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In Vitro Comparison of Rosamicin and Erythromycin Against Urinary Tract Pathogens

THEODORE C. EICKHOFF* AND JOSEPHINE M. EHRET

Division of Infectious Disease, Department of Medicine, University of Colorado Medical Center, Denver, Colorado 80262

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The in vitro activity of rosamicin and erythromycin was compared at various pH values against 311 strains of bacteria representing common urinary tract pathogens. Alkalinization of the media consistently and significantly increased the antibacterial activity of rosamicin against all of the organisms tested. This was also true for erythromycin except when tested against strains of *Proteus*. At pH 8, rosamicin was two- to sixfold more active than erythromycin against *Enterobacteriaceae*. The activity of both antibiotics against *Pseudomonas aeruginosa* was very similar when tested at pH 8. Erythromycin was twice as active as rosamicin at pH 8 against group D streptococci. The activity of both antibiotics was bacteriostatic and inoculum size dependent, regardless of the organism tested or the pH of the test media. The greater activity of rosamicin against *Enterobacteriaceae* warrants clinical investigation.

Rosamicin, a relatively new macrolide antibiotic produced by *Micromonospora rosaria*, shares a number of chemical characteristics with erythromycin and has been shown to have a similar spectrum of antibacterial activity (10, 11). Like erythromycin, it is most active against gram-positive organisms; however, it has been shown to be more active than erythromycin against *Enterobacteriaceae* and *Pseudomonas* (1, 11).

It has been well demonstrated, first by Haight and Finland in 1952 (3) and later by others (5, 7, 12), that alkalinization of culture media enhanced the antibacterial activity of erythromycin. Subsequent clinical studies suggested that erythromycin plus alkalinization of the urine could be effective treatment for many urinary tract infections (4, 6, 13, 14). The increased activity of rosamicin against gram-negative bacilli at neutral or slightly alkaline pH suggested the possibility that it could be more active than erythromycin when used with alkalinization (11). The purpose of this investigation was to assess the relative in vitro activity of rosamicin and erythromycin against common urinary tract pathogens, both gram positive and gram negative, over a range of pH levels readily attainable in urine.

MATERIALS AND METHODS

Bacteria. A total of 311 strains of bacteria was tested. These were distributed as follows: 42 *Escherichia coli*, 42 *Klebsiella*, 42 *Proteus mirabilis*, 42 indole-positive *Proteus*, 36 *Enterobacter*, 42 *Pseudomonas aeruginosa*, 23 *Serratia*, and 42 group D streptococci. These strains were isolated from clinical sources, and most of them were recovered and identified in the Clinical Microbiology Laboratory of Colorado General Hospital, under the direction of L. B. Reller. Many of the strains of indole-positive *Proteus* were provided by S. R. Mostow of the Denver Veterans Administration Hospital.

Antibiotics. Laboratory reference standards were provided by the Schering Corp., Bloomfield, N.J. (rosamicin phosphate, batch number 9825-148I), and Eli Lilly Co., Indianapolis, Ind. (erythromycin, lot number P-86211).

Susceptibility testing method. Isolates were simultaneously tested against rosamicin and erythromycin at pH values of 5, 6, 7, and 8 by means of a microtiter broth dilution technique. All of the tests were performed in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) which had been adjusted to the appropriate pH by the addition of 1 N hydrochloric acid or 1 N sodium hydroxide. A Corning Research pH meter model 12 was used to determine pH values.

Serial twofold dilutions of freshly prepared antibiotics were made in Mueller-Hinton broth at each pH level, and 0.05 ml of each dilution was dropped into the wells of a microtiter plate (Cooke Engineering Co., Alexandria, Va.). Overnight broth cultures of the organisms containing approximately 3.1×10^9 organisms per ml were diluted 10^{-4} in Mueller-Hinton broth at each pH, and 0.05 ml was then added to the diluted antibiotic. The plates were placed on a shaker for 5 min and then incubated overnight at 35°C in ambient air. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic in which there was no visible growth. The minimum bactericidal concentration (MBC) was determined by using an adaptation of the Steers replicator (8) which delivered approximately 0.003 ml from each well of

the microtiter plate to Mueller-Hinton agar (Difco). Plates were incubated overnight at 35°C in ambient air.

