Comparative activity of linezolid against *staphylococci* and *enterococci* isolated in Italy

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The activity of linezolid, a new oxazolidinone, was tested against 862 Gram-positive cocci isolated in Italy and compared with the activities of 12 antibiotics. Overall, MIC_{90}s for linezolid (2–4 mg/L) indicated an in vitro activity comparable to that of vancomycin in methicillin-resistant *Staphylococcus aureus* (4 mg/L), *S. epidermidis* (2 mg/L) and methicillin-susceptible strains. *Enterococcus faecalis* strains were susceptible to linezolid (MIC_{90} 2–4 mg/L), glycopeptides and β-lactams. In *E. faecium*, only glycopeptides (MIC_{90} 2 mg/L) and linezolid (MIC_{90} 2 mg/L) were active. Linezolid was the only drug active against two strains of *Enterococcus* showing a VanA phenotype. Owing to its antibacterial profile, linezolid represents a promising drug for the treatment of Gram-positive infections.

Keywords In vitro activity, linezolid, staphylococci, enterococci

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Resistance to antimicrobial drugs is a global problem. Multiresistant pathogens are distributed worldwide, and the threat of spread of antibiotic resistance is increasing. As a consequence, the efficacy of available antibiotics is decreasing, tipping the balance in favor of multiresistant pathogens [1–3].

For patients infected with these resistant organisms, effective antimicrobial therapy has become exceedingly difficult to practice. As a result, new antimicrobial agents possessing unique mechanisms of action are urgently needed to manage infections caused by these resistant strains. Linezolid is a member of a new class of antibacterials, the oxazolidinones, that are chemically unrelated to currently available agents [4]. Linezolid is available for both oral and parenteral usage, and is highly active against Gram-positive organisms; resistance is seldom selected in vitro [5]. Since the potency of a drug may be influenced by the nature of the epidemiologic environment into which it is introduced, the activity of linezolid was assessed against recently isolated enterococci and staphylococci, isolated from different clinical specimens in high-risk wards and collected in clinical microbiology laboratories in Italy during 1999.

Eight hundred and sixty-two Gram-positive cocci comprising 426 methicillin-resistant *Staphylococcus aureus* (MRSA) strains, 83 methicillin-susceptible *Staphylococcus aureus* (MSSA) strains, 80 methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains, 22 methicillin-susceptible *Staphylococcus epidermidis* (MSSE) strains, 24 coagulase-negative staphylococcal (CoNS) strains, 200 *Enterococcus faecalis* strains and 27 *E. faecium* strains, freshly isolated from clinical specimens, were collected from 29 microbiological laboratories distributed throughout Italy over a 3-month period during 1999. Following a protocol agreed upon by all participants, each center was asked to provide to the reference centers (Laboratories of Microbiology, University of Catania and Genoa, Italy) staphylococcal and enterococcal strains isolated in high-risk wards (hematology, surgery and intensive care units) from the following specimens: blood, urine, prostatic massages, cerebrospinal fluids, catheters, bronchoalveolar lavages, sputa and pus. Only one isolate per patient was accepted and tested.

Repetitive strains were discarded, and all pathogens were re-identified to species level. All strains were stored at −80 °C until use. Along with the clinical isolates, the reference quality control strains *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used.

Susceptibility tests were performed by microdilution, following NCCLS guidelines [6,7]. Antibiotics were supplied by the manufacturers: linezolid and clindamycin (Pharmacia & Upjohn, Milan, Italy), amoxicillin–clavulanate (Smith Kline Beecham, Milan, Italy), vancomycin (Eli Lilly, Sesto Fiorentino, Italy), teicoplanin (Aventis Pharma, Milan, Italy), and ciprofloxacin (Bayer, Milan, Italy). Gentamicin, erythromycin, chloramphenicol, rifampin, streptomycin, oxacillin and penicillin were purchased from Sigma-Aldrich (Milan, Italy).

Overall, staphylococci and enterococci were most frequently isolated from blood cultures (31.3%), followed by urine and lower respiratory tract infection specimens (17.6% and 16.6%, respectively). About 10% of the isolates were associated with intravascular device implants. Methicillin-resistant staphylococci, *Staphylococcus aureus* and coagulase-negative species account for 82.4% of all staphylococcal isolates in high-risk wards in Italy in the period included in the study. Methicillin-resistant strains were more frequently isolated from blood cultures and lower respiratory tract infection specimens. In Tables 1–3, the activities of linezolid and comparator agents against the 523 methicillin-resistant staphylococci, expressed in terms of MIC_{50}, MIC_{90}, and percentages of resistance, are shown. Overall, MIC_{90} values for linezolid (2–4 mg/L) indicate in vitro potency

Table 1 In vitro activity of linezolid compared with 12 antimicrobial agents (mg/L) against 426 methicillin-resistant *Staphylococcus aureus* strains

