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Comparative In Vitro Activities of New 14-, 15-, and 16-Membered Macrolides

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The in vitro activities of several 14-, 15- and 16-membered macrolides were compared with that of erythromycin. In general, 14-membered macrolides such as erythromycin, clarithromycin, and flurithromycin were more active against streptococci and *Bordetella pertussis* than was the 15-membered macrolide azithromycin, which was more active than 16-membered macrolides such as miocamycin and rokitamycin. Clarithromycin was the most active compound against *Streptococcus pyogenes*, pneumococci, *Listeria monocytogenes*, and *Corynebacterium* species. *Legionella pneumophila* was most susceptible to miocamycin, clarithromycin, and rokitamycin. *Branhamella catarrhalis*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae* were most susceptible to azithromycin. Azithromycin and dirithromycin were the most active compounds against *Campylobacter jejuni*. MICs of 16-membered macrolides for strains expressing inducible-type resistance to erythromycin were ≤1 μg/ml, whereas none of the compounds had activity against strains expressing constitutive-type resistance. The MICs of roxithromycin, miocamycin, rokitamycin, and josamycin increased in the presence of human serum, whereas MICs of the other compounds either were unchanged or decreased.

Since its introduction in 1952, erythromycin has been used for treatment of infections caused by staphylococci, *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, and *Bordetella pertussis*. More recently, its use has been expanded to include the treatment of infections caused by *Legionella* spp., *Chlamydia trachomatis*, *Campylobacter jejuni*, and *Ureaplasma urealyticum*.

The renewed interest in erythromycin and the interest in new macrolides have been reviewed elsewhere (14; P. B. Fernandes, Antimicrob. Newsl. 4:25–34, 1987). Recent studies in these areas have focused on improving the chemical, biological, and pharmacokinetic properties of erythromycin. Research into the areas of 14-, 15-, and 16-membered macrolides has led to the discovery of new compounds with improvements in one or more of these properties. New compounds containing a 14-membered lactone ring with chemical modifications to enhance acid stability and prevent anhydro formation include clarithromycin (A-56268/TE-031) (6-*O*-methyl-erythromycin) (9, 15); A-62671 (14-hydroxy-6-*O*-methyl-erythromycin) (P. B. Fernandes and L. A. Freiberg, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 408, 1986); roxithromycin (RU28965) {9-[*O*-(2-methoxyethoxy)methyl-oxime-erythromycin]} (5); erythromycylamine [9-deoxo-(9S)-9-amino-erythromycin] (R. Maier, E. Woitun, B. Wetzel, W. Reuter, H. Goeth, and U. Lechner, U.S. patent no. 4,048,306, 1977); dirithromycin (AS-E 136) {9-deoxo-11-deoxy-9,11-[imino[2-(2-methoxy-ethoxy)ethylidene]-oxy]-(9S)-erythromycin} (Maier et al., U.S. patent no. 4,048,306); and flurithromycin (P-0501A) [(8S)-8-fluoro-erythromycin] (25). A-62671 is the major metabolite of clarithromycin in humans, and dirithromycin is a prodrug of erythromycylamine. Azithromycin (CP-62933/XZ-450) (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) (22), a new 15-membered compound, has nitrogen inserted into the lactone ring, which contributes basicity to the molecule and improves activity against gram-negative bacteria. Compounds containing 16-membered lactone rings include spiramycin (20), josamycin (19, 21, 24), miocamycin (9,3″-diacetyl-3,4″-dipropionyl-leucomycin V) (12), and rokitamycin (TMS-19-Q) (3″-*O*-propionyl-leucomycin A5) (23). Josamycin and spiramycin are currently in use in Europe and Japan, while the other compounds are currently in various stages of clinical development in this country or abroad. The structural differences between these compounds are shown in Fig. 1 and 2.

To compare the in vitro activities of these new compounds with the activities of erythromycin and of each other, we have tested them against the same organisms and by the same methodologies. In this paper, we describe their in vitro activities compared with that of erythromycin. The chemical and pharmacokinetic properties of these compounds and their activities in animal models of infection have been reviewed previously (Fernandes, Antimicrob. Newsl. 4:25–34, 1987).

(This work was presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy [D. J. Hardy, D. M. Hensey, E. J. McDonald, C. Vojtko, and P. B. Fernandes, 27th ICAAC, abstr. no. 225, 1987].)

MATERIALS AND METHODS

Bacterial strains. The strains used were clinical isolates received from several hospitals in the United States. Organisms for quality control were obtained from the American Type Culture Collection, Rockville, Md. *Staphylococcus aureus* RN1389 and RN1551, inducibly and constitutively resistant to erythromycin, respectively, were received from B. Weisblum. *Enterococcus faecalis* JH1 and DS16, genetically characterized as constitutively resistant to erythromycin, were received from D. LeBlanc (13). All strains were identified by standard procedures and maintained frozen at −60°C.