Inoculum size effect. The effect of inoculum size on both the MIC and the MBC at each pH level was evaluated by testing 10 strains from each group of organisms with a 10^{-2} as well as a 10^{-4} dilution of an overnight broth culture.

RESULTS

Figure 1 graphically illustrates the effect of pH on the susceptibility of *E. coli*. At pH 5, only 2% of the 42 strains tested were inhibited by 16 μg of rosamicin per ml; at pH 8 this concentration inhibited 100% of the strains. Similarly, at pH 5, a concentration of 500 μg of erythromycin per ml was not inhibitory for any of the *E. coli* tested; however, at pH 8, 93% were inhibited by 16 μg/ml.

Klebsiella MICs were extremely high at a pH of 5 (Fig. 1); at a pH of 8, 100% of the strains were inhibited by 31 μg of rosamicin per ml, and 83% were inhibited by 31 μg of erythromycin per ml. Testing of *Enterobacter* and *Serratia*, as shown in Fig. 1, revealed similar results.

Alkalinization of the media did not significantly enhance the activity of erythromycin against either *P. mirabilis* or indole-positive strains of *Proteus*; however, rosamicin MICs were appreciably decreased when a pH of 8 was used (Fig. 2). Only 10% of the *P. mirabilis* were inhibited by 250 μg of rosamicin per ml at a pH of 5, but 83% were inhibited by 31 μg/ml when tested at pH 8. Very similar results were obtained with indole-positive *Proteus*. At a pH of 5, only 10% were susceptible to 250 μg of rosamicin per ml; at a pH of 8, 71% of the strains were inhibited by 31 μg/ml.

Although it would appear that rosamicin is more active at pH 5 than pH 6 (Fig. 2), it is difficult to interpret data obtained when testing *P. aeruginosa* at that level of acidity. Organisms whose normal habitat is the gastrointestinal tract (which includes all other groups tested in this study) tolerate a more acid pH (i.e., 4.4) (2). The minimum pH for growth of *P. aeruginosa*

