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In Vitro Activity of Josamycin Against Aerobic Gram-Positive Cocci and Anaerobes

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Josamycin, a new macrolide antibiotic, was compared with ampicillin, erythromycin, and clindamycin in vitro against 25 isolates each of pneumococci, enterococci, *Staphylococcus aureus*, *S. epidermidis*, and nonenterococcal hemolytic streptococci and against 25 anaerobes including 10 *Bacteroides fragilis*. Minimal inhibitory concentration and minimal bactericidal concentration data were obtained for the aerobic organisms, using serial twofold tube dilutions in Mueller-Hinton broth. Minimal inhibitory concentrations were determined for the anaerobes by the agar dilution technique. Josamycin was comparable to erythromycin and clindamycin in activity against the pneumococci, streptococci, and staphylococci and was more active than clindamycin against enterococci. It was somewhat less active than ampicillin against enterococci and *S. epidermidis* and showed its greatest in vitro activity against anaerobes, being comparable to clindamycin.

Josamycin is a new macrolide antibiotic produced by *Streptomyces narbonensis* var. *josamyceticus*. It has been reported to have antibacterial activity similar to that of other macrolides, such as erythromycin, and similar to that of clindamycin. Its antibacterial spectrum includes many of the aerobic gram-positive cocci and mycoplasma, and it has some activity against anaerobic organisms (6).

Josamycin appears to be well tolerated and without serious toxicity. Achievable concentrations in serum after oral administration of josamycin compare favorably with those obtained with erythromycin stearate; the average peak concentration is 1 to 2 μg/ml after a single oral dose of 600 mg (2, 4, 6).

Because of the potentially serious side effects and toxicities of the two currently available antibiotics that have good activity against anaerobes, namely, chloramphenicol and clindamycin, any new antibiotic that shows significant activity against common anaerobic pathogens may be worth investigation. Thus we studied the efficacy of josamycin in vitro against several different groups of organisms isolated from clinical specimens, including aerobic and anaerobic strains, and compared it with erythromycin, clindamycin, and ampicillin.

MATERIALS AND METHODS

Bacterial isolates. We tested 25 isolates each of enterococci, pneumococci, *Staphylococcus aureus*, *S. epidermidis*, and nonenterococcal hemolytic streptococci, which included 11 alpha-hemolytic and 14 group A beta-hemolytic streptococci. Twenty-five anaerobic isolates were also tested (Table 1). All aerobic and anaerobic organisms were isolated from human clinical specimens, and all were considered to be clinically significant pathogens. Enterococci were identified by characteristic appearance on PSE plates and by ability to grow in 6.5% saline and SF Media (Difco). Pneumococci were differentiated from other alpha-hemolytic streptococci by optochin disk susceptibility. Group A streptococci were identified by low-concentration bacitracin disk susceptibility. *S. aureus* isolates were differentiated from *S. epidermidis* by their ability to ferment mannitol anaerobically, by coagulase production, and by acid production on mannitol salt agar aerobically (1). Anaerobes were identified by the Virginia Polytechnic Institute method (7).

Susceptibility testing. Antibiotic susceptibility of the aerobic organisms was determined by serial twofold tube dilutions utilizing Mueller-Hinton broth. The enterococcal and staphylococcal inocula were prepared by using a 1:1,000 dilution of an overnight broth culture, with inoculum preparations averaging 2.3×10^5 organisms/ml. The pneumococcal and hemolytic streptococcal inocula were prepared from overnight cultures in Mueller-Hinton broth and were diluted using a spectrophotometer to give a concentration of 10^5 organisms/ml. A 0.1-ml amount of inoculum was pipetted into each tube containing 1 ml of a serial twofold dilution of the antibiotic. The tubes were incubated overnight, and the lowest concentration showing no turbidity was considered the minimal inhibitory concentration (MIC). An aliquot of 0.03 ml was taken by a calibrated loop from each of the clear tubes, streaked onto blood agar plates,

and incubated overnight. The lowest concentration representing 95% kill or showing less than five colonies on subculture was considered the minimal bactericidal concentration (MBC).

Susceptibility testing of the anaerobic bacteria was performed using the agar dilution technique (7, 8). The inocula were prepared in modified thioglycollate medium enriched with 5 μg of hemin per ml, one μg of $NaHCO_3$ per ml, and 0.5 μg of menadione per ml. The inocula were incubated for 6 h when fast-growing and overnight when slow-growing organisms were under test and then diluted in Brucella broth to a turbidity of a no. 1 MacFarland standard. The inoculum was applied with a modified Steers replicator to plates containing serial twofold dilutions of the antibiotic prepared with 5% laked sheep blood and 0.5 μg of menadione per ml. The plates were incubated at 37 C in GasPak jars, and the MICs were read at 48 h.

