Original Article

Susceptibility to Vancomycin in *Staphylococcus Aureus* Isolated From Patients of Four University-Affiliated Hospitals in Tehran

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ABSTRACT

Background and Objective: **Vancomycin is frequently the antibiotic of choice for the treatment of infections caused by methicillin-resistant *Staphylococcus* aureus (MRSA). For the last years, the incidence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has been increased in various parts of the world. The present study was carried out to determine the presence of VISA and VRSA in Tehran.**

***Materials and Methods*: A total of 164 *S. aureus* strains were isolated from clinical specimens in four university-affiliated hospitals in Tehran from November 2006 to June 2007. Minimum inhibitory concentration (MIC) of vancomycin of isolates was determined by agar dilution method. Vancomycin (6 mg/l) screen agar plate method and E-test were used to confirm presence of resistance to vancomycin. Disc diffusion agar test was also used to detect resistance to other antimicrobial agents.**

Results: **Only one VRSA (MIC 256 mg/l) was detected and three strains with MIC 4 mg/l considered VISA according to recent CLSI breakpoints for vancomycin. Only VRSA strain had shown growth on vancomycin screen agar plate and was also resistant to several antimicrobial agents but susceptible to quinupristin/dalfopristin, linezolid, chloramphenicol, mupirocin and cotrimoxazole. Isolated VISA were also multi-resistant but showed susceptibility to quinupristin/ dalfopristin, linezolid, chloramphenicol and mupirocin.**

Conclusion: **Detection of vancomycin resistance in Iranian *S. aureus* isolates emphasizes the challenges confronted by the infection control specialists in hospitals in Iran as well as causing problems in the treatment of patients with *S. aureus* infections.**

Key words: Vancomycin, *Staphylococcus aureus*

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Introduction

Staphylococcus aureus causes serious infections in both the hospital and the community. The growing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) as a cause of these infections has increased the use of the glycopeptide antibiotic vancomycin over the past 3 decades (1). As a consequence, selective pressure was established that eventually lead to the emergence of strains of *S. aureus* with decreased susceptibility to vancomycin and other glycopeptides (2). In 1997, the first strain of vancomycin-intermediate *S. aureus* (VISA) was reported from Japan (3). Shortly after, two additional cases were reported from United States (4). However, first clinical isolate of vancomycin-resistant *S. aureus* (VRSA) was reported from United States in 2002 (5). Subsequent isolation of VISA and VRSA isolates from United States (6;7) and other countries including Brazil (8), France (9), United Kingdom (10), Germany (11), India (12;13), and Belgium (14) has confirmed that emergence of these strains is a global issue.

The choice of method for susceptibility testing and test conditions are essential considerations in accurately applying a definition of VISA or VRSA for any given isolate. VISA and VRSA isolates are not detected by disc diffusion, whilst vancomycin screen agar plate are only suitable for isolates with a minimum inhibitory concentration (MIC) >6 mg/l. Acceptable methods used to detect these strains are non-automated and include broth or agar dilution and E-test. Also, CLSI has recently lowered breakpoints for vancomycin and strains with MIC of 4-8 mg/l are considered VISA and with MIC ≥ 32 mg/l are VRSA (15-18).

In our previous study in 2003, five out of the 139 of *S. aureus* strains isolated in Tehran had shown a vancomycin MIC of ≥128 mg/l by agar dilution and E-test methods (19). Emaneini et al have recently reported isolation of VRSA from one Iranian patient (20). Keeping these in view we performed this study on 164 *S. aureus* strains for the assessment of current situation of vancomycin resistance in Tehran.

Materials and Methods

S. aureus isolates

A total 164 *S. aureus* were investigated for the period of 8 months from November 2006 to June 2007. The strains were collected from various clinical specimens including pus, urine, wound swabs, catheters, blood, sputum and CSF from the patients of different inpatient and outpatient departments of 4 university-affiliated hospitals in Tehran. The identification of isolates was done according to standard methods (12).

Determination of minimum inhibitory concentration (MIC)

MIC of vancomycin (LKT laboratories, USA) was determined by agar dilution method according to CLSI guidelines (16). Briefly, gradient plates of Mueller-Hinton agar (Merck, Germany) were prepared with vancomycin (0.5–256 mg/l). By direct colony suspension method, 0.5 McFarland equivalent inoculum was prepared in normal saline from 18–24 h agar plate culture. The suspension was further diluted to achieve desired inoculum concentration of 10^5 CFU/ml. All strains were spotted onto gradient plates. Plates were incubated overnight at 35 °C for any visible growth. For strains showing MIC ≥4 mg/l to vancomycin in agar dilution method, E-test (AB Biodisk, Sweden) was used according to manufacture guidelines, to confirm presence of vancomycin resistance. *S. aureus* ATCC 29213 was used as vancomycin susceptible control. Readings were taken according to recent CLSI guideline: MIC≤2 mg/l for vancomycin-susceptible, MIC of 4-8 mg/l for vancomycin-intermediate *S. aureus* (VISA) and MIC≥16 mg/l for vancomycin-resistant *S. aureus* (VRSA) (16).

