ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1988, p. 775–776
0066-4804/88/050775-02$02.00/0

Vol. 32, No. 5

Spectinomycin Disk Zone Diameter as a Predictor of Outcome in Clinical Treatment of Gonorrhea

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Received 3 September 1987/Accepted 3 February 1988

The MICs for 41 *Neisseria gonorrhoeae* strains from patients receiving spectinomycin treatment were determined by the agar dilution method and compared with the zones of inhibition produced by disks containing 100 μg of spectinomycin. Our data demonstrated a good correlation between the two methods. Moreover, a zone of inhibition of ≤15 mm was a good predictor of clinical treatment failures with spectinomycin.

The increasing number of spectinomycin-resistant *Neisseria gonorrhoeae* in various areas of the world threatens the continued use of spectinomycin as an alternative treatment for gonococcal infection (2, 3, 4, 7). While these strains are still rare in the United States, there is recent evidence suggesting some importation (8). A simple and reliable method for monitoring the prevalence and spread of these strains and for directing appropriate antibiotic treatment is needed (1). The agar dilution method is the current technique for determining spectinomycin MICs for the infecting organism. While accurate and reliable, this method is costly, labor intensive, and beyond the capability of many of the laboratories in which spectinomycin-resistant *N. gonorrhoeae* strains are of concern. By comparison, the disk diffusion method is inexpensive, easier, and within the capability of most laboratories. It offers a rapid, more efficient means of conducting surveillance for spectinomycin resistance trends and analysis of spectinomycin treatment failures. The disk diffusion method would clearly be preferable, providing that the results by this technique correlate with those of the agar diffusion method and clinical outcome.

The 41 isolates reported here were collected during a 10-week period in 1983 (8 cultures) and a 2-week period in 1985 (33 cultures) from U.S. military men reporting to U.S. military clinics for sexually transmitted diseases in the Republic of Korea (2). All patients in this report had a positive urethral culture for *N. gonorrhoeae*. The primary treatment for these patients with gonococcal urethritis was an intramuscular injection of spectinomycin (2.0 g). All patients were evaluated after treatment. A patient was considered a spectinomycin treatment failure if he had a positive Gram stain of a urethral specimen or a positive culture for *N. gonorrhoeae* on the follow-up exam and if he denied sexual exposure following initial treatment. Thirteen of the isolates were selected because they were obtained from clinical treatment failures. The remaining 28 isolates were from clinical treatment cures and were selected because they encompassed a spectrum of spectinomycin-susceptible (0.78 to 25 μg/ml) organisms.

Urethral specimens were swabbed onto modified Thayer Martin agar plates, which were incubated in candle extinction jars at 36°C and examined at 24, 48, and 72 h. The presence of *N. gonorrhoeae* was confirmed by Gram stain, oxidase reactivity, and the Phadebact Gonococcus Test Kit (Pharmacia Diagnostics, Piscataway, N.J.). Subcultures of these isolates were suspended in skim milk and stored at −70°C for later antibiotic susceptibility testing.

Spectinomycin hydrochloride for MIC determinations was obtained from The Upjohn Co., Kalamazoo, Mich. The MIC of spectinomycin was determined by the agar dilution method on GC (gonococcal) agar base supplemented with IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.; 5). Twofold dilutions of spectinomycin were tested in the range of 0.09 to 10,000 μg/ml against a standard inoculum of approximately 5×10^4 organisms.

The ability of a 100-μg spectinomycin diagnostic disk (Difco Laboratories, Detroit, Mich.) to inhibit the test strains of *N. gonorrhoeae* was determined in accordance with the procedures outlined by the National Committee for Clinical Laboratory Standards in publication M2-A3 (6). Briefly, *N. gonorrhoeae* was grown overnight on GC base supplemented with IsoVitaleX, and the organisms were suspended in Mueller-Hinton broth to give an optical density similar to a 0.5 McFarland barium sulfate turbidity standard. A sterile, nontoxic swab was dipped into the suspension of organisms, and excess fluid was removed by rotating the swab on the inside wall of the tube above the fluid level. A GC agar plate supplemented with IsoVitaleX was streaked three times with a 60° rotation of the plate between streakings. The plate was allowed to dry for 5 min before a spectinomycin disk was added. Inhibition zone diameters were determined after the cultures were incubated in candle extinction jars for 24 h at 36°C and include the diameter of the disk.

