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In Vitro Activity of Rosamicin, Josamycin, Erythromycin, and Clindamycin Against β-Lactamase-Negative and β-Lactamase-Positive Strains of *Neisseria gonorrhoeae*

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Randomly selected strains of β-lactamase-negative and β-lactamase-positive *Neisseria gonorrhoeae* were tested by an agar dilution method for susceptibility to rosamicin, josamycin, erythromycin, clindamycin, and penicillin G. Rosamicin was more active than erythromycin, which was more active than josamycin or clindamycin; the latter two were similar in their activity. The susceptibility to the macrolide antibiotics and clindamycin was independent of β-lactamase production, but the penicillin minimal inhibitory concentrations were higher in the β-lactamase-positive group because of the enzyme.

Until 1972, *Neisseria gonorrhoeae* exhibited gradually increasing levels of chromosomal resistance to commonly used antimicrobial agents (8, 13), but recent data from a national gonorrhea therapy monitoring program indicated that this pattern of increasing resistance levels had been reversed (6). However, the isolation of β-lactamase-producing (1, 4, 10) and spectinomycin-resistant (11, 18) strains demonstrates the ability of *N. gonorrhoeae* to develop resistance to therapeutic agents. Therefore, currently available antimicrobial agents should be evaluated for potential therapeutic value against gonococcal infections. Erythromycin has been recently studied in clinical trials (3). A newer macrolide, rosamycin, has been recommended as a potentially useful therapeutic agent for *Neisseria* infections (12).

In this study three macrolides (rosamicin, erythromycin, and josamycin), clindamycin, and penicillin G were tested for in vitro activity on β-lactamase-negative (β-lac$^-$) and β-lactamase-positive (β-lac$^+$) strains of *N. gonorrhoeae*.

MATERIALS AND METHODS

Cultures. *N. gonorrhoeae* cultures included 77 recently isolated β-lac$^-$ strains and 54 β-lac$^+$ strains. β-Lactamase production was determined with the chromogenic cephalosporin test (9).

Antibiotics. Antibiotics were obtained from the manufacturers as powders suitable for susceptibility testing. The drugs were dissolved in appropriate solvents, sterilized by membrane filtration (0.22 μm, Millipore Corp., Bedford, Mass.), and frozen at −70°C. Before they were used the antibiotics were thawed and diluted with sterile distilled water, to 10 times the final desired concentration. The drugs were added to molten agar at 50°C (one part of drug solution to nine parts of agar to yield the desired concentration).

Susceptibility tests. The minimal inhibitory concentrations (MICs) were determined by agar dilution; a Steers replicator was used to inoculate the surface of the agar plates. The medium was proteose no. 3 agar (Difco Laboratories, Detroit, Mich.) supplemented with 1% hemoglobin and 1% defined supplement (6, 20). Appropriate concentrations of antibiotics were added to the agar at 50°C.

The inoculum, prepared from overnight growth from chocolate agar plates, was suspended in Mueller-Hinton broth. The turbidity of the bacterial suspension was adjusted with a light-scattering photometer (17) so that each inoculum spot contained approximately 5×10^3 colony-forming units. The inoculated plates were air dried, inverted, and incubated in candle-flame-extinction jars for 24 h at 35°C. MICs were read as the lowest concentration of antibiotic completely inhibiting macroscopic growth.

RESULTS

Results of tests with the 54 β-lac$^+$ *N. gonorrhoeae* isolates are shown in Fig. 1. Rosamicin was the most active antibiotic, with all isolates inhibited by 0.25 μg/ml or less. Erythromycin was the next most active drug, but concentrations of 2.0 μg/ml were required to inhibit all the β-lac$^+$ isolates.

Clindamycin and josamycin were similar in their activities except that josamycin was more active at the lower concentrations. However, 4 μg of each drug per ml was needed to inhibit all the β-lac$^+$ strains. Penicillin was the least active antibiotic against these β-lac$^+$ strains, with MICs ranging from 1 to 32 μg/ml.

The results for the 77 β-lac$^-$ isolates are shown in Fig. 2. Except for the penicillin MICs, results are similar to those obtained with the β-lac$^+$ strains. The macrolide antibiotics generally showed the same relative activity with β-lac$^-$

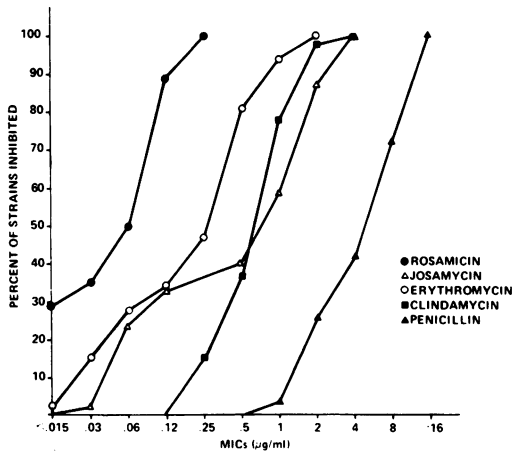

FIG. 1. *Cumulative percentage of 54 β-lac⁺ N. gonorrhoeae strains inhibited by various concentrations of five antibiotics.*

