

# Identification of tigecycline- and vancomycin-resistant *Staphylococcus aureus* strains among patients with urinary tract infection in Iran

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## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of hospital- and community-acquired infections worldwide. Although *S. aureus* rarely accounts for urinary tract infections (UTI), untreated UTI can lead to several complications. For decades vancomycin has been used for the treatment of MRSA infections. This study was performed to assess the *in vitro* activity of vancomycin, tigecycline, linezolid and quinupristin/dalfopristin against MRSA isolates from UTI patients. Thirty MRSA strains from 54 *S. aureus* isolates were isolated from patients with UTI. The antimicrobial susceptibility patterns of the strains were determined by the Kirby-Bauer disk diffusion and broth microdilution methods. PCR assays were used to detect the *vanA* gene. The MRSA isolates resistant to vancomycin were confirmed using the broth microdilution method. The results revealed that the MRSA isolates were 100% susceptible to linezolid and quinupristin/dalfopristin but 93.3% susceptible to vancomycin and tigecycline respectively. The broth microdilution method confirmed two MRSA strains (6.6%) to be resistant to vancomycin and tigecycline. The study identified vancomycin resistance among the MRSA isolates from UTI patients. This vancomycin resistance in MRSA isolates poses a challenge in managing *S. aureus* infections. Our study's results highlight the need to correctly identify patients in whom last-resort therapy such as linezolid and quinupristin/dalfopristin should be administered.

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**Keywords:** Antibiotic resistance, methicillin-resistant *Staphylococcus aureus*, tigecycline, *vanA* gene, vancomycin

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## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major community-associated and hospital-acquired pathogen [1]. Although *S. aureus* accounts for 0.5 to 6% of urinary tract infections (UTI), untreated infection can cause serious complications such as sepsis [2,3].

The emergence of antibiotic resistance in MRSA strains and unavailability of therapeutic options for managing the MRSA infections remain a challenge to healthcare [4]. There is a huge global concern about the increased drug resistance *S. aureus* and development of multiple resistance in several drugs such as penicillins, tetracyclines, macrolides and aminoglycosides [5,6].

The advent of vancomycin, a glycopeptide antibiotic, was considered to be the most reliable therapeutic agent against MRSA infections. However, reports indicated the emergence of vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus* (VRSA) strains [7–9]. Reduction in susceptibility of *S. aureus* strain to vancomycin was first reported in 1997 from Japan [10], while clinical resistance of *S. aureus* to vancomycin was first reported in 2002 from Michigan, USA [11]. So far VRSA strains have been reported from Japan, the United States, France, Korea, South Africa, Brazil and Scotland [12,13]. In Iran

a VRSA strain was identified with a minimum inhibitory concentration (MIC) of  $>512 \mu\text{g/mL}$  for the first time in 2007 [14].

The reduction in susceptibility of MRSA to vancomycin indicated the need for alternative therapies. Thus, the US Food and Drug Administration (FDA) approved linezolid, daptomycin, tigecycline and quinupristin/dalfopristin as treatment options for MRSA infections [8,12]. Tigecycline is the first glycylicycline antimicrobial agent derived from minocycline that is highly active against many multidrug-resistant bacteria, including MRSA. Reports concerning resistance of Gram-positive bacteria including *S. aureus* to tigecycline have been rare [15,16].

The increasing reports concerning reduction in susceptibility of MRSA to vancomycin was found to be an important indicator for determining antibiotic sensitivity. Thus, linezolid, tigecycline and quinupristin/dalfopristin have been introduced as new therapeutic options [5,8]. The aim of this study was to determine the *in vitro* activity of vancomycin, tigecycline and quinupristin/dalfopristin against MRSA isolates recovered from UTI patients.

## Methods

### Bacterial isolates

Thirty nonrepetitive MRSA strains from 54 *S. aureus* isolates from UTI patients were isolated from Sina Hospital, Tehran University of Medical Sciences. These isolates were collected over a period of 9 months from December 2014 to September 2015. *S. aureus* isolates were confirmed using the standard biochemical and microbiologic methods including Gram staining; oxidase, catalase, coagulase and DNase tests; and mannitol fermentation reaction [17].

### Antimicrobial agents and MIC determination

The MRSA strains were identified using cefoxitin (30  $\mu\text{g}$ ) Kirby-Bauer disk diffusion test. Furthermore, the antibiotic susceptibility patterns of the strains for linezolid (30  $\mu\text{g}$ ) and quinupristin/dalfopristin (15  $\mu\text{g}$ ) (MAST, UK) were determined using the same method. The results were interpreted on the basis of Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. For quality controls, *S. aureus* ATCC 25923 was used as a reference strain.