Vancomycin screen agar plate method

In-house vancomycin screen agar plate was prepared by addition of 6 mg/l vancomycin (LKT laboratories, USA) to brain heart infusion (BHI) agar (Merck, Germany). Inoculum suspension was prepared by transferring colonies from overnight growth on nutrient agar plate to sterile saline to produce a suspension that matches the turbidity of a 0.5 McFarland standard. Then, 0.1 ml of this suspension was spread on vancomycin screen agar plate and was incubated for 24 h at 35 °C in ambient air. Any visible growth indicated the vancomycin resistance. In addition, *S. aureus* ATCC 29213 was used as a vancomycin-susceptible control strain.

Disc diffusion agar test

Disc diffusion agar test was carried out using Kirby-Bauer method by following discs: penicillin G (10 U), ampicillin (10 µg), ampicillin-sulbactam (20 µg), oxacillin (1 µg), gentamicin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), tetracycline (30 µg), cotrimoxazole (25 µg),

vancomycin (30 µg), cefoxitin (30 µg), clindamycin (2 µg), rifampicin (5 µg), mupirocin (5 µg), linezolid (30 µg) and quinupristin/dalfopristin (15 µg), all purchased from MAST (UK). Mueller-Hinton agar plates were overlaid with the inoculum (turbidity equivalent to that of a 0.5 McFarland Standard) of the *S. aureus* strains. Zone diameters were measured at 24 h following CLSI criteria (16). *S. aureus* ATCC 25923 was used as reference strain.

Results

MIC for 164 *S. aureus* strains against vancomycin is shown in Table 1. MIC for 97.5% of isolates was ≤2 mg/l. Only one strain was found to be VRSA strains (MIC 256 mg/l). This VRSA strain was isolated from pus of wound of a 36 years old female patient admitted in operation ward. It was not distinguished from vancomycin susceptible *S. aureus* isolates in disc diffusion agar test because it has produced a 20 mm zone of growth inhibition around vancomycin disc. From 164 *S. aureus* strains, only this VRSA strain showed growth on vancomycin screen agar and was resistant to most of the commonly used antimicrobial agents including penicillin G, ampicillin, oxacillin, cefoxitin, clindamycin, ampicillin-sulbactam, ciprofloxacin, gentamicin, erythromycin, rifampicin and tetracycline but susceptible to quinupristin/dalfopristin, linezolid, chloramphenicol, mupirocin and cotrimoxazole (Table 2).

Table 1: Distribution of vancomycin MICs for 164 isolates of *Staphylococcus aureus* as determined by agar dilution method

MIC (mg/l)	No. of strains	Percent
0.5	3	1.8
1	127	77.4
2	30	18.3
4	3	1.8
≥256	1	0.6
Total	**164**	**100**

Table 2: Detailed description of VISA and VRSA (detected by agar dilution method and E-test) including antibiotic susceptibility patterns as determined by disc diffusion method

Strain no.	MIC vancomycin (mg/l)	Strains designed as*	Characterization of patients				Zone vancomycin (mm)	Resistance to	Susceptibility to
			Sex	Age	Specimens	Ward			
31	256	VRSA	Female	36	Wound	Operation	20	PG, AP , OX, FOX, CD, SAM, CIP, G, E, RP, T	SYN, LZD, C , MUP, TS
64	4	VISA	Female	78	Respiratory	Internal	22	PG, AP , OX, FOX, CD, SAM, CIP, G, E, TS, T	SYN, LZD, C , MUP, RP
105	4	VISA	Female	90	Respiratory	ICU	21	PG, AP , OX, FOX, CD, SAM, CIP, G, E, RP, TS	SYN, LZD, C , MUP, T
128	4	VISA	Female	71	Respiratory	ICU	20	PG, AP , OX, FOX, CD, SAM, CIP, G, E, RP, T	SYN, LZD, C , MUP, TS

VA, vancomycin; CD, clindamycin; TS, cotrimoxazole; OX, oxacillin; C, chloramphenicol; RP , rifampicin; PG, penicillin G; LZD, linezolid; SAM, ampicillin-sulbactam; AP, ampicillin; FOX, cefoxitin; SYN, quinupristin/dalfopristin; CIP, ciprofloxacin; G, gentamicin; T , tetracycline; E, erythromycin; MUP, mupirocin.