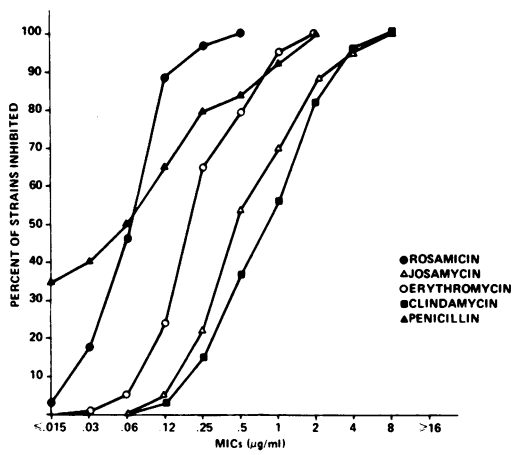

FIG. 2. *Cumulative percentage of 77 β-lac⁻ N. gonorrhoeae strains inhibited by various concentrations of five antibiotics.*

strains as with the β-lac⁺ strains, but with some strains the MICs were 1 dilution higher.

Penicillin was considerably more active than rosamicin against the more sensitive strains, but required 2 μg/ml to inhibit all of the β-lac⁻ strains.

DISCUSSION

The rosamicin MICs that we obtained with the β-lac⁺ and β-lac⁻ strains are quite similar to those obtained for *Neisseria* species by Sanders and Sanders (12), and, as these investigators also found, rosamicin was more active than erythromycin on all strains tested. The erythromycin and clindamycin MICs were similar to those we have obtained previously (16). We are unaware of previous studies on the activity of josamycin on *N. gonorrhoeae*. However, a study comparing josamycin and erythromycin activity on both gram-positive and gram-negative aerobes (15) showed josamycin to be less active than erythromycin against all species tested. In comparing the activities of erythromycin, clindamycin, and josamycin on aerobes, Westerman et al. (19) found that, except against the enterococci, clindamycin was most effective, followed by erythromycin and then josamycin. Our data show that the gonococci are also more sensitive to erythromycin, but the differences between clindamycin and josamycin are minimal.

The presence of a plasmid-mediating β-lactamase in a particular strain did not affect the MICs for the macrolides and clindamycin. Differences between the susceptibility of β-lac⁻ and β-lac⁺ strains to the macrolides and clindamycin would probably be due to differences in their geographic origin. β-Lac⁻ strains collected from patients in the Far East were found to be relatively more resistant to erythromycin than strains from the United States (16). It is likely that the same differences would be found with other macrolides.

In the two decades between 1950 and 1970, gonococci, in general, became more resistant to penicillin (8, 13), but our studies showed that since 1972 this trend has been reversed (6). In these studies it was shown that treatment with penicillin of patients with uncomplicated gonorrhea yielded approximately 97% cures (7). However, efficacy was related to penicillin MICs. Therapy was more likely to fail when patients were infected with strains having relatively high MICs (≥0.5 μg/ml) than when they were infected with strains with lower MICs. Therefore it has been necessary to consider other drugs for alternative therapy against these chromosomally mediated, relatively penicillin-resistant strains. With the discovery of the plasmid-mediated β-lac⁺ strains (1, 4, 10), there was further reason to consider alternative drugs for therapy of patients with gonorrhea caused by these organisms. Brown et al. (3) used erythromycin for therapy of patients with gonorrhea, and they found that for those infected by organisms with erythromycin MICs ≥0.25 μg/ml, the failure rate was an unacceptable 20%. If gonorrhea caused by the organisms in this study had been treated with erythromycin, the failure rate would probably have been unacceptable also.

If equally strong correlation between MIC and efficacy exists for rosamicin, then this drug might be more effective, since only 4 of the 131 strains studied had MICs ≥0.25 μg/ml. Rosamicin could be more effective than erythromycin because tissue and body fluid levels are poten-

tially higher with this drug than with erythromycin. Hoyme et al. (5) and Baumueller et al. (2) reported that in animal and human studies, rosamicin was more concentrated in uretheal and vaginal secretions, and in prostatic tissues and interstitial fluid, than was erythromycin.

MICs of josamycin and clindamycin for these strains, on the other hand, are not lower than the MICs of erythromycin. However, Strausbaugh et al. (14) reported that josamycin was concentrated at higher levels in some body fluids than was erythromycin, even though plasma levels were comparable. It is not known whether josamycin is concentrated in the tissues or interstitial fluids of the genitourinary tract.

Because of its in vitro activity and because of the higher genitourinary tissue levels, rosamicin should be considered for clinical trials for treatment of gonococcal infections.

It would be advantageous to have as much laboratory and clinical knowledge as possible about a variety of alternative drugs, in case a change in recommended therapy has to be made again.

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