Antibacterial agents. Erythromycin, clarithromycin, A-62671, roxithromycin, erythromycylamine, dirithromycin, flurithromycin, azithromycin, and rokitamycin were prepared at Abbott Laboratories, Abbott Park, Ill. The other compounds and their manufacturers were josamycin (Phar-

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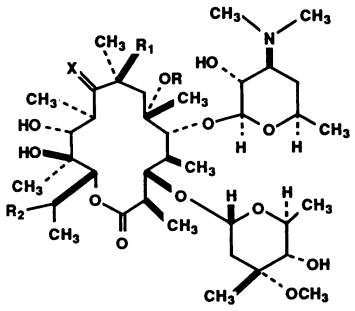
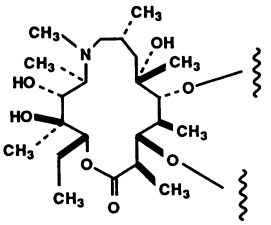


	X	R	R_1	R_2
Erythromycin	O	H	H	H
Clarithromycin	O	CH_3	O	H
A-62671	O	CH_3	H	OH
Flurithromycin	O	H	F	H
Roxithromycin	$NOCH_2O(CH_2)_2OCH_3$	H	H	H



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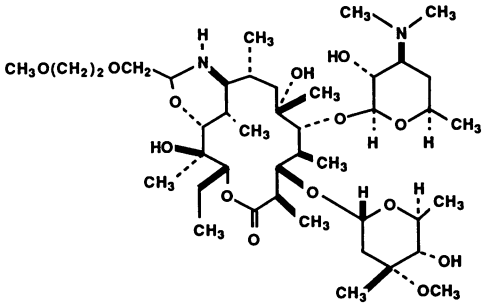

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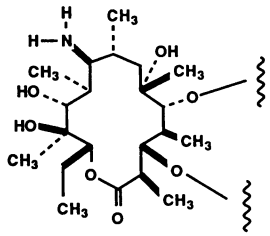
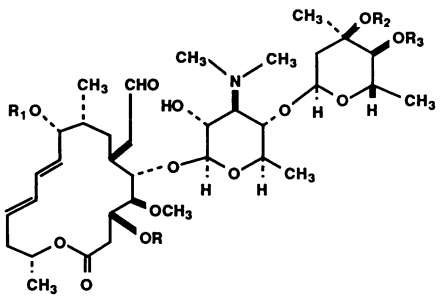


Erythromycylamine

FIG. 1. Chemical structures of 14-membered macrolides and the 15-membered macrolide azithromycin. All compounds have the same amino sugar (desosamine) and neutral sugar (cladinose).

muka, Gennevilliers, France), spiramycin (Specia, Paris, France), and miocamycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan). Stock solutions of clarithromycin containing 1,280 μg of drug per ml were prepared by dissolving 1 mg of compound in 140 μl of methanol and bringing the solution to volume in 0.1 M phosphate buffer (pH 6.8). Stock solutions of other compounds were prepared in methanol.

Susceptibility testing. MICs were determined by the agar or broth dilution method described by the National Committee for Clinical Laboratory Standards (17), with 1×10^4 CFU per spot or 5×10^4 CFU per well, respectively, as the inoculum. Organisms for quality control were included in each assay. Mueller-Hinton agar (MHA), pH 7.3, was used for staphylococci and enterococci. MHA supplemented with 5% (vol/vol) sheep blood was used for streptococci, *Branhamella catarrhalis* and *Corynebacterium* species. *Listeria monocytogenes* was tested in cation-supplemented Mueller-Hinton broth (MHB) supplemented with 3% (vol/vol) lysed horse blood. *B. pertussis* was tested on Bordet-Gengou agar supplemented with 15% (vol/vol) sheep blood and was incubated for 72 h. MHA supplemented with 3% (vol/vol) lysed horse blood and 0.001% NAD was used for *Haemophilus influenzae*. *Neisseria gonorrhoeae* was tested on pro-



	R	R_1	R_2	R_3
Spiramycin	H	Forosamine	H	H
Josamycin	$COCH_3$	H	H	$COCH_2CH(CH_3)_2$
Miocamycin	$COCH_2CH_3$	$COCH_3$	$COCH_3$	$COCH_2CH_3$
Rokitamycin	H	H	$COCH_2CH_3$	$CO(CH_2)_2CH_3$

FIG. 2. Chemical structures of 16-membered macrolides.