RESULTS

Table 2 summarizes the MIC and MBC data on josamycin obtained with all organisms tested. Tables 3, 4, and 5 show the same data for clindamycin, erythromycin, and ampicillin, respectively.

TABLE 1. *Anaerobic organisms tested*

Organism	No. of isolates
Bacteroides fragilis	10
B. capillosus	1
Acidaminococcus fermentans	1
Fusobacterium symbiosum	1
Peptococcus saccharolyticus	3
P. asaccharolyticus	1
Clostridium perfringens	5
C. tertium	1
Clostridium sp.	1
Peptostreptococcus micros	1

Pneumocci. Figure 1 shows the MIC data for 25 isolates of pneumococci. Josamycin showed good activity against 100% of the isolates; they were inhibited by 0.2 μg or less per ml. Clindamycin was the most active of the antibiotics; 100% were inhibited by 0.025 μg/ml. Erythromycin and ampicillin showed very similar results, with 100% of the isolates inhibited by 0.39 μg of ampicillin per ml and 0.78 μg of erythromycin per ml. The MBCs were determined (Tables 2 through 5), and josamycin at 0.39 μg/ml showed bactericidal activity against 100% of the isolates. Clindamycin at 0.05 μg/ml was bactericidal for 100% of the isolates, and ampicillin and erythromycin were bactericidal for 100% of the isolates at concentrations of 0.78 and 1.56 μg/ml, respectively.

Hemolytic streptococci. Eleven isolates of alpha-hemolytic streptococci and 14 isolates of group A beta-hemolytic streptococci were tested. Figure 2 shows the combined cumulative percentage of the organisms inhibited by each of the antibiotics. Josamycin showed good activity against the streptococci, with 100% inhibited by 0.39 μg/ml. Clindamycin and erythromycin were most active, with 100% of the isolates inhibited by 0.05 μg/ml. Ampicillin was intermediate in activity, with 100% of strains susceptible to 0.2 μg/ml. Josamycin and ampicillin in a concentration of 0.78 μg/ml were bactericidal for 100% of the isolates. Clindamycin and erythromycin at 0.2 μg/ml showed bactericidal activity for 100% of the isolates.

Enterococci. With clindamycin, erythromy-

TABLE 2. *Cumulative percentage of aerobic and anaerobic isolates inhibited (MIC) or killed (MBC) by varying concentrations of josamycin*

Isolate	Determination	% Inhibited or killed at concn (μg/ml) of:														
		0.006	0.012	0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.12	6.25	12.5	25	50	>100
Pneumococci	MIC	4			12	84	100									
	MBC				4	44	88	100								
Enterococci	MIC							8	28	76	80				84	100
	MBC								4	20	28	40	48	64	76	100
Streptococci	MIC				4	24	92	100								
	MBC					4	64	92	100							
S. aureus	MIC								28	76	96				100	
	MBC									8	48	72	80	84	88	100
S. epidermidis	MIC						8	12	20	88						100
	MBC									16	68	76		84	88	100
Anaerobes, all	MIC				16		24	36	84	96			100			
B. fragilis	MIC				10		30	50	90				100			

TABLE 3. *Cumulative percentage of various aerobic and anaerobic isolates inhibited (MIC) or killed (MBC) by varying concentrations of clindamycin*

Isolate	Determination	% Inhibited or killed at concn (μg/ml) of:														
		0.006	0.012	0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.12	6.25	12.5	25	50	>100
Pneumococci	MIC	36	72	100												
	MBC	16	44	88	100											
Enterococci	MIC											8	84			100
	MBC													32	40	100
Streptococci	MIC	12	16	76	100											
	MBC	8	16	24	76	88	100									
S. aureus	MIC				12	84	96									100
	MBC				4	16	20	32	48	68	76	92	96			100
S. epidermidis	MIC					88										100
	MBC					8	40	52	64	72	84	88				100
Anaerobes, all	MIC				40	48	64	68	88	96		100				
B. fragilis	MIC				30		60		90	100						

TABLE 4. *Cumulative percentage of various aerobic and anaerobic isolates inhibited (MIC) or killed (MBC) by varying concentrations of erythromycin*

