In Vitro Resistance of *Neisseria gonorrhoeae* to Metronidazole

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Susceptibility of 64 clinical isolates of *Neisseria gonorrhoeae* to metronidazole was determined by the agar dilution technique using Mueller-Hinton and chocolate agar media. All isolates were resistant to metronidazole at 12.5 µg/ml in either medium. This lack of activity of metronidazole against *N. gonorrhoeae* would suggest that the ability to recover *N. gonorrhoeae* in patients being treated with metronidazole for concurrent *Trichomonas vaginalis* infections should not be hampered.

Metronidazole (Flagyl) is an effective oral agent for the treatment of infections caused by *Trichomonas vaginalis* (2, 3). Trichomoniasis is felt to be a sexually transmitted disease, and most female patients present clinically with vaginal discharge. The possibility that concurrent infection with *Neisseria gonorrhoeae* exists must always be considered. It is, therefore, important to ascertain whether metronidazole, in the dosage given for trichomoniasis, will preclude the isolation of *N. gonorrhoeae* or effectively inhibit growth of this organism in vitro. To investigate this possibility, we determined the susceptibility of 64 clinical isolates of *N. gonorrhoeae* to metronidazole by an agar dilution technique.

MATERIALS AND METHODS

Isolation of *N. gonorrhoeae*. All gonococci tested were clinical isolates from the endocervix of women examined at Harbor General Hospital and neighboring clinics. Organisms were cultured on Thayer-Martin medium (17) at 35 to 37 C for 24 to 48 h in candle jars. Isolates were identified by oxidase reaction, typical appearance on Gram stain, and sugar fermentation reactions. Organisms were then stored at 70 C in a mixture of 50% horse serum and 50% Trypticase soy broth until ready for susceptibility testing (19).

Susceptibility testing. Susceptibility of *N. gonorrhoeae* to metronidazole was determined by an agar dilution technique previously described (19). Each gonococcal isolate was tested on both Mueller-Hinton and chocolate agars freshly prepared and enriched with 1% IsoVitalX. Twofold serial dilutions of metronidazole solution (provided by G. D. Searle and Co.) were added. Gonococcal suspensions in Mueller-Hinton broth for inoculation were prepared from a 24-h subculture on chocolate agar enriched

with 1% IsoVitalX and adjusted to a McFarland no. 1 nephelometer standard (1), previously determined to approximate 2×10^8 colony-forming units per ml. A Steers replicating apparatus (14) was used to deliver inocula of approximately 0.0026 ml (5.2×10^3 colony-forming units). Plates were incubated at 35 to 37 C in candle jars and flooded with oxidase (Difco) at 48 h. The minimal inhibitory concentration (MIC) recorded was the least antibiotic concentration that completely inhibited visible growth of any discrete oxidase-positive colonies. A reference strain of *N. gonorrhoeae* with known MIC to metronidazole was included in each determination for reproducibility.

RESULTS

MICs of metronidazole in Mueller-Hinton and chocolate agars against 64 gonococcal isolates, and the cumulative percent of organisms inhibited at each concentration, are summarized in Table 1. On either medium, all 64 isolates were resistant to metronidazole at 12.5 µg/ml. Only 22 isolates (34%) were inhibited at 100 µg/ml on Mueller-Hinton agar, whereas all gonococcal isolates except one were resistant to metronidazole at 100 µg/ml on chocolate agar.

DISCUSSION

Metronidazole has demonstrated effective trichomonacidal activity (3, 6). However, it appears to have little antibacterial activity against *N. gonorrhoeae*. This has important implications. Trichomonal and gonococcal infections may conceivably coexist in patients since both are sexually transmitted. Clinical distinction between these two infections may be difficult. The lack of activity of metronidazole against *N. gonorrhoeae* would suggest that re-

TABLE 1. Susceptibility of 64 isolates of *N. gonorrhoeae* to metronidazole by agar dilution

Medium	No. of isolates susceptible to MIC ($\mu\text{g/ml}$) of:										
	0.2	0.4	0.8	1.6	3.1	6.2	12.5	25	50	100	>100
Mueller-Hinton								2 (3) ^a	9 (17)	11 (34)	42 (100)
Chocolate										1 (1)	63 (100)

^a Numbers in parentheses indicate cumulative percentage of organisms inhibited.

covery of this organism from patients receiving metronidazole therapy for a concurrent trichomonas infection should not be seriously hampered. Additionally, it appears that metronidazole does not have a therapeutic role in the treatment of gonorrhea. Metronidazole is readily absorbed by the oral route, and serum concentrations between 7 and 11.5 $\mu\text{g/ml}$ can be achieved after doses of 250 mg three times daily (16). With larger doses of 1 g four times daily, serum levels can range from 15 to 72.5 $\mu\text{g/ml}$ (4). However, the achievable serum levels of metronidazole at these doses do not approach the relatively high MICs of this drug against gonococcal isolates.

Although metronidazole is inactive against *N. gonorrhoeae* and other aerobic bacteria such as *Staphylococcus aureus* and *Escherichia coli* (2), it is effective against a variety of anaerobic bacteria, including *Bacteroides fragilis* (12, 15, 18). Its effectiveness against *Treponema pallidum* has not been clearly established, and the data are somewhat conflicting (7, 10, 11). The mechanism of action of metronidazole appears to be by interfering with electron transfer in the pyruvate phosphoroclastic reaction involving reduced ferredoxin (8, 9, 13). Phosphoroclastic reactions and ferredoxin are apparently present only in anaerobic and microaerophilic organisms (8, 13). The lack of susceptibility of *N. gonorrhoeae* to metronidazole suggests that this organism: (i) can bypass or does not depend on phosphoroclastic reactions for its metabolism; or (ii) blocks the inhibition of electron transfer by metronidazole.

In spite of the general lack of susceptibility of *N. gonorrhoeae* to metronidazole on both Mueller-Hinton and chocolate agars, there was greater resistance of the organism at the higher MICs on chocolate as compared with Mueller-Hinton medium. The increased numbers of resistant isolates on chocolate agar in part may be explained by this medium's greater ability to promote growth of the gonococci. Inhibitory substances such as toxic fatty acids and trace

metals contained in peptone and agar may be eliminated by the addition of blood or serum to media (5). However, interaction of chocolate agar with metronidazole has not been excluded. The importance of media standardization for future antibiotic susceptibility studies for *N. gonorrhoeae* cannot be overemphasized.

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