TABLE 1. Comparative in vitro activities of macrolides

Taxon (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (16)	Erythromycin	0.06–>128	0.12	>128
	Clarithromycin	0.03–>128	0.06	>128
	A-62671	0.03–>128	0.12	>128
	Roxithromycin	0.12–>128	0.25	>128
	Erythromycylamine	0.06–>128	0.12	>128
	Dirithromycin	0.12–>128	0.25	>128
	Flurithromycin	0.06–>128	0.12	>128
	Azithromycin	0.06–>128	0.12	>128
	Josamycin	0.5–64	1	8
	Spiramycin	0.25–64	1	16
	Miocamycin	0.5–4	1	1
	Rokitamycin	0.25–4	0.5	1
S. aureus, methicillin resistant (13)	Erythromycin	>128–>128	>128	>128
	Clarithromycin	>128–>128	>128	>128
	A-62671	128–>128	>128	>128
	Roxithromycin	>128–>128	>128	>128
	Erythromycylamine	128–>128	>128	>128
	Dirithromycin	128–>128	>128	>128
	Flurithromycin	128–>128	>128	>128
	Azithromycin	>128–>128	>128	>128
	Josamycin	2–>128	>128	>128
	Spiramycin	2–>128	>128	>128
	Miocamycin	1–>128	>128	>128
	Rokitamycin	0.5–>128	>128	>128
S. epidermidis (15)	Erythromycin	0.06–>128	8	>128
	Clarithromycin	0.03–>128	4	>128
	A-62671	0.03–>128	4	>128
	Roxithromycin	0.12–>128	32	>128
	Erythromycylamine	0.12–>128	16	>128
	Dirithromycin	0.06–>128	8	>128
	Flurithromycin	0.06–>128	16	>128
	Azithromycin	0.06–>128	16	>128
	Josamycin	0.25–>128	0.5	>128
	Spiramycin	0.12–>128	1	>128
	Miocamycin	0.25–>128	0.5	>128
	Rokitamycin	0.12–>128	0.25	>128
Streptococcus pyogenes (15)	Erythromycin	0.03–0.06	0.03	0.03
	Clarithromycin	0.015–0.015	0.015	0.015
	A-62671	0.015–0.03	0.015	0.03
	Roxithromycin	0.03–0.06	0.06	0.06
	Erythromycylamine	0.06–0.12	0.12	0.12
	Dirithromycin	0.03–0.12	0.12	0.12
	Flurithromycin	0.03–0.06	0.06	0.06
	Azithromycin	0.03–0.12	0.12	0.12
	Josamycin	0.06–0.25	0.12	0.25
	Spiramycin	0.06–0.12	0.12	0.12
	Miocamycin	0.25–0.5	0.5	0.5
	Rokitamycin	0.12–0.25	0.25	0.25
S. pneumoniae (13)	Erythromycin	0.03–0.03	0.03	0.03
	Clarithromycin	≤0.004–0.015	0.015	0.015
	A-62671	≤0.004–0.015	0.008	0.015
	Roxithromycin	0.03–0.03	0.03	0.03
	Erythromycylamine	0.06–0.12	0.06	0.06
	Dirithromycin	0.06–0.12	0.06	0.12
	Flurithromycin	0.03–0.06	0.03	0.06
	Azithromycin	0.03–0.12	0.06	0.12
	Josamycin	0.03–0.12	0.06	0.12
	Spiramycin	0.015–0.03	0.03	0.03
	Miocamycin	0.12–0.5	0.25	0.5
	Rokitamycin	0.12–0.12	0.12	0.12

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TABLE 1—*Continued*

Taxon (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
S. agalactiae (15)	Erythromycin	0.06–0.06	0.06	0.06
	Clarithromycin	0.03–0.06	0.06	0.06
	A-62671	0.03–0.06	0.06	0.06
	Roxithromycin	0.12–0.25	0.12	0.25
	Erythromycylamine	0.12–0.12	0.12	0.12
	Dirithromycin	0.12–0.25	0.25	0.25
	Flurithromycin	0.06–0.12	0.06	0.12
	Azithromycin	0.12–0.12	0.12	0.12
	Josamycin	0.25–0.25	0.25	0.25
	Spiramycin	0.12–0.25	0.12	0.25
	Miocamycin	0.5–0.5	0.5	0.5
	Rokitamycin	0.12–0.25	0.25	0.25
Streptococcus species, viridans group (13)	Erythromycin	0.015–0.12	0.06	0.06
	Clarithromycin	≤0.008–0.03	≤0.008	0.03
	A-62671	0.015–0.06	0.03	0.03
	Roxithromycin	≤0.008–0.03	0.03	0.03
	Erythromycylamine	0.03–0.12	0.12	0.12
	Dirithromycin	0.06–0.25	0.12	0.25
	Flurithromycin	0.015–0.06	0.03	0.06
	Azithromycin	0.015–0.12	0.06	0.12
	Josamycin	0.06–0.25	0.12	0.25
	Spiramycin	0.03–0.06	0.03	0.06
	Miocamycin	0.12–0.5	0.25	0.25
	Rokitamycin	0.06–0.12	0.12	0.12
Enterococcus species (15)	Erythromycin	0.06–>128	0.5	>128
	Clarithromycin	0.03–>128	0.5	>128
	A-62671	0.12–>128	0.25	>128
	Roxithromycin	0.25–>128	2	>128
	Erythromycylamine	0.25–>128	1	>128
	Dirithromycin	0.12–>128	1	>128
	Flurithromycin	0.25–>128	1	>128
	Azithromycin	0.25–>128	2	>128
	Josamycin	0.25–>128	2	>128
	Spiramycin	0.25–>128	0.25	>128
	Miocamycin	1–>128	2	>128
	Rokitamycin	0.5–>128	1	128
Corynebacterium species (12)	Erythromycin	0.008–16	1	16
	Clarithromycin	0.008–8	0.25	4
	A-62671	0.008–64	2	8
	Roxithromycin	0.015–32	4	16
	Erythromycylamine	0.015–64	1	8
	Dirithromycin	0.03–64	2	8
	Flurithromycin	≤0.015–32	2	16
	Azithromycin	0.008–>128	16	128
	Josamycin	0.06–64	4	32
	Spiramycin	0.06–128	16	128
	Miocamycin	0.12–>128	32	128
	Rokitamycin	0.06–16	8	16
Listeria monocytogenes (14)	Erythromycin	0.25–0.5	0.5	0.5
	Clarithromycin	0.25–0.5	0.25	0.25
	A-62671	0.25–0.5	0.5	0.5
	Roxithromycin	0.5–1	1	1
	Erythromycylamine	1–2	1	1
	Dirithromycin	1–2	2	2
	Flurithromycin	0.25–0.5	0.5	0.5
	Azithromycin	1–2	1	2
	Josamycin	2–2	2	2
	Spiramycin	4–4	4	4
	Miocamycin	2–2	2	2
	Rokitamycin	1–1	1	1