Isolate	Determination	% Inhibited or killed at concn (μg/ml) of:														
		0.006	0.012	0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.12	6.25	12.5	25	50	>100
Pneumococci	MIC	88	60	88	92			96	100							
	MBC		16	52	80	92				100						
Enterococci	MIC					4		24	64	80			84			100
	MBC									4	24	48	68	80		100
Streptococci	MIC		24	76	100											
	MBC		12	24	68	88	100									
S. aureus	MIC					4	28	88				92	96	100		
	MBC						4	8	20	32	52	68	72	84	88	100
S. epidermidis	MIC					12	72	88								100
	MBC					4	8	28		32	44	60	80	88		100
Anaerobes, all	MIC					8	20	24	48	76	96		100			
B. fragilis	MIC								40	50	90		100			

cin, and josamycin, there were significant numbers of resistant organisms at achievable blood level concentrations (Fig. 3). At 1.56 μg/ml, 80% were inhibited by erythromycin and 76% were inhibited by josamycin. Clindamycin was least active, with 8% inhibited by 6.25 μg/ml. All isolates were inhibited by ampicillin at 3.12 μg/ml. At 1.56 μg/ml, erythromycin was bactericidal for only 4% and josamycin was bactericidal for 20% of strains. Clindamycin at that concentration was bactericidal for none of the isolates. Ampicillin was bactericidal for 52% at a concentration of 3.12 μg/ml.

Staphylococci. Figure 4 shows the data obtained with 25 isolates of *S. epidermidis*. Clindamycin was the most active, and 0.1 μg/ml inhibited 88% of strains. At 1.56 μg/ml, 88% of the isolates were inhibited by josamycin and 100% were inhibited by ampicillin. Erythromycin inhibited 88% of the isolates at 0.39 μg/ml. Twelve percent of these isolates were not inhibited by greater than 100 μg of clindamycin, erythromycin, or josamycin per ml. At 1.56 μg/ml, josamycin was bactericidal for 16%, clindamycin for 72%, erythromycin for 32%, and ampicillin for 84% of strains.

Clindamycin was more active in lower concentrations than erythromycin, josamycin, or ampicillin against *S. aureus* (Fig. 5). Ninety-six percent of strains were inhibited by clinda-

TABLE 5. *Cumulative percentage of various aerobic and anaerobic isolates inhibited (MIC) or killed (MBC) by varying concentrations of ampicillin*

Isolate	Determination	% Inhibited or killed at concn (μg/ml) of:														
		0.006	0.012	0.025	0.05	0.10	0.20	0.39	0.78	1.56	3.12	6.25	12.5	25	50	>100
Pneumococci	MIC	16	40	68	80	88	92	100								
	MBC	12	16	56	76	80	92	96	100							
Enterococci	MIC								24	96	100					
	MBC								8	40	52					100
Streptococci	MIC	8	24	64	72	96	100									
	MBC	8	12	56	64	96		100								
S. aureus	MIC					8	24	52	84	92	96		100			
	MBC					4	12	36	56	72		80	84	92	96	100
S. epidermidis	MIC					32	80	88		100						
	MBC					28	48	56	68	84	88	96	100			
Anaerobes, all	MIC				36	40	44	52	56	60	64	68	76	100		
B. fragilis	MIC									10		20	40	100		

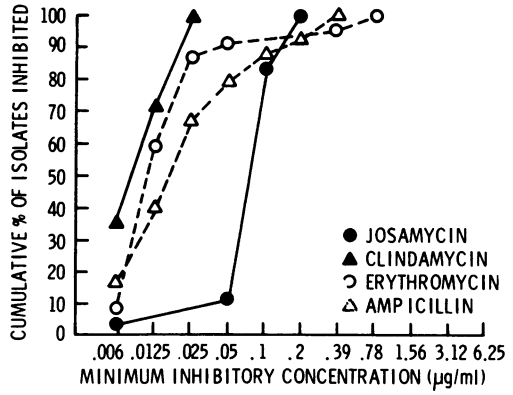

FIG. 1. *Susceptibility of 25 isolates of pneumococci to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

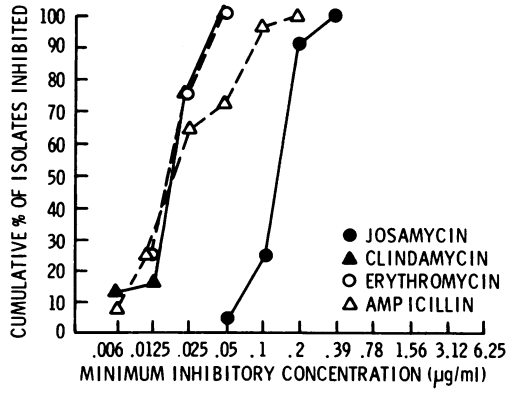

FIG. 2. *Susceptibility of 25 isolates of alpha- and beta-streptococci to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