The minimum inhibitory concentration (MIC) values of vancomycin and tigecycline against the MRSA isolates were determined by broth microdilution method and interpreted using the CLSI and FDA guidelines respectively [18,19]. According to CLSI guidelines, *S. aureus* with vancomycin MICs of  $\leq 2 \mu\text{g/mL}$  were considered to be susceptible, while the definitions for vancomycin-intermediate *S. aureus* and VRSA are changed to MIC of 4 to 8  $\mu\text{g/mL}$  and  $\geq 16 \mu\text{g/mL}$  respectively

[18]. It is noteworthy that medium used for broth microdilution of tigecycline must be freshly prepared. This procedure was repeated three times [20]. The MIC breakpoints used for the susceptibility tests of *S. aureus* to tigecycline and vancomycin (Sigma-Aldrich, USA) were  $\leq 0.5 \mu\text{g/mL}$  and  $\leq 2 \mu\text{g/mL}$  respectively. In this case, *S. aureus* ATCC 29213 was used as a standard strain.

### PCR amplification for *vanA* gene

The PCR assays were used to detect the *vanA* gene. Genomic DNA was extracted from pure cultures of the strains using High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer's instructions.

The primers for detection of *vanA* were: forward, 5'-CAT-GAATAGAATAAAAAGTTGCAATA-3'; and reverse, 5'-CCCCTTTAACGCTAATACGATCAA-3' [21]. PCR was conducted on the summation of all volumes consisting of 25  $\mu\text{L}$  (12.5  $\mu\text{L}$  of 2 $\times$  Hot Star Taq Master Mix, 1  $\mu\text{L}$  of the DNA template, 1  $\mu\text{L}$  of each primer (20 pmol) and 9.5  $\mu\text{L}$  of ddH<sub>2</sub>O) using the Hot Star Taq Master Mix kit (SinaClon, Iran). Settings for the reaction were as follows: initial denaturation step at 94°C for 5 minutes; 30 amplification cycles each for 1 minute at 94°C, 30 seconds at 57°C and 1 minute at 72°C. This was followed by an additional extension step of 10 minutes at 72°C. The PCR product of the *vanA* gene (1030 bp) was electrophoresed on 1% agarose gel containing 1 $\times$  Gel Red DNA stain (Biotium, USA).

## Results

### Antibiotic susceptibility

All 30 isolates were confirmed as MRSA by cefoxitin Kirby-Bauer disk diffusion test. The susceptibility of MRSA strains to linezolid and quinupristin/dalfopristin was 100%, while the susceptibility of the strains to vancomycin and tigecycline was each 93.3%.

In this study, the broth microdilution method at MIC  $>128 \mu\text{g/mL}$  demonstrated two MRSA strains (6.6%) to have resistance for vancomycin. Moreover, using the FDA 2005 cutoff of MIC  $>0.5 \mu\text{g/mL}$  [19] revealed two MRSA strains (6.6%) to be resistant to tigecycline. The tigecycline MIC value for these strains was 1  $\mu\text{g/mL}$ . The MIC values of vancomycin and tigecycline for the MRSA strain susceptibility test are shown in Table 1. Of the four MRSA strains that were resistant to the recent antibiotics, two of them were resistant to vancomycin. The remaining two tigecycline-resistant MRSA strains were susceptible to vancomycin. Overall, this study found that all the strains were susceptible to linezolid and quinupristin/dalfopristin.

**TABLE 1. MIC of vancomycin and tigecycline for MRSA strains**

Antibiotic	MIC ( $\mu\text{g/mL}$ )	Isolates, n (%)
Vancomycin	0.5	14 (46.8)
	1	12 (40)
	2	2 (6.6)
	128	2 (6.6)
Tigecycline	0.125	4 (1.4)
	0.25	21 (70)
	0.5	3 (10)
	1	2 (6.6)

MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.

### Detection of *vanA* gene

In the present study, PCR detection of the *vanA* gene was used to confirm strains considered to be vancomycin resistant by the broth microdilution method. PCR amplification showed that the two VRSA strains contained the *vanA* gene. The nucleotide sequence data that we report here have been submitted to the GenBank sequence database and have been assigned the accession number KU315431.1.

### Description of patients with VRSA infection and VRSA strains

Both patients with VRSA UTI were hospitalized in the urology ward and represent clinical manifestations of UTI including dysuria, frequency and fever. It is noteworthy that one patient was male and the other female, both were older than 35 years and neither had ever had catheters. A more detailed description of the VRSA strains is provided in Table 2.