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TABLE 1—*Continued*

Taxon (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Branhamella catarrhalis (17)[a]	Erythromycin	0.03–0.25	0.12	0.25
	Clarithromycin	0.03–0.25	0.06	0.25
	A-62671	0.03–0.12	0.06	0.12
	Roxithromycin	0.12–1	0.25	1
	Erythromycylamine	0.06–0.25	0.12	0.25
	Dirithromycin	0.12–0.25	0.12	0.25
	Flurithromycin	0.06–0.25	0.12	0.25
	Azithromycin	0.03–0.06	0.03	0.06
	Josamycin	0.25–1	1	1
	Spiramycin	1–4	2	4
	Miocamycin	1–4	2	2
	Rokitamycin	0.12–0.25	0.12	0.25
Neisseria gonorrhoeae (15)[b]	Erythromycin	0.06–1	0.25	0.5
	Clarithromycin	0.06–0.5	0.25	0.5
	A-62671	0.06–1	0.25	0.5
	Roxithromycin	0.12–1	0.5	1
	Erythromycylamine	0.5–2	1	2
	Dirithromycin	0.5–4	2	4
	Flurithromycin	0.12–2	0.5	1
	Azithromycin	0.03–0.06	0.03	0.06
	Josamycin	0.25–2	0.5	2
	Spiramycin	0.25–2	1	1
	Miocamycin	0.5–>4	1	4
	Rokitamycin	0.12–1	0.5	1
Campylobacter jejuni (12)	Erythromycin	0.06–1	0.12	1
	Clarithromycin	0.12–2	0.25	2
	A-62671	0.25–2	0.5	2
	Roxithromycin	0.25–8	1	4
	Erythromycylamine	0.12–1	0.5	1
	Dirithromycin	0.06–0.5	0.25	0.25
	Flurithromycin	0.12–2	0.5	1
	Azithromycin	0.03–0.12	0.06	0.12
	Josamycin	≤0.03–2	0.25	1
	Spiramycin	0.25–2	0.5	2
	Miocamycin	0.5–>4	2	4
	Rokitamycin	0.12–2	1	2
Legionella pneumophila (14)	Erythromycin	0.5–2	1	2
	Clarithromycin	0.12–0.25	0.12	0.25
	A-62671	0.12–0.5	0.25	0.5
	Roxithromycin	0.12–0.5	0.25	0.5
	Erythromycylamine	1–8	4	8
	Dirithromycin	1–16	4	16
	Flurithromycin	0.5–2	1	2
	Azithromycin	0.12–2	0.5	2
	Josamycin	0.5–1	0.5	1
	Spiramycin	8–64	16	64
	Miocamycin	0.12–0.12	0.12	0.12
	Rokitamycin	0.12–0.25	0.25	0.25
Haemophilus influenzae (22)[c]	Erythromycin	0.5–4	4	4
	Clarithromycin	2–8	4	8
	A-62671	1–4	2	4
	Roxithromycin	2–8	4	8
	Erythromycylamine	4–8	8	8
	Dirithromycin	2–8	8	8
	Flurithromycin	4–8	8	8
	Azithromycin	0.12–0.5	0.25	0.5
	Josamycin	4–32	8	16
	Spiramycin	4–16	8	16
	Miocamycin	16–>16	>16	>16
	Rokitamycin	4–16	8	16

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TABLE 1—*Continued*

Taxon (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Bordetella pertussis (18)	Erythromycin	≤0.008–0.06	≤0.008	0.03
	Clarithromycin	≤0.008–0.06	≤0.008	0.03
	A-62671	≤0.008–0.06	0.015	0.06
	Roxithromycin	0.03–0.25	0.03	0.25
	Erythromycylamine	0.015–0.25	0.03	0.03
	Dirithromycin	0.03–0.12	0.03	0.03
	Flurithromycin	0.03–0.06	0.03	0.06
	Azithromycin	0.015–0.12	0.015	0.06
	Josamycin	≤0.015–0.25	≤0.015	0.25
	Spiramycin	0.12–0.5	0.12	0.25
	Miocamycin	0.06–0.25	0.06	0.25
	Rokitamycin	0.03–0.25	0.03	0.12
Bacteroides fragilis (10)	Erythromycin	2–>128	2	4
	Clarithromycin	1–>128	2	2
	A-62671	0.5–>128	1	1
	Roxithromycin	8–>128	16	32
	Erythromycylamine	32–>128	32	32
	Dirithromycin	128–>128	128	>128
	Flurithromycin	4–8	4	8
	Azithromycin	1–>128	2	2
	Josamycin	1–>128	2	8
	Spiramycin	4–>128	8	16
	Miocamycin	1–>128	2	4
	Rokitamycin	0.25–>128	0.25	0.25
Other *Bacteroides* species (10)[d]	Erythromycin	0.12–4	0.25	4
	Clarithromycin	0.06–2	0.25	2
	A-62671	≤0.015–2	0.12	1
	Roxithromycin	0.06–32	0.5	32
	Erythromycylamine	0.12–16	1	16
	Dirithromycin	0.5–64	8	64
	Flurithromycin	0.12–8	2	8
	Azithromycin	0.03–2	0.25	1
	Josamycin	0.06–8	0.25	2
	Spiramycin	0.12–8	0.5	8
	Miocamycin	≤0.015–8	0.12	8
	Rokitamycin	≤0.015–0.5	0.03	0.5
Clostridium perfringens (10)	Erythromycin	1–1	1	1
	Clarithromycin	0.25–0.5	0.5	0.5
	A-62671	0.5–0.5	0.5	0.5
	Roxithromycin	1–2	2	2
	Erythromycylamine	1–2	1	1
	Dirithromycin	2–4	4	4
	Flurithromycin	1–2	2	2
	Azithromycin	0.25–0.25	0.25	0.25
	Josamycin	1–1	1	1
	Spiramycin	2–2	2	2
	Miocamycin	0.5–1	0.5	0.5
	Rokitamycin	0.12–0.12	0.12	0.12
Propionibacterium acnes (12)	Erythromycin	0.03–128	0.03	0.03
	Clarithromycin	0.03–64	0.03	0.03
	A-62671	0.03–>128	0.03	0.06
	Roxithromycin	0.03–64	0.03	0.06
	Erythromycylamine	0.12–>128	0.25	0.5
	Dirithromycin	0.25–1	0.25	0.5
	Flurithromycin	0.03–128	0.06	1
	Azithromycin	≤0.004–4	≤0.004	0.03
	Josamycin	0.06–8	0.12	0.12
	Spiramycin	0.12–4	0.12	0.12
	Miocamycin	0.12–8	0.12	0.12
	Rokitamycin	0.03–2	0.06	0.06