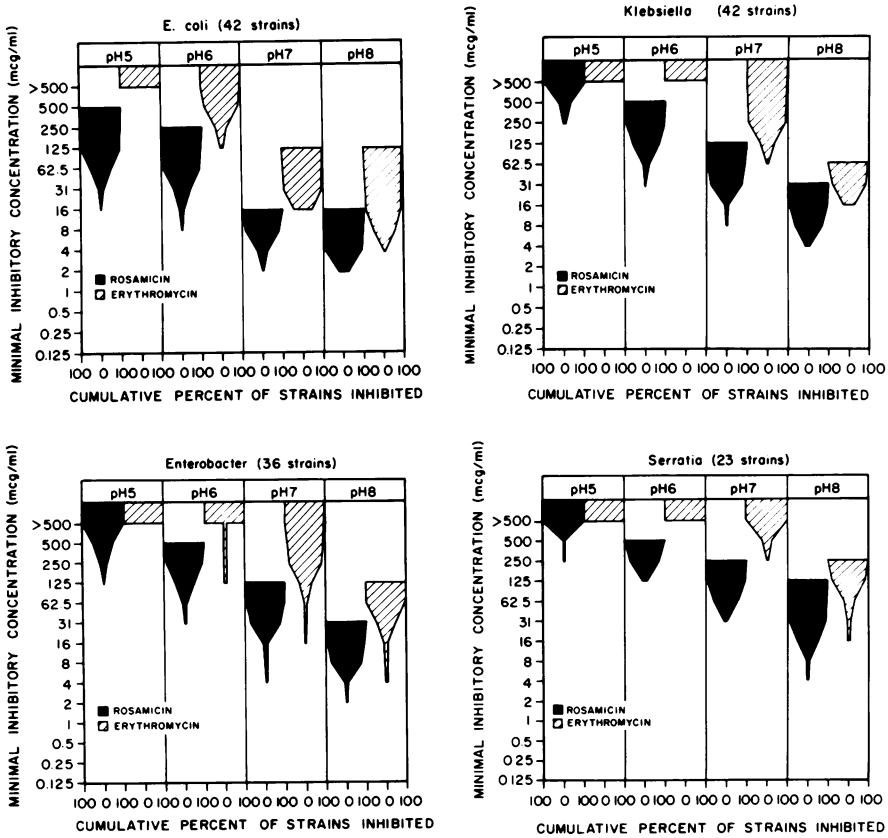

FIG. 1. *Effect of pH on the MIC of rosamicin and erythromycin against E. coli, Klebsiella, Enterobacter, and Serratia.*

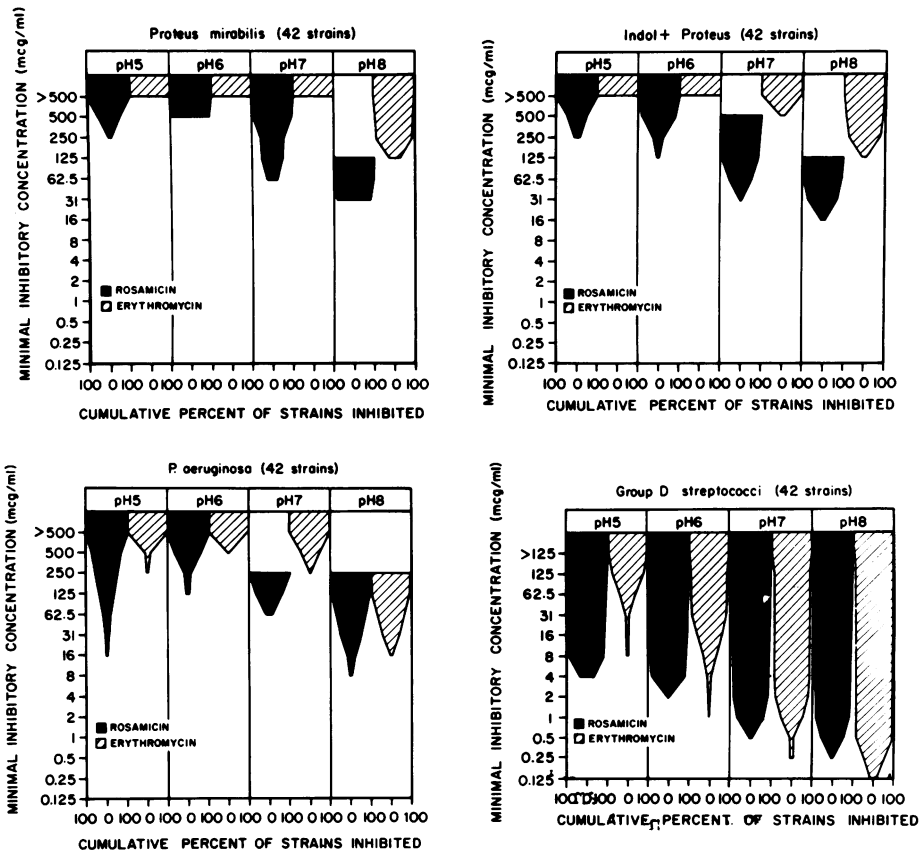

FIG. 2. *Effect of pH on the MIC of rosamicin and erythromycin against P. mirabilis, indole-positive Proteus, P. aeruginosa, and group D streptococci.*

tends to be slightly higher, ranging from 5.6 to 4.4 (2, 9), and indeed a number of strains tested failed to grow at pH 5. Although only strains that visibly grew in the pH 5 broth control were included in these results, it is probable that the effect of acidity on the population was a significant factor. At more alkaline pH values, the pattern of decreasing MICs was again observed, with rosamicin and erythromycin being approximately equal in activity at pH 8. Ninety-five percent of the strains were inhibited by 125 μg of either antibiotic per ml at that pH, and, at a concentration of 31 μg/ml, 50% of the strains were inhibited by rosamicin and 43% were inhibited by erythromycin.

