1997 in many parts of the world [1-3]. Reduced susceptibility to vancomycin is frequently accompanied by acquisition of teicoplanin resistance [1,4]. hVISA is an *S. aureus* isolate with a minimum inhibitory concentration (MIC) for vancomycin within the susceptible range but contain a resistant subpopulation to vancomycin at a frequency of 10^{-5} to 10^{-6} [5]. Although VRSA strains are rare, the prevalence of hVISA/VISA strains is increasing [2, 6].

For testing susceptibility to glycopeptides, the broth microdilution method (BMD) should be used. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines the vancomycin and teicoplanin MIC breakpoint of >2 µg/ml for resistant, and ≤ 2 µg/ml for susceptible [7]. However, *S. aureus* strains with an MIC vancomycin and / or teicoplanin> 1 µg/ml should be tested for

hVISA. In fact, vancomycin treatment failure has been reported even in susceptible strains, which may be attributable to the presence of resistant hVISA subpopulation [5]. The gold standard technique to detect hVISA strains is the population analysis profile area under the curve (PAP-AUC) method, which is time-consuming, expensive, and is unsuitable for routine use in the clinical microbiology laboratories [1,8,9].

Infections caused by multidrug resistant MRSA strains with reduced susceptibility to vancomycin are frequently associated with therapeutic failure and persistent infections justifying the use of alternatives such as linezolid, tigecycline, daptomycin or new generation cephalosporin which have shown potent activity against MRSA in previous publications [1, 10]. In Tunisia, ceftaroline is not commercially available; add to this, there have been no studies focused on prevalence of hVISA among MRSA strains using PAP-AUC method. Therefore, we aimed to determine vancomycin, teicoplanin, linezolid, tigecycline and ceftaroline susceptibility pattern and to investigate the presence and the frequency of hVISA isolates among clinical isolates of MRSA.

Material and Methods

Bacterial Strains

A total of 162 consecutive and non-duplicate MRSA isolates collected between 2017 and 2018 at Sfax university hospital were included in this study. Isolates were collected from various clinical samples, including blood (n=52), skin and soft tissue (n=62), respiratory tract (n=40), and catheter (n=8).

Identification of *S. aureus* isolates was performed using conventional methods. Methicillin resistance was identified by cefoxitin disc according to the EUCAST guidelines [7], and confirmed by the detection of *mec*A gene by PCR [11]. *S. aureus* ATCC 43300 was used as positive control.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities were performed by a disc diffusion method according to the EUCAST criteria [7]. MICs of vancomycin, teicoplanin, linezolid and tigecycline were determined for all of isolates by reference BMD [7]. Ceftaroline MICs were determined for intermediate or resistant strains by the disc method. *S. aureus* ATCC 29213 was used as a control.

Detection of hVISA

Screening for hVISA was performed by various tests on all isolates. *S. aureus* ATCC 29213 (vancomycin-susceptible), *S. aureus* Mu3 (ATCC 700698; hVISA), and *S. aureus* Mu50 (ATCC 700699; VISA) were included as controls.

MHA5T screening agar

Ten microliters of a 2 McFarland inoculum of each strain was inoculated as spot onto the surface of the Mueller-Hinton agar plates with 5 µg/ml teicoplanin. Growth of ≥ 4 colonies after 48 h of incubation at 35°C indicated a positive result [1].

Etestmacromethod

A 2 McFarland inoculum (200µl) was swabbed on Brain-Heart Infusion (BHI) agar and allowed to dry. Vancomycin and teicoplanin Etest strips (BioMérieux) were applied. After incubation for 48 h at 37°C, the MICs were read at complete inhibition. The criteria used to detect hVISA were MICs of ≥8 µg/ml for both vancomycin and teicoplanin or a teicoplanin MIC of ≥12 µg/ml [8].

Etestglycopeptide-resistance detection (GRD)

Etest GRD was performed according to the manufacturer instructions (BioMérieux) using a double-ended Etest strip with vancomycin and teicoplanin. A standard inoculum (0.5 McFarland) was swabbed onto a Mueller-Hinton agar plates with 5% Blood; next a GRD strip was applied. The elliptical zone was read at 24 and 48 h after incubation at 35°C. If either teicoplanin or vancomycin Etest GRD was ≥8 µg/ml, the isolate was considered hVISA[8].

PAP-AUC

PAP-AUC was done as described previously [8, 9]. Briefly, after 24 h incubation in BHI broth, cultures were diluted in saline to 10^{-3} and 10^{-6}, and plated on to BHI plates containing 1, 2,3,4,5 and 6 µg/ml vancomycin. Colonies were calculated after 48 h incubation at 37°C and plotted against vancomycin concentration using GraphPad prism. The ratio of AUC of the test isolate/ AUC of Mu3 was calculated. If ratio < 0.9, the isolate was considered vancomycin susceptible. Ratios of 0.9-1.3 and ≥1.3 were considered positive for hVISA and VRSA respectively [12].