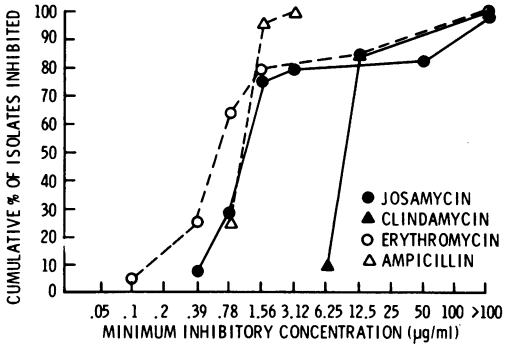

FIG. 3. *Susceptibility of 25 isolates of enterococci to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

mycin at 0.2 μg/ml, whereas 4% were resistant to greater than 100 μg/ml. Erythromycin inhibited 88% of the isolates at 0.39 μg/ml. Josamycin inhibited 76% at 1.56 μg/ml, whereas ampicillin inhibited 92% at 1.56 μg/ml. At a concentration of 1.56 μg/ml, clindamycin was bactericidal for 68%, ampicillin for 72%, erythromycin for 32%, and josamycin for 8%.

Anaerobes. Table 1 shows the identification of the anaerobic strains tested. A heterogeneous group was used, but 10 of the 25 isolates were *Bacteroides fragilis*. Figure 6 and Tables 2 through 5 summarize the antibiotic concentrations required for inhibition of all the anaerobic isolates. Josamycin and clindamycin were the most active, with 96% of the isolates inhibited by 1.56 μg of either antibiotic per ml. The one organism resistant to more than 1.56 μg of clindamycin per ml was a strain of *Clos-*

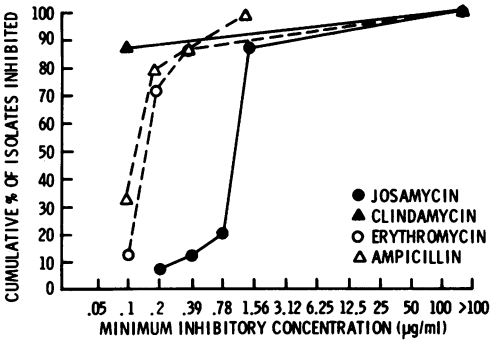

FIG. 4. *Susceptibility of 25 isolates of S. epidermidis to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

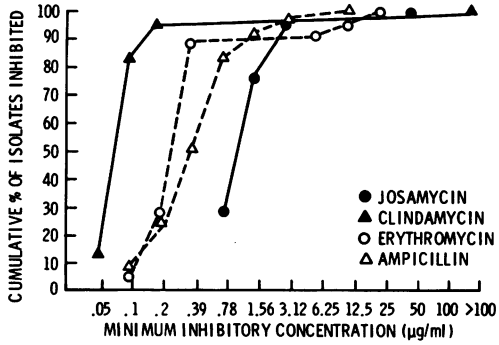

FIG. 5. *Susceptibility of 25 isolates of S. aureus to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

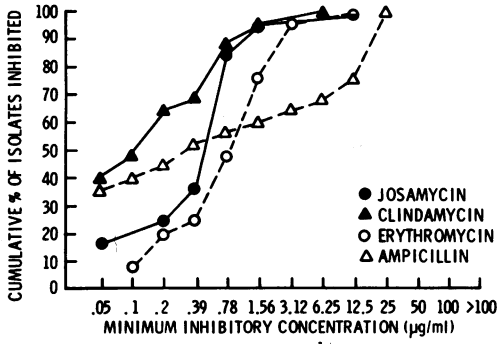

FIG. 6. *Susceptibility of 25 isolates of anaerobes to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

tridium tertium; its MIC to clindamycin was 6.25 μg/ml and its MIC to josamycin was 0.78 μg/ml. Erythromycin was less active; in a concentration of 1.56 μg/ml, it inhibited 76% of the strains. Ampicillin was less active and 40% of the strains were resistant to the same concentration.

