

Antipneumococcal and Antistaphylococcal Activities of Ranbezolid (RBX 7644), a New Oxazolidinone, Compared to Those of Other Agents

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For 260 pneumococcal and 266 staphylococcal strains, ranbezolid MICs ranged from ≤ 0.06 to 4 $\mu\text{g/ml}$. The MICs for pneumococci were similar irrespective of the strains' β -lactam, macrolide, or quinolone susceptibilities, and ranbezolid MICs for coagulase-negative staphylococci were lower than those for *Staphylococcus aureus*. Ranbezolid was bacteriostatic against pneumococci. Ranbezolid MICs were similar to or lower than those of linezolid. Vancomycin and quinupristin-dalfopristin were also very active.

The incidence of pneumococci being resistant to penicillin G and other β -lactams and non- β -lactams has increased worldwide at an alarming rate, including in the United States (1, 5, 9). There is an urgent need for oral compounds for outpatient treatment of respiratory tract infections caused by resistant pneumococci (1, 5, 8). The emergence of methicillin- and quinolone-intermediate, and recently glycopeptide-intermediate, staphylococci, as well as the propensity of these organisms to cause serious systemic infections in immunocompromised hosts, also necessitates other therapeutic modalities (7, 12, 21).

The MICs of linezolid, an oxazolidinone which has been available clinically for the past few years, for pneumococci and staphylococci range between 0.5 and 4 $\mu\text{g/ml}$, irrespective of the organisms' resistance to other agents (2–4, 6, 10, 15, 18). Ranbezolid (RBX 7644; Ranbaxy Research Laboratories, New Delhi, India) is a new parenteral oxazolidinone with enhanced activity against gram-positive aerobes and gram-positive and gram-negative anaerobes.

The present study compared (i) the antipneumococcal activity of ranbezolid with those of linezolid, vancomycin, teicoplanin, quinupristin-dalfopristin, amoxicillin-clavulanate, ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and erythromycin by using MIC and time-kill studies and (ii) the antistaphylococcal activity of ranbezolid with those of linezolid, vancomycin, teicoplanin, and quinupristin-dalfopristin by using an MIC study.

The pneumococci tested comprised 89 penicillin-susceptible, 89 penicillin-intermediate, and 82 penicillin-resistant strains. Of these, 107 were erythromycin resistant. Twenty-six strains were quinolone resistant (levofloxacin MICs of ≥ 8 $\mu\text{g/ml}$). For time-kill studies, 12 penicillin-susceptible, -intermediate, and -resistant strains (four of each), including six macrolide-resistant and two quinolone-resistant strains, were tested. Sixty-eight methicillin-resistant and 65 methicillin-susceptible *Staphy-*

lococcus aureus strains and 69 methicillin-resistant and 64 methicillin-susceptible coagulase-negative staphylococci were examined.

Ranbezolid susceptibility powder was obtained from Ranbaxy Research Laboratories. Other antimicrobials were obtained from their respective manufacturers. For testing with pneumococci, agar dilution was performed by using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood (11). Methicillin MIC plates for staphylococci were incubated for a full 24 h (11).

For time-kill studies, tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (Difco) plus 5% lysed horse blood with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath. The methods employed in this study have been described previously (13, 19, 20).

Time-kill assays were analyzed by determining the number of strains which yielded a change in \log_{10} CFU per milliliter of -1 , -2 , and -3 at 0, 3, 6, 12, and 24 h compared with the counts at time zero. Antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by $\geq 3 \log_{10}$ CFU/ml (99.9%) at each of the time periods, and they were considered bacteriostatic if the inoculum was reduced by 0 to $< 3 \log_{10}$ CFU/ml. The problem of bacterial carryover was addressed by dilution as described previously (13, 19, 20). For erythromycin time-kill testing, only strains for which erythromycin MICs were ≤ 4.0 $\mu\text{g/ml}$ were tested.

Results of agar dilution MIC testing with strains classified by penicillin susceptibility are summarized in Table 1. Ranbezolid MICs (range, 0.06 to 2.0 $\mu\text{g/ml}$; MIC at which 50% of isolates tested are inhibited [MIC₅₀], 0.5 $\mu\text{g/ml}$; MIC at which 90% of isolates tested are inhibited [MIC₉₀], 1.0 $\mu\text{g/ml}$) were usually 2 to 3 dilutions lower than those of linezolid (range, 0.25 to 4.0 $\mu\text{g/ml}$; MIC₅₀, 1.0 $\mu\text{g/ml}$; MIC₉₀, 2.0 $\mu\text{g/ml}$) against all strains. All strains were also susceptible to vancomycin (MICs, 0.125 to 0.5 $\mu\text{g/ml}$), teicoplanin (MICs, ≤ 0.016 to 0.125 $\mu\text{g/ml}$), and quinupristin-dalfopristin (MICs, 0.125 to 1.0 $\mu\text{g/ml}$). The MICs