Results

All MRSA isolates were susceptible to linezolid, tigecycline, vancomycin and quinupristin-dalfopristin (Table 1).

Table1. *Antimicrobial susceptibility of MRSA isolates against evaluated antimicrobial agents.*

Antimicrobial agents	%R	%I	%S
Gentamicin	46.9	0	53.1
Erythromycin	38.3	0	61.7
Clindamycin	32.7	0	67.3
Quinupristin-dalopristin	0	0	100
Ceftaroline[a]	0	2.5	97.5
Chloramphenicol	2.5	0	97.5
Tetracycline	69.2	0	30.8
Tigecycline[a]	0	0	100
Ofloxacin	44.5	0	55.5
Rifampicin	37.7	5.5	56.8
Trimethoprim-sulfamethoxazole	1.2	0	98.8
Fusidic acid	50.6	0	49.4
Vancomycin[a]	0	0	100
Teicoplanin[a]	2.5	0	97.5
Linezolid[a]	0	0	100

S, susceptible; I, intermediate; R, resistant.

[a]Vancomycin, teicoplanin, linezolid, tigecycline and ceftaroline susceptibilities were determined by broth microdilution method.

Of the eight strains classified as resistant or intermediate to ceftaroline by disc diffusion method, four had ceftaroline MIC value of 1 µg/ml (susceptible) and four were in the intermediate category (MIC = 2 µg/ml). The distribution of vancomycin, teicoplanin, linezolid and tigecycline MICs is shown in table 2. The four strains resistant to teicoplanin were susceptible to ceftraoline (MIC of 1 µg/ml) and to vancomycin (MIC of 2 µg/ml).

Table2. *MIC distributions and activities of vancomycin, teicoplanin, linezolid and tigecycline against MRSA strains.*

	No. of isolates with MIC (µg/ml)									
	0.064	0.125	0.25	0.5	1	2	4	MIC_{50}	MIC_{90}	Geometric mean
Vancomycin	0	0	0	30	126	6	0	1	1	0.90
Teicoplanin	0	2	2	61	48	45	4	1	2	0.92
Linezolid	0	0	0	5	70	82	5	2	2	1.45
Tigecycline	9	70	58	25	0	0	0	0.25	0.5	0.19

MIC_{50}: minimum inhibitory concentration which 50% of the strains were inhibited.

MIC_{90}: minimum inhibitory concentration which 90% of the strains were inhibited.

By Etestmacromethod, vancomycin MICs ranged from 2 to 6 µg/ml, teicoplanin MICs ranged from 2 to 12 µg/ml, and one isolate met the criteria of hVISA. By Etest GRD, vancomycin MICs ranged from 1 to 16 µg/ml, teicoplanin MICs ranged from 4 to 32 µg/ml, and 12 isolates met the criteria of hVISA. By MHA5T, 28 isolates met the criteria of hVISA.

The PAP-AUC ratios of the isolates were between 0.32 and 0.98. Only one isolate was hVISA, with a PAP-AUC ratio to Mu3 of 0.98 (figure 1). This strain was also detected by Etest GRD, EtestMacromethod and MHA5T. The hVISA isolate had a vancomycin MIC of 2 µg/ml and teicoplanin MIC of 4 µg/ml.

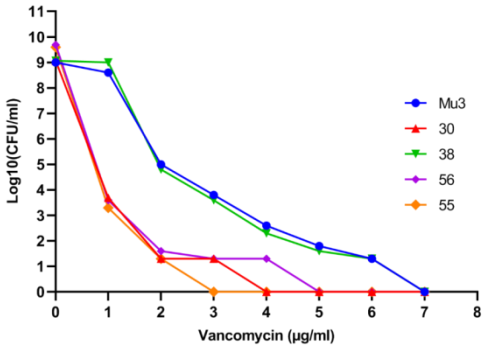

Figure1. *Population analysis profile curves of four isolates.*

One isolate (38) was identified to be hVISA and three isolates were defined vancomycin-susceptible (30, 55, 56) compared to the susceptibility of the Mu3 reference strain.