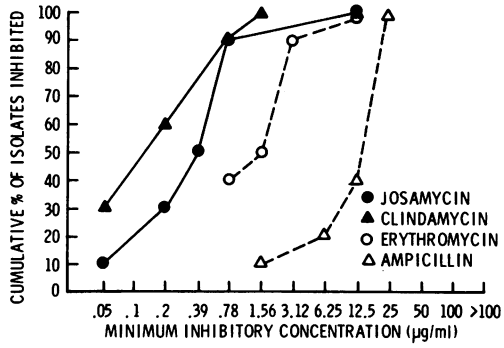

FIG. 7. *Susceptibility of 10 isolates of B. fragilis to varying concentrations of ampicillin, erythromycin, clindamycin, and josamycin.*

Since *Bacteroides* sp. is one of the more common clinical anaerobic pathogens, the data from the 10 *B. fragilis* isolates were extracted and analyzed separately (Fig. 7). Both clindamycin and josamycin were active against *B. fragilis,* and at a concentration of 0.78 μg/ml both inhibited 90% of the isolates. There was one relatively resistant strain that was susceptible to 1.56 μg of clindamycin per ml and 12.5 μg of josamycin or erythromycin per ml, but it was the only *B. fragilis* isolate that was sensitive to 1.56 μg of ampicillin per ml. Erythromycin was less active than clindamycin or josamycin; at 1.56 μg/ml it inhibited 50%. Ampicillin again was less active and only 10% were susceptible to 1.56 μg/ml.

DISCUSSION

Although josamycin has been tested extensively in Japan in vitro and in clinical trials, there has been very little work with this antibiotic in the United States or Europe (2, 3, 4). Its main use in countries where it is available for clinical use has been as an alternative to penicillins for infections caused by gram-positive cocci. However, very little information is available concerning the activity of josamycin against anaerobes either in vitro or in vivo.

Based on our own data, josamycin, however, appears to have good in vitro activity against a variety of aerobic gram-positive cocci. In general, at concentrations of the antibiotic achievable in vivo, josamycin is comparable to clindamycin and erythromycin in its ability to inhibit the growth of pneumococci and other hemolytic streptococci. Josamycin and erythromycin showed comparable activity against enterococci, and, although they both were ineffective against a significant percentage of resistant

strains, they appeared to be far superior to clindamycin in their in vitro activity. Josamycin was comparable to erythromycin and clindamycin in its activity against both *S. aureus* and *S. epidermidis*, although it was somewhat less active than ampicillin against *S. epidermidis* and enterococci. Josamycin, like the other macrolide antibiotics, was primarily bacteriostatic in vitro against aerobic organisms, as shown by the wide separation between MIC and MBC for most isolates.

It is known that the in vitro activity of macrolides in general and erythromycin specifically decreases with lower pH and increasing concentrations of CO_2 (5). The Japanese data suggest that this occurs with josamycin as well (6). Since our anaerobic testing was done under increased CO_2 tension, it is possible that the anaerobic isolates would show greater susceptibility to erythromycin and josamycin under physiological conditions than our data would indicate. Nevertheless, josamycin showed its greatest activity against anaerobic bacteria, being quite similar to clindamycin in vitro against clinically significant anaerobes, including *B. fragilis*. It showed significantly greater activity than either erythromycin or ampicillin against common anaerobic pathogens.

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LITERATURE CITED

1. **Baird-Parker, A. C.** 1965. Subcommittee on Taxonomy of Staphylococci and Micrococci. Int. Bull. Bacteriol. Nomencl. Taxon. 15:107-114.
2. **Bergan, T., and B. Oydvis.** 1972. Pharmacokinetics of josamycin: a new macrolide antibiotic. Pharmacology 7:36-50.
3. **Bergan, T., P. Tolas, and B. Oydvis.** 1972. Influence of food and hepatobiliary disease on the excretion of josamycin. Pharmacology 8:336-343.
4. **Fukushima, K.** 1969. Clinical study of josamycin, p. 750-754. *In* Progress in antimicrobial and anticancer chemotherapy. Proccedings of the Sixth International Congress of Chemotherapy, vol. 1.
5. **Haight, T. H., and M. Finland.** 1952. The antibacterial action of erythromycin. Proc. Soc. Exp. Biol. Med. 81:175-183.
6. **Mitsuhasi, S. (ed).** 1971. Drug action and drug resistance in bacteria. 1. Macrolide antibiotics and lincomycin, p. 41-102. University of Tokyo Press, Tokyo, Japan.
7. **Sutter, V. L., H. R. Attebery, J. E. Rosenblatt, K. S. Bricknell, and S. M. Finegold.** 1972. Anaerobic bacteriology manual, p. 45. The Regents of the University of California.
8. **Sutter, V. L., Y. Y. Kwok, and S. M. Finegold.** 1972. Standardized antimicrobial disc susceptibility testing of anaerobic bacteria. App. Microbiol. 23:268-275.