ORIGINAL ARTICLE

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 *van*A 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.

Keywords: Antibiotic resistance, methicillin-resistant *Staphylococcus aureus*, tigecycline, *van*A 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

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a VRSA strain was identified with a minimum inhibitory concentration (MIC) of >512 μg/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 glycylcycline 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 μg) Kirby-Bauer disk diffusion test. Furthermore, the antibiotic susceptibility patterns of the strains for linezolid (30 μg) and quinupristin/dalfopristin (15 μg) (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 μg/mL were considered to be susceptible, while the definitions for vancomycin-intermediate *S. aureus* and VRSA are changed to MIC of 4 to 8 μg/mL and $\geq$16 μg/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 μg/mL and $\leq$2 μg/mL respectively. In this case, *S. aureus* ATCC 29213 was used as a standard strain.

PCR amplification for *van*A gene

The PCR assays were used to detect the *van*A 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 *van*A were: forward, 5′-CATGAATAGAATAAAAGTTGCAATA-3′; and reverse, 5′-CCCCTTTAACGCTAATACGATCAA-3′ [21]. PCR was conducted on the summation of all volumes consisting of 25 μL (12.5 μL of 2× Hot Star Taq Master Mix, 1 μL of the DNA template, 1 μL of each primer (20 pmol) and 9.5 μL of ddH_2O) 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 *van*A gene (1030 bp) was electrophoresed on 1% agarose gel containing 1× 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 μg/mL demonstrated two MRSA strains (6.6%) to have resistance for vancomycin. Moreover, using the FDA 2005 cutoff of MIC >0.5 μg/mL [19] revealed two MRSA strains (6.6%) to be resistant to tigecycline. The tigecycline MIC value for these strains was 1 μg/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.