Antimicrobial Activity of Linezolid Against Gram-Positive Cocci Isolated in Brazil

Helio S. Sader, Ana C. Gales and Ronald N. Jones

Special Clinical Microbiology Laboratory, Division of Infectious Diseases, Federal University of São Paulo, São Paulo, SP, Brazil; Medical Microbiology Division, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa, USA

The new oxazolidinone linezolid and other antimicrobial agents used to treat Gram-positive infections were tested against 1,585 Gram-positive cocci; 1,260 staphylococci and enterococci isolates from patients hospitalized in Brazilian hospitals, and 325 *S. pneumoniae* isolates for patients with community acquired infections. Susceptibility testing was performed using broth microdilution according to NCCLS procedures. Linezolid was the most active compound and the only drug that inhibited 100% of the isolates at the susceptible breakpoint (≤ 4 mg/mL). Resistance to vancomycin was very rare (99.9% susceptibility), and both quinupristin/dalfopristin and gatifloxacin were active against approximately 90% of the strains evaluated. All other compounds inhibited less than 65% of the isolates. The excellent *in vitro* Gram-positive activity by linezolid, in this study, indicate that this compound may represent an important therapeutic option for the treatment of infections caused by these pathogens in Brazil.

Key Words: Gram-positive cocci, oxazolidinones, antimicrobial resistance, linezolid, vancomycin resistance, nosocomial infections.

Gram-positive organisms have developed a broad range of mechanisms to evade antimicrobial agents. Of particular concern has been the emergence of glycopeptide-resistant enterococci and glycopeptide-intermediate *Staphylococcus aureus* [1-3]. Oxacillin resistance among *S. aureus*, and penicillin resistance among *Streptococcus pneumoniae* and viridans group streptococci, also represent important therapeutic problems in some patients [4-6]. In addition, coagulase-negative staphylococci (CoNS) has recently become an important cause of nosocomial infections, and this pathogen has presented high rates of resistance to oxacillin and glycopeptides [7].

As a result, new antimicrobial agents are being developed, and older compounds re-evaluated, as potential alternatives for the management of infections due to multiply resistant Gram-positive microorganisms. Everninomicins [8], streptogramins [9, 10]; glycopeptide derivatives [11]; carbapenems [12]; daptomycin [13], and the oxazolidinones [14] are all in various stages of development for the treatment of Gram-positive infections.

Linezolid is a member of the oxazolidinone class of synthetic antibacterial agents that inhibit bacterial protein synthesis through a unique mechanism. In contrast to other inhibitors of protein synthesis, the oxazolidinones act early in the translation by preventing the formation of a functional initiation complex [15]. Consequently, linezolid is not expected to show cross-resistance with existing antimicrobial agents. Linezolid has remarkably

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Address for correspondence: Prof. Hélio S. Sader, MD. Laboratório Especial de Microbiologia Clínica, Disciplina de Doenças Infecciosas e Parasitárias, Universidade Federal de São Paulo – EPM. Rua Botucatu, 740. São Paulo, SP. Zip Code: 04023-063 – Brazil. Phone: (55 11) 5081-2819 / 5571-5180 / 5576-4393. Fax: (55 11) 5543-3013 / 5571-5180.
E-mail: heliosader@uol.com.br

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consistent inhibitory activity against staphylococci, enterococci, and pneumococci, with MICs of 1-4 mg/mL [16]. It also has moderate activity against *Bacteroides* spp. and *Moraxella catarrhalis* (MIC 8 mg/mL), but other Gram-negative bacteria are resistant as a result of endogenous efflux mechanisms [17].

The objective of this study was to evaluate the *in vitro* activity of linezolid against Gram-positive cocci isolated in Brazil in the 3-year period previous to the commercialization of the drug in this country.

Materials and Methods

The isolates were collected and tested as part of the ongoing SENTRY Antimicrobial Resistance Surveillance Program [5]. A total of 1,260 Gram-positive cocci were collected from nosocomial infections during the period of January, 1997, to December, 1999, in 4 Brazilian medical centers. In addition, 325 *S. pneumoniae* isolates from community acquired respiratory infections were collected and tested in the same period of time and included in the study. The participating centers included the Laboratório Especial de Microbiologia Clínica / São Paulo Hospital (LEMC – coordinator center for SENTRY Latin America), São Paulo; Laboratório Médico Santa Luzia, Florianópolis; Laboratório Lâmina, Rio de Janeiro (1997 and 1998 only); and Hospital de Clínicas de Porto Alegre, Porto Alegre (1999 only).

The isolates were consecutively collected according to the site of infection including bloodstream, lungs, wound and soft tissue, and urinary tract infections in hospitalized patients; and community acquired respiratory tract infection. Only isolates judged to be the cause of infection by local physicians were included in this study. The isolates recovered in the participant clinical microbiology laboratories were shipped to the University of Iowa College of Medicine (Iowa City, Iowa, USA), the monitoring laboratory.