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TABLE 1. MICs (micrograms per milliliter) obtained by agar dilution of agents for 260 strains classified by penicillin susceptibility

Drug	Type of strain ^a	MIC range	MIC ₅₀	MIC ₉₀
Penicillin	S	≤0.008–0.06	0.016	0.06
	I	0.125–1.0	0.25	1.0
	R	2.0–16.0	2.0	4.0
Ranbezolid	S	0.06–1.0	0.25	1.0
	I	0.125–2.0	0.25	0.5
	R	0.06–2.0	0.5	1.0
Linezolid	S	0.25–4.0	1.0	2.0
	I	0.25–2.0	1.0	2.0
	R	0.5–4.0	1.0	2.0
Vancomycin	S	0.125–0.5	0.25	0.25
	I	0.125–0.5	0.25	0.25
	R	0.125–0.5	0.25	0.25
Teicoplanin	S	≤0.016–0.125	0.06	0.06
	I	≤0.016–0.125	0.06	0.06
	R	≤0.016–0.125	0.06	0.06
Quinupristin-dalfopristin	S	0.125–1.0	0.5	0.5
	I	0.125–1.0	0.5	0.5
	R	0.125–1.0	0.5	1.0
Amoxicillin-clavulanate	S	0.016–0.5	0.03	0.125
	I	0.03–4.0	0.25	2.0
	R	0.125–16.0	4.0	8.0
Ciprofloxacin	S	1.0–>32.0	2.0	4.0
	I	0.5–32.0	2.0	4.0
	R	0.5–>32.0	2.0	16.0
Levofloxacin	S	0.5–32.0	1.0	2.0
	I	0.5–16.0	1.0	2.0
	R	0.5–16.0	1.0	16.0
Gatifloxacin	S	0.25–8.0	0.5	1.0
	I	0.125–8.0	0.5	1.0
	R	0.125–8.0	0.5	4.0
Moxifloxacin	S	0.06–4.0	0.25	0.5
	I	0.06–4.0	0.125	0.25
	R	0.03–4.0	0.125	2.0
Erythromycin	S	≤0.008–>64.0	0.06	>64.0
	I	≤0.008–>64.0	0.06	>64.0
	R	≤0.008–>64.0	2.0	>64.0

^a S, penicillin susceptible; I, penicillin intermediate; R, penicillin resistant.

of β-lactams and macrolides increased with those of penicillin G. The results for all drugs tested, except penicillin G and amoxicillin-clavulanate, were similar when strains were analyzed according to erythromycin susceptibility. Moxifloxacin yielded the lowest quinolone MICs against quinolone-susceptible and -resistant strains.

The results of time-kill analysis are presented in Table 2. Ranbezolid and linezolid were mainly bacteriostatic, although bactericidal activity (99.9% killing) was detected against nine strains at four times the MIC after 24 h, with slower killing at earlier time periods. Vancomycin, teicoplanin, quinupristin-dalfopristin, amoxicillin-clavulanate, ciprofloxacin, gatifloxacin, and moxifloxacin were bactericidal against 11 of the 12 strains tested at the MIC after 24 h. Quinupristin-dalfopristin had the best kill kinetics of all drugs tested, with bactericidal

activity against 10 of the 12 strains tested at two times the MIC after 3 h and against all 12 strains at two times the MIC after 24 h. Erythromycin was bactericidal against 8 of 9 erythromycin strains, with MICs of ≤4.0 μg/ml at two times the MIC after 24 h. Regrowth was rarely found.

The MICs for staphylococci are listed in Tables 3 and 4. The MIC₅₀ and MIC₉₀ of ranbezolid against *S. aureus* were 2 and 2 μg/ml, respectively. By contrast, ranbezolid MICs against coagulase-negative strains were lower, with an MIC₅₀ of 0.125 μg/ml and an MIC₉₀ of 1 μg/ml. Linezolid MICs, especially against coagulase-negative strains, were often 1 to 2 dilutions higher than those of ranbezolid. Vancomycin MICs were low against all strains, but teicoplanin was much less active against coagulase-negative strains. Quinupristin-dalfopristin was equally active against all strains. The lower ranbezolid MICs for