Antimicrobial	Range	MIC_{50}	MIC_{90}	% R
Linezolid	0.5–4	2	4	–
Penicillin	0.06 to >4	>4	>4	99.3
Imipenem	<0.5 to >64	64	>64	89.4
Amoxicillin–clavulanate	<0.5 to >64	64	>64	99.5
Vancomycin	<1–2	<1	2	0
Teicoplanin	<1–8	2	8	0
Gentamicin	<0.5 to >64	>64	>64	97.4
Erythromycin	<0.25 to >32	>32	>32	90.3
Clindamycin	<0.25 to >32	>32	>32	98.1
Ciprofloxacin	<0.25 to >32	>32	>32	93.9
Chloramphenicol	2 to >128	16	128	68.5
Rifampin	<0.5 to >64	8	>64	57.3
Co-trimoxazole	<0.5 to >64	<0.5	16	11.7

R, resistance.

Table 2 In vitro activity of linezolid compared with 12 antimicrobial agents (mg/L) against 80 methicillin-resistant *Staphylococcus epidermidis* strains

Antimicrobial	Range	MIC_{50}	MIC_{90}	% R
Linezolid	0.5–4	2	2	–
Penicillin	1 to >4	>4	>4	100
Imipenem	<0.5 to >64	32	64	78.7
Amoxicillin–clavulanate	<0.5 to >64	32	64	88.7
Vancomycin	<1–4	<1	2	0
Teicoplanin	<1–8	4	8	0
Gentamicin	<0.5 to >64	64	>64	80.0
Erythromycin	<0.25 to >32	>32	>32	95.0
Clindamycin	<0.25 to >32	>32	>32	95.0
Ciprofloxacin	<0.25 to >32	32	>32	73.7
Chloramphenicol	<1 to >128	64	>128	57.5
Rifampin	<0.5 to >64	2	>64	52.5
Co-trimoxazole	<0.5–64	1	64	45.0

R, resistance.

Table 3 In vitro activity of linezolid compared with 12 antimicrobial agents (mg/L) against 17 methicillin-resistant coagulase-negative *Staphylococcus* strains

Antimicrobial	Range	MIC_{50}	MIC_{90}	% R
Linezolid	0.5–4	1	4	–
Penicillin	1 to >4	>4	>4	100
Imipenem	<0.5 to >64	>64	>64	64.7
Amoxicillin–clavulanate	<0.5 to >64	32	64	70.6
Vancomycin	<1–2	<1	2	0
Teicoplanin	<1–64	4	8	5.8
Gentamicin	<05 to >64	>64	>64	94.1
Erythromycin	0.5 to >32	>32	>32	94.1
Clindamycin	<0.25 to >32	>32	>32	64.7
Ciprofloxacin	0.25 to >32	32	>32	94.1
Chloramphenicol	<1 to >128	4	128	41.2
Rifampin	0.5 to >64	1	>64	41.2
Co-trimoxazole	<0.5 to >64	16	64	52.9

R, resistance.

comparable to that of vancomycin and teicoplanin in MRSA (2 and 8 mg/L), MRSE (2 and 8 mg/L) and MRCoN strains (2 and 8 mg/L). Methicillin-resistant staphylococci were significantly resistant to all antibiotics tested, with the exception of glycopeptides and linezolid. Among MRSA strains, 90.3% were resistant to erythromycin, 68.5% to chloramphenicol, 97.4% to gentamicin, 93.9% to ciprofloxacin and 11.7% to co-trimoxazole. Comparable data, with slight variations in the percentages of resistance to co-trimoxazole and chloramphenicol, were obtained for MRSE and MRCoN strains. Linezolid showed excellent activity against the 112 strains of methicillin-susceptible staphylococci, with MIC_{90} values 2–4 mg/L (data not shown). Against the same isolates, the remaining antibiotics also demonstrated good inhibitory activity.

The *E. faecalis* strains included in the study were generally susceptible to glycopeptides and β-lactams and showed an MIC_{90} value of 4 mg/L for linezolid (Tables 4 and 5). The strains were characterized by high-level resistance to streptomycin (62.5%) and gentamicin (46%), and were resistant to chloramphenicol and rifampin. Against *E. faecium*, only glycopeptides (MIC_{90} 2 mg/L) and linezolid (MIC_{90} 2 mg/L) were active. Over 92.6% of the strains were resistant to all β-lactams, and 40.7% to chloramphenicol; 55.5% displayed high-level resistance to streptomycin, and 22.2% to gentamicin. One strain of *E. faecalis* and one strain of *E. faecium*, both showing a VanA phenotype (confirmed by PCR), were susceptible only to linezolid.