## Discussion

During the past few decades, MRSA has evolved as one of the major causes of nosocomial and community-acquired infections worldwide. Although infections caused by *S. aureus* include a low proportion of UTIs, *S. aureus* should not be underestimated as a causative agent because untreated infection can lead to serious complications [2,22]. Vancomycin was the main antibiotic of choice for treating serious MRSA infections. However, a reduction in susceptibility of MRSA to vancomycin has been reported recently from many countries including the United States, Japan, France, Korea, South Africa, Brazil and Scotland

[12,13]. VRSA strains tend to be multidrug resistant against many currently available antimicrobial drugs. This has led to limited options for the treatment of resistant infections and has posed a major public health challenge [5,23].

Clinical features of patients with VRSA UTI, including presence of urinary symptoms, catheterization and prior vancomycin use, have been considered in several studies [24,25]. In one study, one VRSA strain was obtained from a urine sample and the urinary tract catheter of an elderly patient in long-term care in New York in 2004 [24]. In another study, both patients with VRSA infection had a history of receipt of vancomycin [20]. In addition, another study showed that *S. aureus* isolates with reduced susceptibility to vancomycin emerged after its unbalanced use in cases of MRSA infection [25]. In the current study, both patients with VRSA UTI represent clinical manifestations of UTI and had a history of vancomycin therapy, although neither had had a catheterization experience.

In Iran the first VRSA strain to vancomycin at MIC >512  $\mu\text{g/mL}$  was isolated from a diabetic patient in 2007 [14]. The current study identified 6.6% (2/30) of the MRSA strains to be resistant to vancomycin at MIC  $\geq$  128  $\mu\text{g/mL}$ . The PCR assay for *vanA* also demonstrated that the two VRSA strains contained the *vanA* gene. A recent study in Pakistan reported that 9.8% of strains of *S. aureus* were resistant to vancomycin [12]. Another study using MIC of >512  $\mu\text{g/mL}$  reported a VRSA strain containing the *vanA* gene [9].

Effective treatment options for infections caused by MRSA included linezolid, daptomycin, tigecycline and quinupristin/dalfopristin [8,12]. Tigecycline was the first glycolcycline antimicrobial agent to be highly active against many multidrug-resistant bacteria, including MRSA. So far, reports concerning Gram-positive bacteria, including *S. aureus* resistance to tigecycline, have been rare [15,16]. However, a study in Libya reported tigecycline resistance in 3.6% of the MRSA and methicillin-susceptible *S. aureus* strains they assessed [26].

Another study demonstrated that 2% of MRSA and 3% of methicillin-resistant coagulase-negative staphylococci were resistant to tigecycline [27]. A study in Iran reported a tigecycline-resistant MRSA strain for the first time in 2013 [28]. The current study demonstrated 6.6% MRSA strains with MIC of 1  $\mu\text{g/mL}$  resistance to tigecycline. These strains were believed to be susceptible to vancomycin, linezolid and

**TABLE 2. Description of vancomycin-resistant *Staphylococcus aureus* strains**

Strain No.	Specimen/Sex	MIC vancomycin ( $\mu\text{g/mL}$ )	<i>vanA</i> gene	Prior vancomycin use	Resistant	Susceptible	Ward
VRSA 1	Urine/male	> 128	+	Yes	VAN, FOX	LZD, SYN, TIG	Urology
VRSA 2	Urine/female	> 128	+	Yes	VAN, FOX	LZD, SYN, TIG	Urology

FOX, ceftioxin; LZD, linezolid; SYN, quinupristin/dalfopristin; TIG, tigecycline; VAN, vancomycin.

quinupristin/dalfopristin. Although we found that all strains were susceptible to linezolid and quinupristin/dalfopristin, two of these MRSA strains were found to be resistant to vancomycin and sensitive to tigecycline. Additionally, two other MRSA strains were resistant to tigecycline. Thus, our study found that linezolid and quinupristin/dalfopristin can be used as alternative therapy for the treatment of MRSA infections.

In conclusion, the results of our study confirm the occurrence of *vanA* gene-positive VRSA and tigecycline-resistant *S. aureus* in Iran. Reports concerning *S. aureus* resistance to tigecycline have been rare. However, the detection of two MRSA strains resistant to tigecycline in the current study hints at the potential for other resistant strains in the future. The decrease in susceptibility of MRSA to vancomycin emphasizes the need for alternative therapies. Clinicians need to correctly identify patients in whom last-resort therapy such as linezolid and quinupristin/dalfopristin should be administered.

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## Conflict of Interest

None declared.

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