TABLE 2. Kill kinetic results

Drug	Dose	No. of strains with indicated change in log ₁₀ CFU/ml ^a at:											
		3 h			6 h			12 h			24 h		
		-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3
Ranbezolid	4× MIC	7	2	0	11	3	1	12	11	3	12	12	9
	2× MIC	6	0	0	7	1	0	12	8	2	12	11	5
	MIC	4	0	0	5	0	0	10	5	0	10	8	3
Linezolid	4× MIC	6	2	0	11	4	0	12	10	1	12	12	9
	2× MIC	5	0	0	8	2	0	12	6	1	12	12	6
	MIC	3	0	0	3	1	0	9	5	0	11	7	2
Vancomycin	4× MIC	11	4	0	12	7	0	12	12	8	12	12	12
	2× MIC	11	4	0	12	6	0	12	12	8	12	12	12
	MIC	10	2	0	12	4	0	12	12	7	10	11	10
Teicoplanin	4× MIC	7	0	0	9	0	0	12	9	1	12	12	10
	2× MIC	6	0	0	7	0	0	12	8	1	12	12	10
	MIC	4	0	0	6	0	0	12	8	1	11	10	7
Quinupristin-dalfopristin	4× MIC	12	12	10	12	12	10	12	12	12	12	12	12
	2× MIC	12	12	10	12	12	10	12	12	12	12	12	12
	MIC	12	12	9	12	12	10	12	12	8	11	7	7
Amoxicillin-clavulanate	4× MIC	12	4	0	12	6	1	12	12	7	12	12	11
	2× MIC	12	4	0	12	6	1	12	12	7	12	12	11
	MIC	8	3	0	12	3	1	12	12	4	12	12	10
Ciprofloxacin	4× MIC	12	4	0	12	10	0	12	12	9	12	12	11
	2× MIC	11	1	0	12	8	0	12	12	8	12	12	11
	MIC	7	0	0	12	1	0	12	12	6	10	10	6
Levofloxacin	4× MIC	11	3	0	12	10	0	12	12	8	12	12	11
	2× MIC	11	2	0	12	9	0	12	12	8	12	12	11
	MIC	6	0	0	11	2	0	11	10	3	12	10	6
Gatifloxacin	4× MIC	12	4	0	12	10	0	12	12	8	12	12	11
	2× MIC	12	3	0	12	8	0	12	12	7	12	12	11
	MIC	7	0	0	12	3	0	12	10	4	12	12	7
Moxifloxacin	4× MIC	12	3	0	12	8	0	12	12	8	12	12	11
	2× MIC	10	0	0	12	6	0	12	12	7	12	12	11
	MIC	5	0	0	9	1	0	10	9	2	12	11	9
Erythromycin ^b	4× MIC	6 ^c	1	0	8	2	0	9	9	4	9	9	9
	2× MIC	6	0	0	7	2	0	9	8	2	9	9	8
	MIC	4	0	0	5	0	0	8	6	0	7	6	2

^a -1, 90% killing; -2, 99% killing; -3, 99.9% killing.

^b Only nine strains for which erythromycin MICs were ≤4.0 μg/ml were tested.

TABLE 3. Summary of MICs (micrograms per milliliter) for *S. aureus* strains

Drug	Methicillin-resistant <i>S. aureus</i> (n = 68)			Methicillin-susceptible <i>S. aureus</i> (n = 65)		
	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀
Ranbezolid	0.25–4	2	4	0.5–4	2	2
Linezolid	0.5–4	2	4	1–4	2	4
Vancomycin	0.5–2	1	1	0.5–2	1	1
Teicoplanin	0.125–16	0.5	1	0.125–4	0.5	1
Quinupristin-dalfopristin	0.125–1	0.5	1	≤0.06–1	0.5	0.5

coagulase-negative strains compared to those for *S. aureus* and the relative inactivity of teicoplanin against coagulase-negative staphylococci are both noteworthy. Oxazolidinone MICs were not influenced by the methicillin susceptibility of staphylococcal strains.

Ranbezolid is a new oxazolidinone with expanded activity against gram-positive cocci, fastidious gram-negative rods, and anaerobes (A. Rattan, A. Mehta, B. Das, M. Pandya, P. Bhatija, T. Mathur, S. Singhal, R. Sood, S. Malhotra, A. Yadav, A. Ray, R. Rao, and S. Rudra, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1288, 2002; D. Hoellman, L. Ednie, M. Jacobs, A. Rattan, and P. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1289, 2002; L. M. Kelly, D. Hoellman, M. Jacobs, A. Rattan, and P. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1290, 2002; L. Ednie, M. Jacobs, A. Rattan, and P. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1291, 2002; A. Rattan, M. Pandya, P. Bhatija, T. Mathur, R. Dhar, B. Das, and A. Mehta, 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1294, 2002). The results of the present study confirm the recently reported low MICs of ranbezolid against pneumococci and staphylococci (see above-cited abstracts). As is the case with other oxazolidinones (4), ranbezolid was bacteriostatic against most strains tested.

