Emergence of High-Level Vancomycin-Resistant *Staphylococcus aureus* in the Imam Khomeini Hospital in Tehran

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Key Words

Methicillin-resistant *Staphylococcus aureus* • Vancomycin •
Vancomycin-resistant *Staphylococcus aureus* • *vanA* gene

Abstract

Objective: The objective of the study was to investigate the prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA). **Materials and Methods:** Three hundred and fifty-six *S. aureus* isolates from the Imam Khomeini hospital in Tehran, Iran, were evaluated for methicillin and decreased vancomycin susceptibility by the microbroth dilution method. The *mecA*, *vanA* and *vanB* genes were targeted by polymerase chain reaction. **Results:** Of the 356 isolates, 149 (41.85%) *S. aureus* strains were resistant to methicillin. Two strains of methicillin-resistant *S. aureus* were VRSA strains. One isolate, Teaching Hospital-1 (TEH-1), had a vancomycin minimum inhibitory concentration (MIC) of 64 µg/ml and was susceptible to teicoplanin while the other isolate (TEH-2) had a vancomycin and teicoplanin MIC of 512 and >256 µg/ml, respectively, and was positive for the *vanA* gene. **Conclusion:** This report shows that the emergence of VRSA in Iran warrants active microbiological surveillance and careful monitoring of vancomycin therapy.

Introduction

Treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections is becoming increasingly more complicated due to the emergence of various types of antimicrobial resistance. Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA [1, 2]. The first clinical isolate of vancomycin-resistant *S. aureus* [VRSA; minimum inhibitory concentration (MIC) ≥ 32 µg/ml] was reported from the United States [1]. In this study, we investigated the vancomycin susceptibility pattern of *S. aureus* isolated from the Imam Khomeini hospital in Tehran, Iran, by the microbroth dilution method.

Materials and Methods

Clinical Isolates

Three hundred and fifty-six *S. aureus* isolates were evaluated in this study. These strains were isolated from different clinical samples received in the Department of Microbiology over a period of 1 year (2005). Identification of isolates was done by conventional methods.

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Table 1. The antibiotic susceptibility pattern of VRSA strains

Isolate	MIC, µg/ml																
	AMK	AMP	CFZ	CFX	CIP	CLI	CRO	CZX	DOX	ERY	GEN	IMP	LZD	OFX	PEN	RIF	TET
TEH-1	64	16	16	16	8	<0.25	32	32	<2	<0.5	64	64	<0.5	8	16	≥128	<2
TEH-2	≥512	≥32	≥256	≥256	≥128	≥64	≥512	≥256	<0.5	512	≥512	≥256	2	128	≥16	4	<4

AMK = Amikacin; AMP = ampicillin; CFZ = cefazolin; CFX = cefotaxime; CIP = ciprofloxacin; CLI = clindamycin; CRO = ceftriaxone; CZX = ceftizoxime; DOX = doxycycline; ERY = erythromycin; GEN = gentamicin; IMP = imipenem; LZD = linezolid; OFX = ofloxacin; PEN = penicillin; RIF = rifampin; TET = tetracycline.

Antimicrobial Susceptibility Testing

The MICs of oxacillin, vancomycin (Sigma-Aldrich, Germany), teicoplanin, amikacin, ampicillin, cefazolin, cefotaxime, ciprofloxacin, clindamycin, ceftriaxone, ceftizoxime, doxycycline, erythromycin, gentamicin, imipenem, linezolid, ofloxacin, penicillin, rifampin, and tetracycline (ADATAB, Mast Group Ltd., UK) were determined by the microbroth dilution method using the cation-adjusted Mueller-Hinton broth according to the National Committee for Clinical Laboratory Standards (now Clinical Laboratory Standards Institute) guidelines [3]. *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 strains were used as vancomycin-susceptible controls while resistant *Enterococcus* strains [*E. faecalis* E206 (*vanA* positive) and *E. faecium* E278 (*vanB* positive), kindly provided by Dr. Edet Udo] as vancomycin-resistant controls.

