Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to Vancomycin, Teicoplanin, Linezolid, Pristinamycin and Other Antibiotics

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* is a major nosocomial pathogen worldwide. Vancomycin is the traditional drug of choice, but decreasing susceptibility to vancomycin and other glycopeptides has been reported since 1996.

Objectives: To test the *in vitro* activity of linezolid (oxazolidinone) and other antimicrobial agents against MRSA isolates recovered from hospitalized patients.

Methods: We tested 150 MRSA isolates recovered from hospitalized patients. The minimal inhibitory concentration of vancomycin, teicoplanin, pristinamycin (quinupristin-dalforistin) and linezolid was determined by the Etest method. Susceptibility to other antibiotics was tested by the disk diffusion method.

Results: All isolates were sensitive to vancomycin, teicoplanin, pristinamycin, and linezolid. The MIC_{90} was 2.0 μg/ml for vancomycin and teicoplanin (range 0.5–2.0 μg/ml and 0.125–2.0 μg/ml, respectively), and 0.5 μg/ml for pristinamycin and linezolid (range 0.125–0.75 μg/ml and 0.125–0.5 μg/ml, respectively). Of the other antibiotics, fusidic acid showed the best *in vitro* activity, with 96.7% susceptibility, associated with trimethoprim/sulfamethoxazole (85.8%) and minocycline (84%). Penicillin was associated with the lowest susceptibility (1.3%), associated with ofloxacin (3%) and erythromycin (14%). An increase in the minimal inhibitory concentration value of vancomycin was associated with a significant decrease in resistance to TMP-SMZ ($P < 0.01$) and an apparent increase in resistance to other antibiotics.

Conclusion: The excellent *in vitro* activity of linezolid and its reported *in vivo* effectiveness renders it an important therapeutic alternative to vancomycin in the treatment of MRSA infection.

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Methicillin-resistant *Staphylococcus aureus* is a major nosocomial pathogen worldwide [1]. In some institutions, it was found to play a significant role in up to 50% of nosocomial *S. aureus* infections [2,3]. The National Nosocomial Infection Surveillance Systems of the Centers for Disease Control reported that *S. aureus* methicillin resistance in hospitals in the United States increased from 2.4% in 1975 to 29% in 1991, with a higher degree of resistance in intensive care units [4,5]. More recent data from 1990 through 1997, published by INSPEAR (International Networks for the Study and Prevention of Emerging Antimicrobial Resistance) [6], documented a 260% rise in the rate of infection in participating hospitals.

The continuing increase in the prevalence of nosocomial MRSA infections has become a major therapeutic challenge. Vancomycin is the traditional drug of choice, but decreasing susceptibility to vancomycin and other glycopeptides has been reported since 1996 [7]. The long anticipated development of vancomycin resistance in *S. aureus* has now occurred, including vancomycin intermediate *S. aureus* [8] and vancomycin-resistant *S. aureus* [9].

An important clinical difference exists between the VISA and VRSA strains in relation to vancomycin. The finding that the VISA strain emerges in patients with MRSA infection after frequent vancomycin treatments over several months, while VRSA emerges in patients not treated with vancomycin, indicates that prolonged vancomycin treatment for MRSA is associated with emerging VISA infection [10].

These findings have prompted researchers to seek new antimicrobial agents for the treatment of MRSA infections. Some agents, like linezolid, appear to be good potential alternatives, providing clinicians the possibility to select an antimicrobial agent with a different mechanism of action in order to reduce resistance to glycopeptides by Gram-positive microorganisms [11]. The aim of the present study was to compare the *in vitro* activity of linezolid and other antimicrobial agents against MRSA isolates recovered from hospitalized patients.

Materials and Methods

The study was conducted at Rabin Medical Center, a major university-affiliated tertiary care facility (900 beds) in central Israel. A total of 150 MRSA clinical isolates were recovered from 150 hospitalized patients: 115 isolates from wounds, 26 from blood, 4 from synovial fluid, 3 from peritoneal fluid, and 2 from cerebrospinal fluid. Isolates were identified with the Slidex Staph-Kit (bioMerieux, Marey-Etoile, France) or the Pastorex Staph slide agglutination test (Sanofi Diagnostics Pasteur, Paris, France); findings were confirmed by DNase (DNase test agar Hy-Laboratories, Rehovot, Israel) or the API-Staph test (ID 32 STAPH, bioMerieux).

