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NOTES

In Vitro Susceptibility of 30 Strains of *Chlamydia trachomatis* to Rosamicin

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A total of 13 of 30 clinical isolates of *Chlamydia trachomatis* were susceptible in vitro to 0.01 μg of rosamicin per ml. Only two of these strains were susceptible to tetracycline or erythromycin at this level. The results suggest that rosamicin may be useful for the treatment of chlamydial urethritis.

Rosamicin is a new macrolide antibiotic, chemically similar to erythromycin, which has been found to be active against anaerobic bacteria, including *Bacteroides fragilis, Peptococcus,* and *Clostridium* (15, 19), and some aerobes, including *Staphylococcus aureus, Staphylococcus epidermidis,* and enterococci (16). The antibacterial spectrum and the high levels of the drug which were found in vaginal secretions and tissues of dogs and rats and in prostates of humans after experimental administration of the antibiotic (3, 8) suggested that rosamicin might be useful in the treatment of genitourinary infections.

Chlamydia trachomatis causes urethritis in males and cervicitis in females and can be recovered from 30 to 50% of symptomatic individuals (7). Because of the difficulties in successfully treating nongonococcal urethritis (mostly caused by *C. trachomatis*), it was of interest to test the susceptibility of freshly isolated strains of *C. trachomatis* to rosamicin. For comparison, the susceptibility of these strains to tetracycline, penicillin, and erythromycin was also determined.

A total of 30 fresh clinical isolates of *C. trachomatis* were tested: 20 from male urethral specimens, 7 from cervical and vaginal material, 2 from the respiratory tract, and 1 from the eye.

Rosamicin was supplied by Schering Corp., Bloomfield, N.J. Erythromycin and penicillin G were purchased from Eli Lilly & Co., Indianapolis, Ind., and tetracycline-HCl was obtained from Lederle Laboratories, Chicago, Ill. Stock solutions of all drugs were adjusted to 1,000 μg/ml, according to their stated potency. Rosamicin and erythromycin were initially dissolved in 2.5 ml of 95% ethyl alcohol before water was added to achieve the appropriate concentration of each.

In vitro susceptibility studies were performed by the method of Ridgway et al. (13). Briefly, McCoy cell monolayers were infected with 100 to 400 inclusion-forming units per cover slip prepared by dilution in antibiotic-free cell culture medium (Eagle minimal essential medium, powdered medium, K. C. Biological, Lenexa, Kan.). The cell culture vials were centrifuged, and the inoculum was removed and replaced with Eagle minimal essential medium containing the test antibiotic (18). Each concentration of antibiotic was tested in four cell culture vials. After 48 h of incubation, the cell cultures were stained with iodine to identify chlamydial inclusion bodies. The minimal inhibitory concentration (MIC) was the lowest concentration of antibiotic preventing the appearance of any inclusion bodies in the cell monolayer.

The high level of activity of rosamicin for strains of *C. trachomatis* is shown in Table 1. Rosamicin was distinctly more active than tetracycline and all strains were inhibited by 0.1 μg/ml. Whereas almost half of the *C. trachomatis* strains were susceptible to rosamicin at 0.01 μg/ml, this concentration inhibited only 7% of the strains tested in cell cultures containing tetracycline or erythromycin.

Susceptibility studies of *C. trachomatis* to antibiotics have been performed in ovo (9, 17) and more recently in cell cultures. In four of the reports, cell cultures were used with 9 to 30 strains of *C. trachomatis* (1, 5, 11, 20), whereas in other studies more limited antibiotic susceptibility data with only one laboratory strain of the organism were presented (2, 4, 12, 13). Despite differences in techniques used in the in

TABLE 1. *In vitro susceptibility of 30 clinical isolates of Chlamydia trachomatis to 4 antibiotics*

Antibiotic	Cumulative % susceptible at increasing MIC (μg/ml)[a]				
	0.01	0.1	0.5	1	2
Rosamicin	43	100			
Tetracycline	7	83	100		
Erythromycin	7	27	97		100
Penicillin	3	13	97		100

[a] MIC, Minimal inhibitory concentration. For penicillin, units per milliliter.

vitro cell assay described in these reports, the MICs of tetracycline, erythromycin, and penicillin were very similar to ours. In this regard, Kuo et al. (11) found that of 16 strains (15 immunotypes) of *C. trachomatis,* 15 were inhibited by 0.05 μg of rosamicin per ml, the lowest level of antibiotic tested in their study. Our data are in complete agreement with their results in that all 30 strains were inhibited by 0.1 μg of rosamicin per ml. However, we found almost half of the *C. trachomatis* strains tested to be susceptible to the very low concentration 0.01 μg/ml. On the other hand, unlike others (4, 10), we did not observe the development of morphologically abnormal or abortive inclusions when *C. trachomatis* strains were inhibited by penicillin. The use of very high-titered inocula of *C. trachomatis* in other studies, rather than the inoculum of 100 to 400 inclusion-forming units per cover slip used in the present study, most likely accounts for their results.

In our study, no attempt was made to identify *C. trachomatis* strains by serotype. This does not seem to be important since Treharne et al. (20) failed to demonstrate significant variations in antibiotic susceptibility results with nine different serotypes of *C. trachomatis.* Interestingly, all five strains that had MICs >0.1 μg of tetracycline per ml or >5 μg of erythromycin or penicillin per ml came from the urethra.

Because of the remarkable activity of rosamicin against *C. trachomatis* observed in this study and against *Neisseria gonorrhoeae* observed by Sanders and Sanders (14) and the possibility that high drug levels are attained in the urethra (6), clinical trials of this agent in males with chlamydial and gonococcal urethritis are warranted.

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