Antimicrobial susceptibility testing was performed at the coordinating laboratory using broth microdilution methods as described by the National Committee for Clinical Laboratory Standards [18]. Antimicrobial agents were obtained from respective manufacturers as laboratory grade powder and included the oxazolidinone linezolid; the streptogramin quinupristin-dalfopristin; the glycopeptides vancomycin and teicoplanin; the b-lactams penicillin, ampicillin, and oxacillin; the fluoroquinolones ciprofloxacin and gatifloxacin; the macrolide erythromycin; the aminoglycoside gentamicin; and other compounds such as clindamycin and trimethoprim-sulfamethoxazole. The enterococci were tested against gentamicin only to detect high level resistance (MIC >500 mg/mL). The susceptibility breakpoint used for linezolid was ≤4 mg/mL [14]. Breakpoints for all other compounds followed the NCCLS standards [18]. Quality control measures were utilized by testing *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Results

The Gram-positive microorganism most frequently isolated during the period of the study was *S. aureus* (852 strains, 53.8%), followed by *Streptococcus pneumoniae* (325 strains, 20.5%) coagulase-negative staphylococci (CoNS, 261 strains, 16.5%) and *Enterococcus* spp. (147 strains, 9.3%). All other Gram-positive species were isolated only occasionally and were not included in the analysis. The vast majority of *S. pneumoniae* isolates were collected from community acquired respiratory infections, while staphylococci and enterococci were collected from hospitalized patients.

Linezolid was the only compound active against 100% of the strains tested. One isolate (*Enterococcus* spp.) was resistant to vancomycin (99.9% susceptibility), and 32 isolates (2.0%) were considered non-susceptible to teicoplanin (Tables 1 and 2). The majority of isolates with non-susceptible teicoplanin MICs were CoNS (Table 1). Quinupristin/dalfopristin was very active against staphylococci and pneumococci (>99% susceptibility), but this compound showed poor activity against *Enterococcus* spp. (3.4% susceptibility) due to intrinsic resistance among *E. faecalis*.

Thirty-four percent of *S. aureus* isolates were resistant to oxacillin, and the vast majority of oxacillin-resistant *S. aureus* (ORSA) isolates showed cross-resistance to clindamycin, ciprofloxacin, trimethoprim/

sulfamethoxazole, and gentamicin. The rate of oxacillin resistance was much higher among CoNS (>80%), and only linezolid and vancomycin were active against more than 90% of the strains evaluated.

Penicillin was active against 76.3% of *S. pneumoniae* isolates at the susceptible breakpoint (MIC ≤0.06 mg/mL). The majority of penicillin non-susceptible strains showed low level resistance (MIC 0.12-1 mg/mL) and only 3.4% of isolates showed high level resistance (MIC ≥2 mg/mL). Trimethoprim-sulfamethoxazole showed poor activity against pneumococci (MIC_{90}, 4 mg/mL) and inhibited only 50.8% of isolates at the susceptible breakpoint.

Discussion

The emergence of antimicrobial resistance among Gram-positive species has been rapid and alarming [19]. The staphylococci have always demonstrated a remarkable ability to develop resistance to each new molecular entity of antimicrobials, and to spread effectively among patients, institutions, and communities [20]. Resistance to penicillinase-resistant penicillins (PRPs, oxacillin or methicillin) has become widespread in *S. aureus* and CoNS at many medical centers and in most nations [19, 21]. Staphylococci that are resistant to PRPs are frequently resistant to other antimicrobials including the cephalosporins, tetracyclines, macrolides, lincosamides, aminoglycosides, and sulfonamides. Although initially very susceptible to fluoroquinolones, oxacillin-resistant staphylococci (ORS) strains have demonstrated a rapid development of resistance to these agents, particularly when they have been used as monotherapy [22].

The glycopeptides, vancomycin or teicoplanin, remain the preferred therapy for serious infections with ORS. At the present time, high-level resistance has not been reported in a clinical isolate of *S. aureus*, although this vancomycin-resistance genome of *Enterococcus* has been passed into a strain of ORSA [23]. *S. aureus* strains with reduced susceptibility to teicoplanin have been reported regularly from Europe and the USA [19]. Resistance to teicoplanin is usually encountered among *S. haemolyticus* and *S. epidermidis* [7]. Vancomycin resistance is uncommon, but low level resistance has been recently described in isolates of *S. aureus* in the USA and Japan [1].

Vancomycin-resistant enterococci (VRE) began to be recognized in the late 1980s, and has been reported worldwide [2, 19]. Increasing prevalence of VRE have been documented among patient populations at risk, institutions, and geographic areas and very few therapeutic options remain for treatment of multiply-resistant enterococci [2, 21]. Glycopeptide resistance rates are still very low among enterococci in Brazil, and vancomycin was active against the vast majority of Gram-positive cocci evaluated in the present study (Table 2). However, several hospitals have already reported cases of VRE infections, and both intra- and inter-hospital dissemination of this pathogen has been documented in Sao Paulo [3, 10, 24]. In addition, the vast majority of VRE reported in Sao Paulo are also resistant to teicoplanin [10].