MRSA = methicillin-resistant *Staphylococcus aureus*
TMP-SMX = trimethoprim/sulfamethoxazole

VISA = vancomycin intermediate *S. aureus*
VRSA = vancomycin-resistant *S. aureus*

The minimal inhibitory concentration of vancomycin, teicoplanin, pristinamycin and linezolid was tested by the Etest method (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions, on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA). Susceptibility to trimethoprim/sulfamethoxazole, minocycline, chloramphenicol, rifampicin, imipenem, tetracycline, clindamycin, gentamicin, erythromycin, ofloxacin and penicillin (all from Difco) was tested by the disk diffusion method on Mueller-Hinton agar, according to the procedures established by the National Committee of Clinical Laboratory Standards [12]. For fusidic acid a breakpoint of ⩾21 mm was used to define susceptibility [13]. *S. aureus* ATCC 25923 was used for quality control. Plates were incubated at 30°C to define methicillin resistance (using oxacillin 1 μg/disk), and at 37°C for other antibiotics for 18 hours. The chi-square test was used for statistical analysis.

Results

According to the criteria of the National Committee for Clinical Laboratory Standards, all isolates were sensitive to vancomycin, teicoplanin, pristinamycin and linezolid. The distribution of MIC values for the four antibiotics, the minimal concentrations at which 50% and 90% of the isolates were inhibited (MIC_{50}, MIC_{90}), and the MIC range are given in Table 1. All isolates were inhibited by ⩽0.5 μg/ml linezolid. In this concentration pristinamycin inhibited 95.3%, teicoplanin 26%, and vancomycin only 7.3% of all isolates.

Table 2 shows the susceptibility to the other antibiotics: 3.3% of isolates were resistant to fusidic acid, 14% to TMP-SMX and 16% to minocycline. An increase in the MIC values of vancomycin was associated with a significant decrease in resistance to TMP-SMX ($P < 0.01$) and an apparent increase in resistance to all the other antibiotics.

Table 1. Distribution of MIC values for vancomycin, teicoplanin, pristinamycin, and linezolid

	No. of isolates (%)			
MIC (μg/ml)	Vancomycin	Teicoplanin	Pristinamycin	Linezolid
⩽0.5	11 (7.3)	39 (26)	143 (95.3)	150 (100)
0.75–1.0	59 (39.3)	37 (24.7)	7 (4.7)	0
1.5–2.0	80 (52.2)	74 (49.3)	0	0
MIC_{50}	1.5	1.0	0.38	0.38
MIC_{90}	2.0	2.0	0.5	0.5
Range	0.5–2.0	0.125–2.0	0.125–0.75	0.125–0.5

Table 2. Susceptibility of 150 MRSA isolates to 12 antibiotics and prevalence of resistance according to the MIC of vancomycin

			Resistance (%) according to MIC of vancomycin		
Antibiotic	μg/disk	Resistance (%) (n=150)	⩽0.5 μg/ml (n=11)	0.75–1.0 μg/ml (n=59)	1.5–2.0 μg/ml (n=80)
Fusidic acid	10	3.3	0	1.7	5.0
TRP-SMX	25	14.0	63.6*	16.9*	5.0*
Minocycline	30	16.0	0	10.2	22.5
Chloramphenicol	30	43.3	36.4	40.7	46.2
Rifampicin	5	51.3	0	30.5	73.7
Imipenem	10	67.3	18.2	50.8	86.2
Tetracycline	30	80.0	63.6	72.9	87.5'
Clindamycin	2	81.3	27.3	76.3	92.5
Gentamicin	10	86.7	36.4	86.4	93.7
Erythromycin	15	87.3	54.5	79.7	97.5
Ofloxacin	5	94.0	54.5	93.2	100.0
Penicillin	10**	1.3	90.9	98.3	100.0

* $P < 0.01$
** Units.