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TABLE 1—*Continued*

Taxon (no. of strains)	Compound	MIC (μg/ml)		
		Range	50%	90%
Peptococcus-Peptostreptococcus species (12)	Erythromycin	0.03–>128	0.5	4
	Clarithromycin	0.015–>128	0.25	4
	A-62671	0.008–>128	1	4
	Roxithromycin	0.03–>128	1	32
	Erythromycylamine	0.03–>128	2	>128
	Dirithromycin	0.25–>128	2	128
	Flurithromycin	0.03–>128	1	16
	Azithromycin	0.03–>128	0.25	2
	Josamycin	0.12–16	0.25	8
	Spiramycin	0.06–32	0.25	32
	Miocamycin	0.008–16	0.25	8
	Rokitamycin	0.03–8	0.12	1

[a] Includes 15 β-lactamase-positive and 2 β-lactamase-negative strains.
[b] Includes 1 β-lactamase-positive and 14 β-lactamase-negative strains.
[c] Includes 11 type b and 11 non-type b strains and 7 β-lactamase-positive and 15 β-lactamase-negative strains.
[d] Includes four strains of *Bacteroides thetaiotaomicron*, two strains of *Bacteroides vulgatus*, and one strain each of *Bacteroides bivius*, *Bacteroides disiens*, *Bacteroides loescheii*, and *Bacteroides melaninogenicus*.

teose no. 3 agar supplemented with 1% bovine hemoglobin and 1% (vol/vol) Kellogg supplement. *Legionella pneumophila* was tested on buffered charcoal-yeast extract agar. *N. gonorrhoeae* and *L. pneumophila* were tested in the presence of 5% CO_2 and were incubated for 24 h; *H. influenzae* was tested in an ambient atmosphere. *C. jejuni* was tested on MHA and incubated in a microaerophilic atmosphere in gas jars (Campy-Pak system, BBL Microbiology Systems, Cockeysville, Md.). Anaerobic bacteria were tested on Wilkins-Chalgren agar by the dilution method described by the National Committee for Clinical Laboratory Standards (18), with 2×10^5 to 6×10^5 CFU per spot as the inoculum. All plates were incubated at 35°C.

Effect of serum and pH on in vitro activity. The effects of 50% human serum and pHs of 6.5, 7.2, and 8.0 on the in vitro activity of macrolides were determined by a broth microdilution method as previously described (9). To separate the effect of pH from the effect of serum, the pH of the serum was adjusted to 7.3 before testing. Staphylococci, streptococci, and *H. influenzae* were tested in MHB, brain heart infusion broth, and MHB supplemented with 3% (vol/vol) lysed horse blood and 0.001% NAD, respectively.

Time-kill studies. The bactericidal activities of macrolides over time against *S. aureus* 553 and *H. influenzae* 1435 (type b) were determined in MHB and brain heart infusion broth supplemented with 5% Fildes enrichment (Difco Laboratories, Detroit, Mich.), respectively. A 1-ml portion of log-phase cells was added to 250-ml flasks containing 19 ml of broth to yield final cell densities of approximately 5×10^5 to 1×10^6 CFU/ml. Compounds were added to separate flasks before inoculation to achieve final concentrations of four or eight times the MIC. Separate flasks without compound served as growth controls for the organisms. Flasks were incubated at 37°C. At 0.5, 1, 2, 4, 6, and 24 h, samples (0.5 ml) were aseptically removed and serially diluted in 10-fold increments in 0.1% Trypticase soy broth (BBL) for counting of viable cells on drug-free agar; to eliminate the effect of drug carry-over, a minimum dilution of 1:200 was used.