Detection of *mecA*, *vanA* and *vanB* Genes by Polymerase Chain Reaction

Genes encoding the methicillin and vancomycin resistance determinants, *mecA*, *vanA* and *vanB*, were investigated by polymerase chain reaction using specific primers [4]. PCRs were performed in a 50-µl volume consisting of: 1× PCR buffer, 3.5 mM MgCl₂ (2.5 mM MgCl₂ for *mecA*), 0.5 µg/ml of each primer, 2.5 U *Taq* DNA polymerase, 0.2 mM dNTP mix and 3 µl of DNA template (10 µg/ml). The PCR conditions consisted of a predenaturation step at 94°C for 5 min, followed by 30 cycles of 45 s at 94°C, 45 s at 54°C (58°C for *mecA*) and 45 s at 72°C. A final extension step was performed at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1.5% agarose gel. DNA bands were visualized by staining with ethidium bromide and photographed under UV illumination.

Results

Of the 356 *S. aureus* isolates, 149 (41.85%) strains were resistant to methicillin. PCR showed that all MRSA strains contained the *mecA* gene. All MRSA strains were susceptible to vancomycin, while two strains of MRSA were vancomycin resistant. The Teaching Hospital-1 (TEH-1) strain was isolated from a 42-year-old woman with a soft tissue wound and the TEH-2 strain from a

67-year-old diabetic man with a post-heart surgery wound.

The TEH-1 isolate was resistant to vancomycin (MIC 64 µg/ml), susceptible to teicoplanin and negative for *vanA* and *vanB* genes. The TEH-2 strain was resistant to vancomycin and teicoplanin with an MIC of 512 and >256 µg/ml, respectively, and was positive for the *vanA* gene.

The antibiotic susceptibility of VRSA strains is described in table 1. TEH-1 was not only susceptible to tetracycline, linezolid and doxycycline like TEH-2 but was also susceptible to erythromycin, clindamycin and cefazolin. TEH-1 was found to express reduced susceptibility to cefotaxime, ceftriaxone and ceftizoxime.

Discussion

The emergence and increase of the prevalence of vancomycin-resistant enterococci caused a significant alarm among the medical community in Iran [2]. Since the vancomycin-resistant genes (*vanA* and *vanB*) are transmissible to other bacterial species, in particular *S. aureus*, the emergence of vancomycin resistance in clinical staphylococci has become a great concern [5]. Therefore, comprehensive data concerning the endemic prevalence and susceptibility patterns of staphylococci in various health institutions are necessary for the control and appropriate treatment of patients with staphylococcal infections.

The rate of MRSA prevalence in the present study (41.85%) is lower than in other reports of MRSA prevalence (60%) in Iranian hospitals [2], but similar to the rate (40.61%) reported from India [6]. The lower prevalence of MRSA in this study, compared to other reports from Iran, may be related to using PCR and MIC methods in our study instead of the disk agar diffusion (in full) method.

In this study, we found two MRSA strains which were vancomycin resistant. These VRSA strains were isolated from the wound specimens of 2 different patients. Both VRSA isolates were also resistant to several other antimicrobials (table 1): the TEH-2 isolate was also resistant to cefazolin, clindamycin, ceftriaxone, ceftizoxime, and erythromycin, while TEH-1 had shown reduced susceptibility to cefotaxime, ceftriaxone, and ceftizoxime.

The emergence of glycopeptide resistance is of great concern. Though the first case of VRSA was reported in the USA, few other countries have reported reduced susceptibility of *S. aureus* to glycopeptides [1, 5, 6].

The existence of vancomycin-resistant staphylococci in a hospital could pose a serious challenge to the clinicians in finding an alternative treatment. The development of antibiotic resistance in developing countries like Iran seems to be highly related to the irrational antibiotic usage due to its easy availability at the drugstores without prescription. This emergence of VRSA may also be due to the selective pressure of vancomycin, a glycopeptide which is currently the main antimicrobial agent available to treat life-threatening infections with MRSA.

In the current study, one of the VRSA strains (TEH-1) was negative for the *vanA/vanB* genes by PCR. Therefore, the absence of the *vanA/vanB* genes in one of the strains indicates an alternative mechanism of vancomycin resistance such as vancomycin affinity trapping [7]. It is noteworthy to point out that discovering VRSA among Iranian staphylococcal isolates may represent an important development of MRSA resistance to antibiotics.

Conclusion

This report shows that the emergence of VRSA in Iran warrants active microbiological surveillance and careful monitoring of vancomycin therapy.

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