The MICs for 41 *N. gonorrhoeae* strains for which we have well-documented clinical evaluations and follow-ups were determined by the agar dilution method and compared with the zones of inhibition produced by disks containing 100 μg of spectinomycin (Fig. 1). Those strains for which MICs were equal to or less than 25 μg/ml all showed 20-mm or greater zone diameters. All but one patient from whom these 29 strains were isolated were cured by spectinomycin. The culture from the patient who failed to be cured by spectinomycin treatment had an MIC of 1.56 μg/ml and a disk zone diameter of 25 mm. Twelve strains for which MICs were equal to or greater than 250 μg/ml were also evaluated by the disk diffusion method. The largest zone for these strains was

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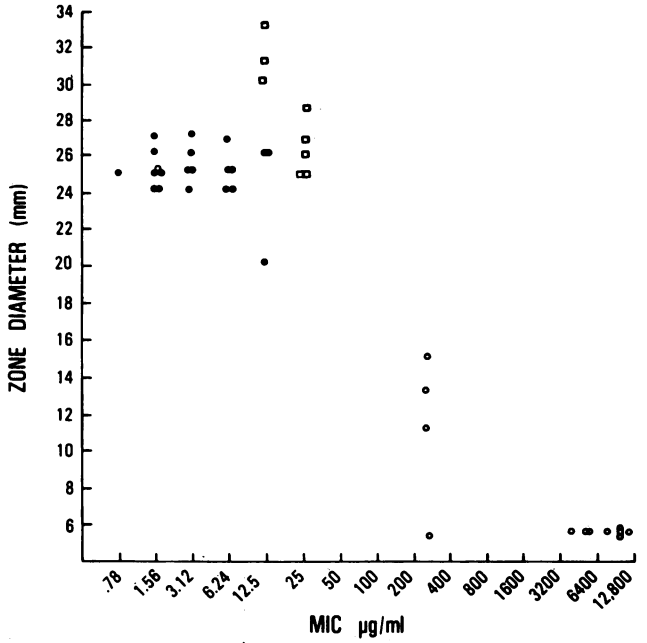

FIG. 1. Comparison of zone diameters with MICs. Symbols: ●, patients treated and cured with spectinomycin (1985); ○, patients treated and not cured with spectinomycin (1985); □, patients treated and cured with spectinomycin (1983). Zone diameters were produced by 100-μg spectinomycin identification disks after 24 h of incubation at 36°C. MICs were determined by using 10^4 organisms as the inoculum and were read after 24 h of incubation at 36°C. Regression line: slope, −0.00210; intercept, 23.8380; correlation, 0.6239.

15 mm, but nine of these strains had confluent growth adjacent to the disk. Treatment with spectinomycin failed in all of the patients from whom these strains were isolated. No *N. gonorrhoeae* strains for which MICs were between 50 and 200 μg/ml were available for testing.

We conclude that the disk diffusion method for determining *N. gonorrhoeae* susceptibility to spectinomycin correlates strongly with the agar dilution method and clinical outcome. A zone of 15 mm or less around a 100-μg spectinomycin disk can be used to detect spectinomycin-resistant *N. gonorrhoeae*. Because of its simplicity, we recommend the disk diffusion method to conduct surveillance for spectinomycin resistance or analyze *N. gonorrhoeae* spectinomycin treatment failures. When the disk diffusion test is performed, care must be taken to adhere to the established protocol, because variations in inoculum size or medium constituents can markedly affect the zone size obtained (6).

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