RESULTS

In vitro potency. The ranges of MICs of each of the compounds tested and the MICS required to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the test strains are presented in Table 1. The MIC_{90}s of 14- and 15-membered compounds for streptococci, *B. pertussis*, *Branhamella catarrhalis*, *Listeria monocytogenes*, and *Propionibacterium acnes* were generally within 1 to 2 twofold dilutions of those of erythromycin. The MIC_{90}s of clarithromycin, A-62671, roxithromycin, miocamycin, and rokitamycin for *L. pneumophila* were 2 to 4 twofold dilutions lower than that of erythromycin. The MIC_{90}s of clarithromycin for *Corynebacterium* species were 2 twofold dilutions lower than that of erythromycin. The MIC_{90}s of azithromycin for *N. gonorrhoeae* and *H. influenzae* were 3 twofold dilutions lower than those of erythromycin. The MIC_{90}s of dirithromycin and azithromycin for *C. jejuni* were 2 and 3 twofold dilutions lower, respectively, than that of erythromycin. MIC_{90}s of miocamycin and rokitamycin for methicillin-susceptible staphylococci were at least 7 twofold dilutions lower than that of erythromycin. MIC_{90}s of rokitamycin for anaerobes were 2 to 4 twofold dilutions lower than that of erythromycin.

The MICs of all compounds for three strains of *Streptococcus pyogenes*, two strains of *E. faecalis*, and one strain each of *S. aureus* and *Streptococcus pneumoniae* expressing constitutive-type resistance to erythromycin were ≥128 μg/ml. However, MICs of the 16-membered macrolides josamycin, spiramycin, miocamycin, and rokitamycin for three strains of *Streptococcus pyogenes* and one strain of *S. aureus* expressing inducible-type resistance to erythromycin were ≤1 μg/ml. The MICs of 14-membered macrolides and azithromycin for strains expressing inducible-type resistance ranged from 1 to 16 μg/ml.

Effect of serum. MICs of macrolides for two strains each of *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *H. influenzae* and for one strain of *Streptococcus pyogenes* were determined by a broth microdilution method in medium supplemented with 50% human serum; results for four strains are presented in Table 2. Compared with MICs determined in medium without serum, the MICs of roxithromycin, miocamycin, and rokitamycin were increased 1 to 4, 1 to 3, and 1 to 3 twofold dilutions, respectively. The MICs of josamycin were unchanged or increased 1 to 2 twofold dilutions, while the MICs of erythromycin, clarithromycin, A-62671, and flurithromycin were unchanged or decreased 1 to 2 twofold dilutions in the presence of serum. MICs of azithromycin were unchanged

or decreased 2 to 6 twofold dilutions, and those of spiramycin, erythromycylamine, and dirithromycin were decreased 1 to 4, 2 to 5, and 2 to 5 twofold dilutions, respectively.

Effect of pH. MICs of macrolides for one strain each of *S. aureus* and *Streptococcus pyogenes* were determined at pHs 6.5, 7.2, and 8.0. Compared with MICs determined at pH 7.2, MICs of miocamycin, josamycin, rokitamycin, and A-62671 were the same or 1 twofold dilution lower when tested at pH 8.0; MICs of erythromycin, clarithromycin, roxithromycin, flurithromycin, and spiramycin were 1 to 2 twofold dilutions lower when tested at pH 8.0; and MICs of erythromycylamine, azithromycin, and dirithromycin were 3 to 4 twofold dilutions lower when tested at pH 8.0. MICs of flurithromycin, josamycin, miocamycin, and rokitamycin tested at pH 6.5 were the same or 1 twofold dilution higher than those tested at pH 7.2; MICs of clarithromycin were 1 to 2 twofold dilutions higher; MICs of erythromycin, A-62671, roxithromycin, and spiramycin were 2 twofold dilutions higher; and MICs of erythromycylamine, azithromycin, and dirithromycin were 2 to 3 twofold dilutions higher.

Time-kill studies. The effects of macrolides over time on the viability of logarithmic-phase cultures of *S. aureus* 553 and *H. influenzae* 1435 were determined (Table 3). Rokitamycin and miocamycin were slowly bactericidal for *S. aureus* 553 (>6 h for 99.9 and 99% reduction, respectively, in viable counts). The other compounds had a bacteriostatic effect on strain 553. Rokitamycin was bactericidal (>99.9% reduction in viable counts) for *H. influenzae* 1435 within 2 h at four and eight times the MIC; A-62671, erythromycylamine, azithromycin, josamycin, spiramycin, and miocamycin were bactericidal against this strain within 4 h at four times the MIC; and clarithromycin was bactericidal within 4 h at eight times the MIC. Erythromycin, clarithromycin, roxithromycin, dirithromycin, and flurithromycin were bactericidal within 6 h at four times the MIC for strain 1435.

DISCUSSION

Because of strain variations and differences in test methodologies, it is sometimes difficult to compare the activities of new compounds on the basis of individual reports from the literature. In general, MICs from dilution susceptibility tests performed in agar are approximately 1 to 2 twofold dilutions lower than MICs from tests performed in the comparable broth medium. Different formulations of media, however, can produce results which differ to an even greater degree. Other variables affecting results include pH, osmolarity, presence of divalent cations, and redox potential of the medium used (R. M. Cozens and O. Zak, Antimicrob. Newsl. **4**:93–100, 1987). At least partly because of these variables, MIC_{90}s of erythromycin for *H. influenzae* have been reported to range from 2 to 16 μg/ml (7, 9). In our tests, MHA supplemented with lysed horse blood and NAD was used for susceptibility testing of *H. influenzae*. Although the current recommendation for testing the susceptibility of *H. influenzae* uses the broth equivalent of this medium (17), *Haemophilus* test medium may be recommended in the future (11). When tested in this medium, MIC_{90}s of erythromycin and clarithromycin for *H. influenzae* were the same or 1 twofold dilution lower than values obtained with either MHB or MHA supplemented with lysed horse blood and NAD (11; our unpublished observation). The MICs of macrolides for *L. pneumophila* are likewise medium dependent. It has been reported that charcoal in growth medium adversely affects the activity of macrolides, rifampin, and quinolones (2, 4, 8). The MIC_{90}s of erythromycin, clarithromycin, and roxithromycin for *L. pneumophila* when tested in a broth medium without charcoal were 0.25, ≤0.06, and 0.25 μg/ml, respectively (10), whereas MICs in the buffered charcoal-yeast extract agar used in our tests were 1 to 3 twofold dilutions higher.