In this study, the activity of linezolid was tested and compared with that of other useful drugs

Table 4 In vitro activity of linezolid (mg/L) compared with 12 antimicrobial agents against 200 *Enterococcus faecalis* strains

Antimicrobial	Range	MIC_{50}	MIC_{90}	% R	% HLR
Linezolid	0.5–4	1	4	–	–
Ampicillin	<0.5–64	1	4	7.5	–
Imipenem	<0.5 to >64	0.5	4	8.0	–
Vancomycin	<1–128	<1	4	1.0	–
Teicoplanin	<1–4	<1	<1	0	–
Erythromycin	0.5 to >32	32	>32	95.0	–
Clindamycin	2 to >32	>32	>32	100	–
Ciprofloxacin	0.25 to >32	4	>32	72.0	–
Chloramphenicol	2–128	8	64	45.5	–
Rifampin	0.25 to >32	2	16	44.5	–
Amoxicillin–clavulanate	<1–32	<1	2	2.5	–
Streptomycin	64 to >2048	–	–	–	62.5
Gentamicin	<8 to >1024	–	–	–	46.0

R, resistance; HLR, high-level resistance.

Table 5 In vitro activity of linezolid (mg/L) compared with 12 antimicrobial agents against 27 *Enterococcus faecium* strains

Antimicrobial	Range	MIC_{50}	MIC_{90}	% R	% HLR
Linezolid	0.5–4	2	2	–	–
Ampicillin	<0.5 to >64	64	64	62.9	–
Imipenem	<0.5 to >64	64	>64	62.9	–
Vancomycin	>1 to >128	<1	2	3.7	–
Teicoplanin	<1–16	<1	2	3.7	–
Erythromycin	2 to >32	>32	>32	100	–
Clindamycin	0.5 to >32	>32	>32	96.3	–
Ciprofloxacin	1 to >32	>32	>32	92.6	–
Chloramphenicol	2–64	8	32	40.7	–
Rifampin	<0.25–32	8	16	92.6	–
Amoxicillin–clavulanate	<1–128	8	32	51.8	–
Streptomycin	64 to >2048	–	–	–	55.5
Gentamicin	<8 to >1024	–	–	–	22.2

R, resistance; HLR, high-level resistance.

against a large number of staphylococci (635) and enterococci (227) isolated from high-risk wards by 29 microbiology laboratories, from all over Italy during 1999, minimizing multiple inclusion of local epidemic strains. Our study, performed in a restricted period of time (3 months), confirmed once more that the frequency of methicillin-resistant multiresistant staphylococci in Italy is an increasing problem. Methicillin-susceptible staphylococci were inhibited by the majority of antibiotics tested, while only linezolid, vancomycin and teicoplanin were active against methicillin-resistant strains. It is noteworthy that erythromycin and clindamycin were not uniformly active against MSSA, indicating that the constitutive mechanism of resistance to these drugs was more common among MRSA [8]. Vancomycin-intermediate *Staphylococcus aureus* (VISA) was not detected, and the frequency of hetero-VISA was not assessed in this study, but, importantly, linezolid has been shown by other authors to be active against vancomycin-intermediate staphylococci [9,10].

Vancomycin-resistant enterococci are still rare (0.5%) in Italy. During this survey, only one strain of *E. faecium* and one strain of *E. faecalis*, possessing the VanA phenotype and genotype, were isolated. However, our data highlight the general problem of treating enterococcal infections because of increasing resistance to several commonly prescribed antibiotics [10,11].

In Europe, vancomycin-resistant enterococci have a smaller impact than in the USA [1–10]. The extensive use of glycopeptides is responsible for their emergence, but differences in the prevalence of resistant strains noted in various countries cannot be explained so easily [12,13].

In conclusion, linezolid exhibited good activity against staphylococci and enterococci (MIC_{90} values of 1–4 mg/L), irrespective of their resistance to other drugs. These results are in agreement with data from other authors [4,14,15], in which the spectrum of activity and the unique mechanism of action of linezolid conferring absence of cross-resistance to other antimicrobial classes were underlined.

Information on the breakpoint for susceptibility of this drug is still not officially available, but the tentative value of >4 mg/L for resistance supported by pharmacokinetic data places all our strains in the susceptibility range [16].

Although the activity of glycopeptides against Gram-positive cocci seems quite reassuring in the collection of strains studied (98.5% and 99% of susceptible staphylococci and enterococci, respectively), and this situation seems to be confirmed by other local studies [10,17], this condition does not leave room for complacency, since the evolution of resistance cannot be easily predicted. As a consequence, evaluation of new drugs is essential. Among new anti-Gram-positive molecules, linezolid seems attractive because of its potential for wider use, being an oral agent active not only against MRSA, VISA and vancomycin-resistant enterococci, but also against important community-acquired pathogens such as *Streptococcus pneumoniae* (including penicillin-resistant strains) and *Streptococcus pyogenes* susceptible and resistant to macrolides [5].

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