Other antimicrobial agents that could be used as empiric therapy for Gram-positive infections were evaluated. The newer quinolone, gatifloxacin, showed a similar general spectrum to that of quinupristin/dalfopristin (Table 2). However, ORS shows some resistance to this compound, and the gatifloxacin clinical efficacy against infections caused by ciprofloxacin-resistant staphylococci still must be evaluated in large clinical trials.

Another important therapeutic concern has been the increasing rates of b-lactam resistance among *S. pneumoniae* and other streptococci. Penicillin resistance remains relatively low in Brazil. However, some recent studies have shown an important increase in prevalence of penicillin-resistant strains, which usually show cross-resistance with other compounds, except the newer quinolones [6, 25].

As a result of the increase in antimicrobial resistance among Gram-positive bacteria, new compounds have been developed. Linezolid is one of the most promising drugs [17]. In the present study, linezolid was the only compound that showed *in vitro* activity against 100% of the tested organisms at the susceptible breakpoint (Tables 1 and 2). The other new compound evaluated, quinupristin/dalfopristin, had poor activity against *E.*

Table 1. Antimicrobial activity and spectrum of linezolid and other antimicrobial agents used to treat Gram-positive infections according to the species

Antimicrobial agents	Pathogen (no. tested)							
	S. aureus (852)		CoNS (261)		*Enterococcus* spp. (147)		*S. pneumoniae* (325)	
	MIC_{50}/MIC_{90}	% Susc.	MIC_{50}/MIC_{90}	% Susc.	MIC_{50}/MIC_{90}	% Susc.	MIC_{50}/MIC_{90}	% Susc.
Linezolid	2/4	100.0	1/2	100.0	2/2	100.0	1/1	100.0
Quinupristin/dalfopristin	0.25/0.5	99.8	0.25/1	98.9	8/>8	3.4	0.5/0.5	100.0
Penicillin	16/>32	9.2	16/>32	8.1	2/16	89.1	0.03/0.25	76.3
Ampicillin	16/>16	9.9	16/>16	12.6	1/4	98.0	NT	NT
Oxacillin	0.5/>8	66.0	>8/>8	19.9	>8/>8	NA	NT	NT
Erythromycin	1/>8	45.3	>8/>8	39.1	>8>8	6.1	0.25/1	88.0
Clindamycin	0.25/>8	66.5	>8/>8	46.4	>8/>8	NA	≤0.06/0.25	95.7
Ciprofloxacin	0.5/>2	65.6	2/>2	49.8	2/>2	49.0	1/2	NA
Gatifloxacin	0.12/4	89.3	0.5/2	92.7	0.5/>4	73.5	0.25/0.5	100.0
Trimethoprim/ sulfamethoxazole	≤0.5/>2	68.0	2/>2	40.2	≤0.5/2	76.9	0.5/4	50.8
Tetracycline	≤4/>8	62.3	≤4/>8	75.1	>8/>8	35.4	≤2/>16	65.2
Gentamicin	1/>16	64.8	16/>16	41.4	≤500/>1000	72.8	NT	NT
Teicoplanin	1/2	99.8	2/16	88.9	0.25/0.5	99.3	NT	NT
Vancomycin	1/1	100.0	1/2	100.0	1/2	99.3	0.25/0.5	100.0

Abbreviations: NA: There are no breakpoints defined by NCCLS [ ]; NT: Not tested.

Table 2. Antimicrobial activity and spectrum of linezolid and other antimicrobial agents against all Gram-positive cocci evaluated

Antimicrobial agents	Gram-positive cocci (1585)		
	MIC_{50} (mg/mL)	MIC_{90} (mg/mL)	% Susceptible
Linezolid	2	4	100.0
Quinupristin / dalfopristin	0.5	1	90.7
Gatifloxacin	0.25	2	90.6
Penicillin	8	>32	30.1
Ampicillin	8	>16	32.1
Erythromycin	0.5	16	49.4
Tetracycline	4	16	62.4
Trimethoprim / sulfamethoxazole	0.5	4	60.7
Vancomycin	1	2	99.9

faecalis, which is the most frequently isolated *Enterococcus* species in Brazilian hospitals. In addition, resistance to quinupristin/dalfopristin has been demonstrated among *E. faecium* isolated in Brazilian hospitals [3, 10].

In addition to the excellent *in vitro* Gram-positive activity reported in this study and in several other investigations, intravenous and oral linezolid has produced high rates of clinical success in clinical trials involving hospitalized patients with skin or soft tissue and pneumonia [26]. Preliminary clinical data also indicate that twice daily intravenous or oral linezolid 600 mg is as effective as intravenous vancomycin in the treatment of patients with hospital-acquired pneumonia and in those with infections caused by ORS. Linezolid dosing at 600 mg twice daily produced >85% clinical/ microbiological cure in VRE infections [26]. Nevertheless, more data are needed on efficacy in immunosuppressed patients and for other conditions, notably endocarditis, where bactericidal activity may be necessary.

The results of our study, coupled with the results on clinical efficacy demonstrated in other studies [17, 26], indicate that linezolid may represent an excellent therapeutic option to treat infections due to Gram-positive cocci in Brazil; in particular those caused by multiresistant strains.

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