We have observed that the addition of 5% defibrinated sheep blood to MHA for testing of streptococci and other fastidious organisms can result in elevation of pH from 7.3 to 7.6. At pH 7.6 in this medium after 16 to 18 h of incubation, MICs of azithromycin and dirithromycin for streptococci

TABLE 2. Effect of serum on in vitro activity of macrolides

Organism and compound	MIC (μg/ml)		Twofold-dilution difference
	Broth	Broth + serum	
Staphylococcus aureus 553			
Erythromycin	0.12	≤0.03	≥−2
Clarithromycin	0.12	≤0.03	≥−2
A-62671	0.25	0.12	−1
Roxithromycin	0.25	2	3
Erythromycylamine	0.5	0.03	−4
Dirithromycin	1	0.03	−5
Flurithromycin	0.5	0.12	−2
Azithromycin	0.25	≤0.004	≥−6
Josamycin	1	1	0
Spiramycin	2	0.25	−3
Miocamycin	2	8	2
Rokitamycin	1	2	1
Streptococcus pyogenes EES61			
Erythromycin	0.008	0.008	0
Clarithromycin	0.008	0.004	−1
A-62671	0.06	0.06	0
Roxithromycin	0.06	0.5	3
Erythromycylamine	0.12	0.03	−2
Dirithromycin	0.12	0.015	−3
Flurithromycin	0.06	0.03	−1
Azithromycin	0.03	0.002	−4
Josamycin	0.06	0.12	1
Spiramycin	0.12	0.015	−3
Miocamycin	2	4	1
Rokitamycin	0.25	2	3
Streptococcus pneumoniae 698			
Erythromycin	0.03	0.015	−1
Clarithromycin	0.03	0.015	−1
A-62671	0.06	0.03	−1
Roxithromycin	0.12	1	3
Erythromycylamine	0.12	0.008	−4
Dirithromycin	0.12	0.015	−3
Flurithromycin	0.06	0.06	0
Azithromycin	0.12	0.004	−5
Josamycin	0.12	0.25	1
Spiramycin	0.12	0.06	−1
Miocamycin	0.25	2	3
Rokitamycin	0.25	2	3
Haemophilus influenzae 1435			
Erythromycin	4	1	−2
Clarithromycin	4	2	−1
A-62671	4	1	−2
Roxithromycin	8	16	1
Erythromycylamine	8	0.25	−5
Dirithromycin	8	0.25	−5
Flurithromycin	4	1	−2
Azithromycin	1	0.12	−3
Josamycin	16	32	1
Spiramycin	64	16	−2
Miocamycin	32	>64	>1
Rokitamycin	8	64	3

TABLE 3. Effect of macrolides over time on viability

Organism and compound	Log decrease in viable counts after exposure for[a]:				
	1 h	2 h	4 h	6 h	24 h
Staphylococcus aureus 553					
Erythromycin	0.6 (0)	0.6 (0.2)	0.08 (0.9)	NT[b]	0.08 (0.3)
Clarithromycin	0.06 (0.3)	0.1 (0.5)	0.2 (−0.4)	NT	0.6 (1.5)
A-62671	−0.1 (−0.1)	−0.2 (−0.1)	−0.06 (0)	−0.3 (0.1)	−0.4 (−0.4)
Roxithromycin	−0.02 (0.08)	0.04 (0.09)	0 (−0.04)	0.1 (0.1)	1.8 (1.8)
Erythromycylamine	−0.1 (−0.07)	−0.1 (−0.07)	−0.01 (−0.1)	0.03 (0.2)	−0.4 (−0.4)
Dirithromycin	0.2 (0.16)	0.2 (0.2)	0.3 (0.3)	0.4 (0.5)	−0.4 (0.2)
Flurithromycin	0.2 (0.2)	0.2 (0.2)	0.2 (0.2)	0.4 (0.4)	0.4 (0.6)
Azithromycin	0.01 (0.04)	0.4 (0.4)	1.3 (1.6)	2.0 (1.9)	<0.6 (<0.6)
Josamycin	0.1 (0.4)	0.6 (0.9)	1.7 (1.9)	2.3 (2.5)	0.6 (0.2)
Spiramycin	0.03 (0.08)	0.1 (0.1)	0.07 (0.01)	0.5 (0.2)	NT (2.4)
Miocamycin	0.2 (0.2)	0.5 (0.3)	1.2 (1.3)	1.4 (1.5)	2.9 (2.9)
Rokitamycin	1.9 (1.7)	1.9 (2.0)	2.3 (2.5)	2.5 (2.7)	>3.6 (>3.6)
Haemophilus influenzae 1435					
Erythromycin	0.03 (0.03)	0.7 (0.7)	2.4 (2.9)	3.7 (>4)	>4 (>4)
Clarithromycin	0.17 (0.1)	1.2 (0.7)	2.3 (3.4)	3.8 (>4)	>4 (>4)
A-62671	0.09 (0.1)	1.8 (1.6)	3.6 (4.0)	>4 (>4)	>4 (>4)
Roxithromycin	0.08 (0.2)	1.1 (1.5)	1.7 (2.1)	3.1 (3.9)	>4 (>4)
Erythromycylamine	0.4 (0.4)	2.4 (2.1)	4.0 (3.4)	>4 (>4)	>4 (>4)
Dirithromycin	0.4 (0.4)	1.5 (1.0)	2.7 (2.2)	>3 (>3)	>4 (>4)
Flurithromycin	0.1 (0.1)	0.7 (0.6)	2.2 (2.2)	>3 (>3)	>4 (>4)
Azithromycin	0.1 (0.4)	1.5 (2.4)	>4 (>4)	>4 (>4)	>4 (>4)
Josamycin	0.6 (1.2)	2.3 (2.7)	>4 (>4)	>4 (>4)	>4 (>4)
Spiramycin	0.06 (0.4)	0.5 (1.2)	3.0 (3.8)	>4 (>4)	>4 (>4)
Miocamycin	0.2 (0.3)	1.9 (1.9)	4.0 (3.7)	>4 (>4)	>5 (>5)
Rokitamycin	2.0 (2.9)	3.5 (3.9)	>4 (>4)	>5 (>5)	>5 (>5)