Group D streptococci (Fig. 2) were the most susceptible organisms tested and the only group which demonstrated lower MICs to erythromycin than to rosamicin at pH 8. At a concentration of 0.5 μg/ml, only 56% of the strains were inhibited by rosamicin whereas 85% were inhibited by erythromycin. Again, increasing alkalinity significantly decreased MICs for both antibiotics.

MBC data are not shown, but MBC values remained consistently high for both antibiotics, regardless of the organism or pH used.

Table 1 demonstrates the dramatic increase in geometric mean MICs obtained when the inoculum size was increased 100-fold. This was evident for both antibiotics, all organisms tested, and at each pH when MIC values were such as to permit comparison.

DISCUSSION

These results indicate that, like other macrolide antibiotics, the antibacterial activity of rosamicin was consistently and significantly increased by increasing the pH of the media, even when tested against *Proteus*. This is an advantage over erythromycin which, as demonstrated by the data in this study and earlier by Sabath et al. (7), does not show significant activity against most strains of *Proteus*, even when

TABLE 1. *Effect of inoculum size on in vitro activity of rosamicin and erythromycin*[a]

Strain tested	Inoculum[b]	pH 5		pH 6		pH 7		pH 8	
		10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}	10^{-2}	10^{-4}
Serratia	Rosamicin	>500	>500	>500	287	102	82.4	29.4	18.2
	Erythromycin	>500	>500	>500	>500	>500	>500	353.6	124.9
Enterobacter	Rosamicin	>500	>500	>500	177	500	31	44	10.5
	Erythromycin	>500	>500	>500	>500	>500	203	144	33.4
P. mirabilis	Rosamicin	>500	435	>500	500	>500	467	>500	33
	Erythromycin	>500	>500	>500	>500	>500	>500	>500	233
Indole-positive *Proteus*	Rosamicin	>500	>500	>500	500	177	71.7	67	35.8
	Erythromycin	>500	>500	>500	>500	>500	>500	467	213
E. coli	Rosamicin	379	67	467	44	203	6.5	89	3.2
	Erythromycin	>500	>500	>500	354	500	20.9	466	7.5
P. aeruginosa	Rosamicin	308.5	170	>500	>500	435	165	109	95
	Erythromycin	>500	>500	>500	>500	>500	>500	250	35.8
Klebsiella	Rosamicin	>500	>500	>500	102	203	27	88	7.5
	Erythromycin	>500	>500	>500	>500	>500	177	>500	25.5
Enterococci	Rosamicin	41	7.5	36	3.7	27	0.87	6.9	0.47
	Erythromycin	154	67	32	8.6	15.8	0.9	11.9	0.25

[a] Geometric mean MIC for 10 strains.
[b] Dilution of overnight culture in Mueller-Hinton broth.

tested at pH 8. Although erythromycin did demonstrate enhancement of antibacterial activity by alkalinization when tested against the other *Enterobacteriaceae* in this study, rosamicin at pH 8 was the more active antibiotic, with MICs two- to sixfold lower than those of erythromycin. The activity of both antibiotics against *P. aeruginosa* at pH 8 was very similar and, again, significantly improved over activity at lower pH values. MICs against group D streptococci were also decreased by alkalinization, but unlike the gram-negative bacilli studied, erythromycin appeared to be more active than rosamicin at an alkaline pH.

Previous studies by Sabath and his colleagues (6) have demonstrated that alkalinization of urine has no effect on the amount of erythromycin excreted in urine in normal volunteers and that the urine concentration of erythromycin varied from 33 to 368 μg/ml after four oral doses of 1.0 g of erythromycin estolate at 8-h intervals. In a subsequent clinical study, Zinner et al. found (14) that erythromycin plus alkalinization eradicated the infecting organism during therapy in 73% of 37 patients with chronic bacteriuria and that urine cultures remained negative 3 weeks after treatment in 17 patients.

The in vitro data provided here suggest that, if pharmacokinetic studies prove it to be similar to other macrolides in terms of urinary concentration and toxicity, rosamicin could be a useful agent in the treatment of urinary tract infections, particularly those caused by members of the *Enterobacteriaceae*. Clinical investigation of rosamicin would then be warranted.

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