DISCUSSION

Vancomycin remains as the only widespread therapeutic preference of serious MRSA infections although new anti-staphylococcal antibiotics such as linezolid and tigecycline have been developed. However, treatment failure may occur even when MRSA is susceptible to vancomycin [5,13,14]. Recently a phenomenon of gradual increase in the value of vancomycin MIC over time was reported in literature as MIC creep. It was described as one of the suspected causes of vancomycin treatment failure [15]. The studies reporting vancomycin creep have shown conflicting results. There are reports of increased MIC over the time [16,17], but other studies did not confirm these findings in MRSA [18,19]. A systematic review and meta-analysis did not report an increase in vancomycin MIC, suggesting that vancomycin continues to be the treatment of choice of MRSA infections [20]. The proportion of MRSA isolates with vancomycin MIC >1 µg/ml was 26% in the USA, 18% in Asia, and 17% in Europe. The pooled means of vancomycin MIC were 1.12 µg/ml in Europe, 1.17% in Asia and 1.37% in USA [20]. In Tunisia, only one multicenter study, conducted between 2011 and 2012, evaluated the activity of glycopeptides on MRSA by determination of MICs by BMD [21].By comparing the results of this multicenter study with the present study, we observed an increase in the geometric means for vancomycin MIC (0.73to 0.90 µg/ml) and for teicoplanin MIC (0.49 to 0.92 µg/ml), accompanied by an increase in the percentage of strains with vancomycin MIC > 1µg/ml (from 1.5% to 3.7%).

Our study documented the presence of 0.6% hVISA and 2.5% teicoplanin resistance amongst MRSA isolates. The previously study in Tunisia reported hVISA and teicoplanin resistance prevalence of 0.8% [21]. No resistance to vancomycin was noted in our study and in Tunisia [21]. VRSA due to the acquisition of the *vanA* gene from enterococci are currently very low. To date, few cases of VRSA have been reported from different countries such as the United States, India and Iran [6]. The prevalence of hVISA/VISA varied geographically. The differences between studies may be explained by the use of different screening methods of hVISA and VISA strains. Add to this,confirmation of hVISA strains by the reference method is not performed in many studies [3,19,22,23]. A systematic review and meta-analysis [2] showed that the prevalence of hVISA/VISA isolates increased gradually from 4.68/2.05 % before 2006 to 7.01/7.93 in 2010-2014.

The PAP-AUC is the reference method to detect hVISA. However, this method is time-consuming, expensive and is not applicable in routine. Various screening strategies have been investigated for detection of hVISA. Several studies showed low sensitivity but good specificity (> 92%) with EtestGRD and Etestmacromethod. However, agar screening plates with vancomycin or teicoplanin were highly sensitive but less specific [3,8,12,19,22-24]. In our study, false positive results have been found with Etest GRD and MHA5T. This result could be related to the rarity of strains with vancomycin MIC ≥2 µg/ml, since there are studies in which hVISA strains were more commonly found among the isolates having vancomycin MICs of 2 µg/ml [22].

Heteroresistance to vancomycin should be considered and investigated in case of clinical failure while using vancomycin to treat severe MRSA infection, and newer agents can be used as alternative if available. Linezolid and tigecycline are popular choices for the treatment of MRSA infections [10]. Li et al reported that the efficacy of linezolid should be better than that of vancomycin in the treatment of MRSA infections [25]. A systematic review and meta-analysis showed that linezolid and tigecycline have the best effect on MRSA with very low resistance (<1%) [10]. In our study, all the MRSA isolates were susceptible to linezolid and tigecycline. The MIC_{50}, MIC_{90} and the mean MIC were similar to those reported in the previously study from Tunisia [21].

Ceftaroline is a fifth-generation broad-spectrum cephalosporin that has activity against MRSA. It is reported to be non-inferior to vancomycin against MRSA [26,27]. In several studies around the globe, ceftaroline has an excellent in vitro activity against *S. aureus* isolates. Although MIC_{50} and MIC_{90} are significantly higher for MRSA. Susceptibility of MRSA to ceftaroline was 99.5% in the United States, 94% in Europe, 92.3% in Africa/West Asia, 84.4% in

South America and 75.9% in Asia-Pacific [27]. In our study, 97.5% of the MRSA isolates were susceptible to ceftaroline. This was expected finding for us, as ceftaroline is not commercially available in Tunisia.

CONCLUSION

This is the first study in Tunisia investigating the prevalence of hVISA among MRSA strains by PAP-AUC method. We have demonstrated that the prevalence of hVISA and teicoplanin resistance are low. However, it is essential to test for hVISA especially for strains having teicoplanin or vancomycin MIC ≥ 2 µg/ml. Susceptibility to linezolid and tigecycline was higher than that of ceftaroline. These antibiotics should be kept as alternative therapy for critical cases of MRSA infections. Continuous and regular monitoring of MICs at local and regional level is necessary to guide clinician in their empiric antibiotic selection.

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