Effect of \( pH \) on the Activity of Erythromycin Against 500 Isolates of Gram-Negative Bacilli

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Erythromycin was found to be a more effective inhibitor of gram-negative bacilli in alkaline medium than in neutral or acid medium. A definite effect was noted with all of 500 recent clinical isolates of *Escherichia coli*, *Klebsiella-Enterobacter*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* studied, but it was most striking with *E. coli*. At \( pH \) 8.5 all strains, except for 14% of those of *P. mirabilis*, were inhibited by concentrations of erythromycin readily achieved in urine with common therapeutic doses.

Studies on streptomycin (1, 7) and later on erythromycin (3) indicated that their antibacterial activities vary markedly with the \( pH \) of the medium; these two antibiotics have significantly lower minimal inhibitory concentrations (MIC) at high \( pH \) than in medium with neutral or low \( pH \). The \( pH \) effect on erythromycin activity was first demonstrated against *Streptococcus pyogenes* and *Sarcina lutea* (3) and later against staphylococci (2). It was shown that alkaline \( pH \) also enhances the activity of erythromycin against gram-negative organisms (6, 9). Zagar (9) and recently Sabath et al. (5) demonstrated that erythromycin at \( pH \) 8 was up to 300 times more active against *Escherichia coli* than at \( pH \) 6. Volunteers, taking 3 g of erythromycin estolate together with 12 to 15 g of sodium bicarbonate daily, produced urines sufficiently alkaline with enough erythromycin to inhibit the growth of *E. coli* in dilutions as high as 1:128 (5).

Because enhancement of the activity of erythromycin against gram-negative bacilli by alkaline \( pH \) has been well established and has potential clinical application, a large number of strains of gram-negative bacilli commonly found in urinary infections was tested to determine the proportion that are susceptible to erythromycin at levels of \( pH \) readily attainable in the urine.

**MATERIALS AND METHODS**

Two hundred strains of *E. coli* and 100 each of *Klebsiella-Enterobacter*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, all recent isolates from urinary tract infections, were tested at \( pH \) 7 and 8.5 by a broth-dilution method as previously described (5). Ten strains of *E. coli* were also tested at several \( pH \) intervals between \( pH \) 5.5 and 8.0 to determine more precisely the point at which the antibacterial activity of erythromycin shows the greatest change. Both the MIC and the minimal bactericidal concentration (MBC) were determined by using techniques and criteria previously described (5). The erythromycin used was generously supplied by Eli Lilly & Co., Indianapolis, Ind.

**RESULTS**

The results (Table 1) indicate that at \( pH \) 8.5 all the strains of *E. coli* were inhibited by 3.125 \( \mu g \) of erythromycin per ml, a concentration easily attainable in urine with therapeutic doses of erythromycin. The strains of *Klebsiella-Enterobacter* were less sensitive. Only 60% of them were inhibited by 3.12 \( \mu g \) of erythromycin per ml, but 95% were inhibited by 12.5 \( \mu g \)/ml, a concentration also readily achieved in urine (4, 10). Most strains of *Pseudomonas* were more resistant, but 12 of those tested were sensitive to 6.25 \( \mu g \)/ml and all were inhibited by 50 \( \mu g \)/ml, a concentration achieved in urine by all subjects taking 3.0 g of erythromycin estolate in 24 hr (4). All strains of *Proteus* were resistant to 12.5 \( \mu g \)/ml, but 90% of them were inhibited by 100 \( \mu g \)/ml or less, concentrations found in the urine of some subjects during therapy (4).

The results (Fig. 1A) indicate that between \( pH \) 6 and 7 the inhibitory activity of erythromycin increases sharply. Further increase in activity was also noted up to \( pH \) 8. The effect of \( pH \) on the MBC did not parallel the effect on MIC; for 4 of the 10 strains studied, the MBC was identical at \( pH \) 5.5 and 8 (Fig. 1B).
EFFECT OF pH ON ERYTHROMYCIN

Table 1. Numbers of strains susceptible to erythromycin at pH 7 and pH 8.5

| Organism                  | No. of strains tested | pH of test | No. of strains inhibited by indicated concn
|----------------------------|-----------------------|------------|----------------------------------------
| **Escherichia coli**       | 200                   | 7.0 8.5    | >200 200 100 50 25 12.5 6.25 3.12 1.56 0.78 0.39 0.19 0.09 0.04
| **Klebsiella-Enterobacter**| 100                   | 7.0 8.5    | 9 12 40 107 32 12 27 77 74 4 6
| **Pseudomonas aeruginosa** | 100                   | 7.0 8.5    | 30 25 8 2 10 25 35 20 5
| **Proteus mirabilis**      | 100                   | 7.0 8.5    | 25 44 12 19 39 21 28 12

* Expresses as micrograms per milliliter.

Fig. 1. A, Effect of pH on the minimum inhibitory concentration (MIC) of erythromycin for 10 strains of E. coli. B, Effect of pH on the minimum bactericidal concentration (MBC) of erythromycin for the same 10 strains of E. coli.

DISCUSSION

Williamson and Zinnemann (8) tested 347 strains of E. coli and found 50% of the strains were inhibited by 10 μg of erythromycin per ml (pH 6). The strains reported here showed greater resistance; none were inhibited by 10 μg/ml, even at pH 7. However, they were much more sensitive in alkaline medium. In a small clinical study, Zinner et al. (10) found that 71% of 24 patients cleared their urine of bacteria with erythromycin-alkali treatment. The results of the present study suggest that most isolates of E. coli and Klebsiella-Enterobacter from patients with bacteriuria might be expected to be sensitive to erythromycin in sufficiently alkaline urine. Because strains of Pseudomonas and especially Proteus had higher MIC, even in alkaline urine, larger doses of erythromycin would appear to be indicated for therapeutic trials of urinary tract infections due to those organisms. Nevertheless, alkalinization of the medium clearly increased the antibacterial activity of erythromycin against virtually all the strains studied.

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LITERATURE CITED

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