* According to CLSI (2006)

Three strains of *S. aureus* had vancomycin MIC of 4 mg/l and considered VISA according to recent CLSI breakpoints for vancomycin (16). However, these strains were not distinguished from vancomycin-susceptible *S. aureus* isolates in disc diffusion test because they had shown ≥20 mm zone of growth inhibition around vancomycin disc. All isolated VISA were resistant to penicillin G, ampicillin, oxacillin, cefoxitin, clindamycin, ampicillin sulbactam, ciprofloxacin, gentamicin and erythromycin but susceptible to quinupristin/dalfopristin, linezolid, chloramphenicol and mupirocin and susceptibility to other antimicrobial agents were different among these strains. The isolated VISAs were isolated from respiratory specimens of female patients admitted in different wards with various ages (Table 2).

Discussion

Infections caused by methicillin-resistant *S. aureus* have been associated with high morbidity and mortality rates (18). Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but unfortunately, decreases in vancomycin susceptibility of *S. aureus* and isolation of vancomycin-intermediate and resistant *S. aureus* were recently reported from many countries (3-14). Until now, two reports from Iran were published about VISA and VRSA. In our previous study in 2003 (19), five out of the 139 *S. aureus* strains isolated in Tehran were VRSA (MIC ≥128 mg/l). Emaneini et al. have recently reported isolation of one VRSA strain in a teaching hospital in Tehran (20). In this study, one of 164 *S. aureus* strains was VRSA (MIC ≥256 mg/l). There has been increasing evidence that strains with a vancomycin MIC of 4 mg/l behave similar in the clinical setting to VRSA strains as clinical failure generally results if treatment with vancomycin is continued (18;21;22). So, the previous breakpoints for vancomycin (≤4 mg/l (S); 8-16 mg/l (I); ≥32 mg/l (R) (15) have recently been revised (≤2 mg/l (S); 4-8 mg/l (I); ≥16 mg/l (R)) (16). Therefore, three isolated *S. aureus* strains in this study with MIC 4 mg/l have been considered VISA.

Isolation of VRSA and VISA in Tehran calls for the implementation of a regional and nationwide surveillance system to monitor presence of these strains in other regions in Iran. Unfortunately, VISA and VRSA isolates are not detected by disc diffusion agar test and automated methods did not accurately identify these strains. Broth or agar dilution methods and E-test are standard methods for detection of VISA and VRSA (17-18), although discrepancies between their results have been reported (22;23). Vancomycin screen agar plate are only suitable for isolates with MIC> 6 mg/l and is unsuitable in view of the lower CLSI susceptibility breakpoint of 2 mg/l (18). The Center for Disease Control and Prevention (CDC) recommends that laboratories use MIC method plus vancomycin screen agar for detection of VISA and VRSA (17). Therefore, we used different methods to identify vancomycin resistance in *S. aureus*.

On the other hand, VISA and VRSA tend to be multi-drug resistant against a large number of currently available antimicrobial agents, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy and increase in morbidity and mortality (18). In the present study, isolated VRSA and VISA showed resistance to a wide range of different antimicrobial agents but retained susceptibility to linezolid, quinupristin/dalfopristin, chloramphenicol and mupirocin. Isolated VRSA was also sensitive to cotrimoxazole. Sensitivity of isolated VRSA to linezolid and quinupristin/dalfopristin; two recently approved antimicrobials by the Food and Drug Administration (FDA) has also been shown in other studies (6;7;21;24;25) and linezolid was used for treatment of clinical infection of VRSA (6). Also, most VISA and VRSA strains isolated in other studies (12;24;26) and this study were susceptible to cotrimoxazole, which for the time being, may represent adequate therapy for skin and soft tissue infections caused by these strains. However, VRSA isolated in our previous study (19) and some of isolated VRSA in other studies has shown *in vitro* resistance to cotrimoxazole (21;26). Also, widespread use of these drugs will surely lead to resistance and newer therapeutic modalities are urgently needed.

Conclusion

The importance of discovering vancomycin resistance in *S. aureus* isolates underscores the fact that physicians should include vancomycin susceptibility tests in strategies for managing patients with S. aureus infections and use of an active infection control policy to prevent the spread of VRSA in the healthcare facilities.

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