[a] Determined after exposure to four times the MIC of each compound; log decrease after exposure to eight times the MIC is given in parentheses.
[b] NT, Not tested.

were 1 to 2 twofold dilutions lower than those at pH 7.3 after 24 h of incubation; MICs of erythromycin for streptococci under these two conditions were the same. The results presented here for streptococci were obtained in medium that was adjusted to pH 7.3 and were recorded after 24 h of incubation. Tests performed under these conditions produce results which, in general, agree with the results of others (3, 22).

New 14- and 15-membered macrolides had MICs which were generally within 2 twofold dilutions of those of erythromycin for streptococci, *B. pertussis*, and *P. acnes*. Sixteen-membered compounds were generally less active against streptococci and *B. pertussis*. Clarithromycin was the most active compound against *Streptococcus pyogenes*, pneumococci, *Listeria monocytogenes*, and *Corynebacterium* species. Miocamycin, clarithromycin, and rokitamycin were the most active compounds against *L. pneumophila*. Azithromycin was the most active compound against *Branhamella catarrhalis*, *N. gonorrhoeae*, and *H. influenzae*. Azithromycin and dirithromycin were the most active compounds against *C. jejuni*. Miocamycin and rokitamycin were the most active compounds against methicillin-susceptible *S. aureus*. Rokitamycin was the most active compound against anaerobes other than *P. acnes* and was, in general, the most active of the 16-membered compounds.

Only the 16-membered macrolides were active against strains expressing resistance of the inducible type. All compounds were inactive against strains expressing constitutive-type resistance to macrolides. In addition, all strains of methicillin-resistant *S. aureus* were also resistant to 14- and 15-membered macrolides. Five (38%) of thirteen methicillin-resistant *S. aureus* strains had inducible-type resistance to macrolides and were susceptible to 16-membered compounds, whereas the other eight (62%) of these strains had constitutive-type resistance to macrolides. Of the methicillin-susceptible *S. aureus*, 14 (88%) of 16 strains were susceptible to 14-, 15-, and 16-membered macrolides, whereas 2 (12%) of the strains had constitutive-type resistance. None of the methicillin-susceptible *S. aureus* had inducible-type resistance to macrolides.

The activities of roxithromycin, miocamycin, and rokitamycin were decreased by 1 to 4 twofold dilutions in the presence of 50% human serum, whereas the activity of josamycin was unchanged or decreased 1 to 2 twofold dilutions. The decreased activity of roxithromycin has been reported to result from saturable binding (82 to 93% at concentrations of roxithromycin ranging from 1 to 5 μg/ml) of the compound to acid α1-glycoprotein (1) with an affinity which exceeds that of erythromycin and of clarithromycin (6). The activities of erythromycin, clarithromycin, A-62671, and flurithromycin were generally enhanced to the same degree in serum, while the activities of erythromycylamine, dirithromycin, and azithromycin were enhanced to an even greater degree. Spiramycin was the only 16-membered macrolide with enhanced activity in serum. Whether these in vitro observations have significance in vivo remains unclear.

The bactericidal activity of rokitamycin has been related to the propionyl moiety at the 3″ position. Although rokitamycin was bactericidal in vitro, it is rapidly converted in vivo to metabolites that are bacteriostatic (16). Whether the observed in vitro bactericidal activities of any of these compounds against *H. influenzae* 1435 and streptococci (9, 22) have in vivo significance will depend on their pharmacokinetic properties at sites of infection.

The new compounds have spectra of activity that are similar to that of erythromycin. In general, the 14-membered

macrolides were as active or more active than erythromycin, whereas the 15-membered macrolide azithromycin was less active against gram-positive bacteria but more active against gram-negative bacteria. The 16-membered macrolides were generally less active than erythromycin. Advantages in pharmacokinetics of these compounds, however, may result in significant improvements in in vivo efficacies compared with the activity of erythromycin. Clinical trials will determine whether these compounds are more effective than erythromycin in treatment of infections in humans.

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