The results of MIC and time-kill studies of other compounds tested against pneumococci are similar to those described previously, with quinupristin-dalfopristin having the most rapid bactericidal activity, followed by quinolones, β -lactams, and macrolides (2, 3, 6, 9, 10, 13–20).

The results of this study indicate a potential role for ranbezolid in the treatment of pneumococcal and staphylococcal infections. However, interpretation of these in vitro results

TABLE 4. Summary of MICs (micrograms per milliliter) for coagulase-negative staphylococci

Drug	Coagulase-negative, methicillin-resistant staphylococci (n = 69)			Coagulase-negative, methicillin-susceptible staphylococci (n = 64)		
	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀
Ranbezolid	≤0.06–4	0.25	1	≤0.06–1	0.125	0.5
Linezolid	1–4	2	4	0.5–4	1	2
Vancomycin	0.125–4	2	2	0.125–2	1	2
Teicoplanin	≤0.06–>16	4	16	0.06–8	1	4
Quinupristin-dalfopristin	0.125–2	0.25	0.5	≤0.06–1	0.25	0.5

must be complemented by toxicity and pharmacokinetic-pharmacodynamic studies before the drug can be recommended for clinical testing.

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REFERENCES

- Appelbaum, P. C. 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. 15:77–83.
- Cercenado, E., F. Garcia-Garrote, and E. Bouza. 2001. In-vitro activity of linezolid against multiply resistant gram-positive clinical isolates. J. Antimicrob. Chemother. 47:77–81.
- Cuny, C., and W. Witte. 2000. In vitro activity of linezolid against staphylococci. Clin. Microbiol. Infect. 6:328–333.
- Diekema, D. J., and R. N. Jones. 2000. Oxazolidinones: a review. Drugs 59:7–16.
- Friedland, I. R., and G. H. McCracken, Jr. 1994. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. 331:377–382.
- Henwood, C. J., D. M. Livermore, A. P. Johnson, D. James, M. Warner, A. Gardiner, and the Linezolid Study Group. 2000. Susceptibility of gram-positive cocci from 25 UK hospitals to antimicrobial agents including linezolid. J. Antimicrob. Chemother. 46:931–940.
- Hiramatsu, K. 1998. The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. Am. J. Med. 104:7S–10S.
- Jacobs, M. R., and P. C. Appelbaum. 1995. Antibiotic-resistant pneumococci. Rev. Med. Microbiol. 6:77–93.
- Jacobs, M. R., S. Bajaksouzian, A. Zilles, G. Lin, G. A. Pankuch, and P. C. Appelbaum. 1999. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 US surveillance study. Antimicrob. Agents Chemother. 43:1901–1908.
- Livermore, D. M. 2000. Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? J. Antimicrob. Chemother. 46:347–350.
- National Committee for Clinical Laboratory Standards. 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard. NCCLS publication no. M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Nichols, R. L. 1999. Optimal treatment of complicated skin and soft tissue infections. J. Antimicrob. Chemother. 44:19–23.
- Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum. 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. 38:2065–2072.
- Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum. 1995. Comparative activity of ampicillin, amoxicillin, amoxicillin/clavulanate and cefotaxime against 189 penicillin-susceptible and -resistant pneumococci. J. Antimicrob. Chemother. 35:883–888.
- Patel, R., M. S. Rouse, K. E. Piper, and J. M. Steckelberg. 1999. In-vitro activity of linezolid against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. 34:119–122.
- Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum. 1992. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. 36:856–859.
- Spangler, S. K., M. R. Jacobs, G. A. Pankuch, and P. C. Appelbaum. 1993. Susceptibility of 170 penicillin-susceptible and -resistant pneumococci to six oral cephalosporins, four quinolones, desacetylcefotaxime, Ro 23–9424 and RP 67829. J. Antimicrob. Chemother. 31:273–280.
- Stevens, D. L., L. G. Smith, J. B. Bruss, M. A. McConnell-Martin, S. E. Duvall, W. M. Todd, and B. Hafkin. 2000. Randomized comparison of linezolid (PNU-100766) versus oxacillin-dicloxacillin for treatment of complicated skin and soft tissue infections. Antimicrob. Agents Chemother. 44:3408–3413.
- Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum. 1996. MIC and time-kill study of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-susceptible and -resistant pneumococci. Antimicrob. Agents Chemother. 40:362–366.
- Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum. 1997. Antipneumococcal activity of BAY 12–8039, a new quinolone, compared with activities of three other quinolones and four β -lactams. Antimicrob. Agents Chemother. 41:2786–2789.
- Voss, A., D. Milatovic, C. Wallrauch-Schwarz, V. T. Rosdahl, and I. Braveny. 1994. Methicillin-resistant *Staphylococcus aureus* in Europe. Eur. J. Clin. Microbiol. Infect. Dis. 13:50–55.