Discussion

Methicillin-resistant *S. aureus* is a growing medical concern. During the last two decades the rates of infection caused by MRSA increased among hospitalized patient in most developing countries. The prevalence of MRSA in our center is relatively high, ranging from 26% to 31% in the last 5 years. An increase in the MIC values of vancomycin in this period was not detected. The decreasing susceptibility to vancomycin reported in the literature [7] and the high prevalence of MRSA isolates have alerted researchers to the need for alternative antimicrobial agents.

In the present study both linezolid and pristinamycin showed the best *in vitro* activity of all antibiotics tested [Table 1], although all isolates were sensitive to vancomycin (breakpoint ⩽4 μg/ml), teicoplanin, pristinamycin and linezolid. All isolates were inhibited by ⩽0.5 μg/ml linezolid. In this concentration pristinamycin inhibited 95.3%, teicoplanin 26%, and vancomycin only 7.3% of all isolates.

The MIC_{50} and MIC_{90} for both pristinamycin and linezolid were 0.38 and 0.5 μg/ml, for teicoplanin 1.0 and 2.0 μg/ml and for vancomycin 1.5 and 2.0 μg/ml, respectively. Pristinamycin, an antimicrobial agent active against Gram-positive pathologies, has been used successfully in both nosocomial and community infections. However, several practical issues complicate the use of pristinamycin, one of which is the limited number of approved indications [14]. Others include the fact that it must be given intravenously, generally by deep catheter, to avoid venous irritation. It also affects the clearance of medications via the cytochrome P450 system and has the potential for major drug interactions. A variable number of patients experience myalgia and/or arthralgia that can be severe enough to require dose reduction or administration of opiate analgesics. Linezolid is a new antimicrobial agent that is primarily active against Gram-positive pathogens [15]. It inhibits formation of the 70S initiation complex by binding to the 50S ribosomal subunit near the interface with the 30 subunit [16]. This mechanism is unique, and no cross-resistance between oxazolidinones and other protein-synthesis inhibitors has been reported. Treatment with linezolid has been clinically and microbiologically proven to be as effective as standard vancomycin therapy for patients with MRSA infections [17]. Good results were also reported in orthopedic patients [18] and in children [19]. The concentrations of linezolid in plasma and other body sites is much higher than the MIC value [20]. The drug can be also be given orally, and is well tolerated.

MIC = minimal inhibitory concentration

If one considers the properties of both agents, linezolid would be found to be the more versatile of the drugs. Regarding other antibiotics, fusidic acid, TMP-SMX and minocycline showed the best activity [Table 2]. Of the 150 isolates, only 3.3% were resistant to fusidic acid. Fusidic acid can be used for MRSA infections, including bacteremia. Its adverse reactions depend on the route of administration. Thrombophlebitis and reversible jaundice have been noted with the intravenous formulation [21].

Resistance to TMP-SMX was detected in 14% of isolates. In two other studies we previously reported an increase in the susceptibility of MRSA to TMP-SMX over time. In one study, susceptibility increased from 12% in 1991 to 80% in 1997 [2]. In the second study [22], susceptibility of MRSA isolates recovered from the bloodstream increased progressively from 31% in 1988 to 92% in 1997 ($P < 0.0001$). Several factors may have influenced the emergence of TMP-SMX-sensitive MRSA, including the reduced usage of this drug in our center as confirmed by our pharmacy records [22].

A recent multicenter report from several hospitals in Belgium showed an increase in TMP-SMX susceptibility among MRSA isolates [23]. The relatively uniform activity of vancomycin and TMP-SMX against MRSA strains was previously reported *in vitro* [24] and *in vivo* [25]. Our finding that an increase in the MIC values of vancomycin was associated with a significant decrease in resistance to TMP-SMX ($P < 0.01$) is interesting and needs further investigation.

In conclusion, the excellent *in vitro* activity of linezolid noted here in a large number of isolates and its reported *in vivo* effectiveness in the treatment of MRSA infections make this agent an important therapeutic alternative to vancomycin. In order to prevent widespread use that might accelerate the emergence of resistance, its use should be limited to patients infected with vancomycin-resistant strains or to those who cannot tolerate vancomycin. This is the first report on the *in vitro* susceptibility of MRSA isolates to linezolid from Israel.

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