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Antimicrobial Susceptibility Testing of *Neisseria gonorrhoeae* and Implications for Epidemiology and Therapy

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INTRODUCTION

Gonorrhea is a disease that has been recognized since antiquity and is still a common bacterial infection. In 1991, there were 586,638 confirmed cases in the United States reported to the Centers for Disease Control (20), making it the most commonly reported bacterial infection in this country. After many failed efforts and false leads, effective curative therapy has now been available for about 60 years. As is the case with most bacterial infections, the strategy of developing in vitro tests to predict the likely clinical and microbiologic outcome of treatment has been going on almost as long as effective treatment has been available. Yet despite the large number of culture-documented cases of gonorrhea, in vitro susceptibility testing is seldom performed. A College of American Pathologists report published in 1988 showed that clinical laboratories were very successful in growing and identifying *Neisseria gonorrhoeae*, but only 633 of 2,017 institutions responding to the questionnaire (of nearly 8,000 laboratories surveyed) reported doing any susceptibility testing (54). Most laboratories do nothing more than check for the presence of β-lactamase enzyme. Many reasons for the failure to do routine susceptibility testing on *N. gonorrhoeae* have been proposed. I have determined 14 reasons why in vitro testing of *N. gonorrhoeae* has been avoided by many clinical laboratories. (i) Tests are not available. (ii) Tests are available but unreliable. (iii) Tests are available and reliable but impractical. (iv) Susceptibility to specific antibiotics is predictable from knowledge of the β-lactamase phenotype. (v) Tests are too expensive. (vi) Tests of susceptibility are unnecessary because they are poorly predictive of response to therapy. (vii) Tests are unnecessary because a return of symptoms always heralds treatment failure. (viii) Even in research laboratories, resources for studying *N. gonorrhoeae* are better spent in other areas. (ix) Tests are unnecessary because patterns of resistance are stable. (x) Tests are unnecessary because regional monitoring can anticipate changes in susceptibility. (xi) Tests are unnecessary because treatment is always initiated before culture results come back. (xii) Tests are unnecessary because therapy is over before results can be available. (xiii) Tests are unnecessary because treatment is always the same. (xiv) Susceptibility testing is of no epidemiologic value. These reasons fall into three broad categories: the technology for testing is unavailable or fundamentally flawed, the results of testing have no meaningful correlation with the outcome of treatment, and the choice of treatment strategy is not likely to be influenced by test results even when the results are consistent and highly predictive of outcome. I will address these issues and focus on how in vitro susceptibility testing might contribute to the selection or modification of treatment schedules.

PREANTIBIOTIC TREATMENT OF GONORRHEA

Gonorrhea, especially when it involves the male urethra, can be a highly symptomatic disease. Before effective antimicrobial agents were developed, the treatments for gonor-

rhea were largely mechanical. Irrigation and the passage of urethral sounds (stiff metal rods) were the most applied forms of therapy. Since almost all gonococcal infections will ultimately subside irrespective of treatment, there was some perceived benefit to such therapies. Even after sulfonamides were marketed, mechanical treatments were still widely advocated as alternative or combined therapy. In the late 1930s and early 1940s, the notion of low-pressure irrigation gained favor (87). The high-pressure irrigation systems used previously had led to an unacceptable incidence of ascending infection of the prostate, seminal vesicles, and epididymis. Although infections of the rectum and female genitals were less well appreciated, there were comparable systems of irrigation developed for them as well. Pharyngeal gonorrhea was very poorly understood, and no specific treatments for it were described as late as 1943. Some of the irrigation techniques involved the instillation of antimicrobial substances such as detergents and antiseptics, but there was no systematic way to determine the killing power of these agents in vitro. Controlled clinical studies were not performed.

AST

The concept of in vitro testing of antimicrobial agents against bacteria dates back to the 1920s and the first paper in which Alexander Fleming described the antimicrobial properties of penicillin (37). While the theory of comparing in vitro susceptibility with clinical outcome is appealing, it was virtually unheard of to include data from antimicrobial susceptibility testing (AST) in the earliest papers on sulfonamides or penicillin. In fact, in some papers that describe sulfonamide therapy, the workers used culture confirmation of *N. gonorrhoeae* in only the minority of patients studied (20). The first description of penicillin AST for *N. gonorrhoeae* appeared in 1940, before the commercial introduction of penicillin, and the precursors of modern AST were, in general, developed pari passu with antimicrobial agents. For example, the concept of using paper disks impregnated with antimicrobial agents was first presented in 1944 (101).

THE SEARCH FOR AST

The quest for reproducible, standardized ASTs did not follow an easy path (98). In an early description of different AST methods, Jackson and Finland showed the importance of making fundamental choices regarding inoculum size, duration of incubation, and endpoints in deciding on quantitative susceptibility determination (51). It was also clear that many of the tests showed comparable results in most settings, but for certain antimicrobial agents or certain species of bacteria, there was great variance. Without an independent standard of clinical significance, it would be difficult to choose among the discrepant tests. For example, it was not until the important work of Bauer et al. in the 1960s that there came to be agreement on the standardization of conditions for the Bauer-Kirby disk diffusion test, which bears their name (4). Prior to that, there was no agreement on whether to read the results qualitatively or quantitatively. For many antibiotics there was initially a choice of disks of differing antimicrobial contents, and the inconsistency in the antimicrobial content of these commercial disks was extreme, irrespective of the stated potency. Even with the Bauer-Kirby standardization, the disk diffusion test originally was useful only for bacteria that grow rapidly in an aerobic atmosphere on the specified test medium.

By the 1970s, there was general agreement on the conditions of AST for common bacterial pathogens, and the American College of Pathologists was able to measure the accuracy of clinical laboratories in determining the susceptibility of these bacteria by comparing their results with those obtained in reference laboratories. Because of the frequency of gonorrheal infections, a substantial amount of data was developed for studying susceptibility of *N. gonorrhoeae* via a number of in vitro tests (76). Currently, agar dilution and disk diffusion testing are most commonly done (89), but despite the historical troubles with broth media for growing *N. gonorrhoeae*, good results have been reported with some broth microdilution test modifications (40, 95).

Benefits and Problems of AST

In general, the benefits and problems of AST have been widely discussed. As reviewed by Greenwood in 1981, several fundamental issues about extending the results of such testing to management decisions for individual patients are still unresolved (43). He points out that the MIC is actually a misnomer (since inhibitory effects can usually be measured well below the MIC) and may not distinguish between rapid, complete killing and slow, partial inhibition. Furthermore, most other ASTs are designed to correlate with the MIC rather than an independent clinical measure, and test conditions are adjusted so that this correlation is maximized. Most of Greenwood's general objections still hold true today, but less so for *N. gonorrhoeae* than for any other microorganism. In a two-part study published in 1976, the Cooperative Study Group compared treatment outcomes for several different therapeutic regimens including intramuscular penicillin-oral probenecid, oral ampicillin-probenecid, tetracycline, and spectinomycin (52, 62). On the basis of these results, partially displayed in Table 1, and their correlation with MICs, a rough outcome-based breakpoint system was developed. The lowest antimicrobial concentration that predicts a ≥95% success rate with standard therapy is the breakpoint for the highly susceptible range, the concentrations that predict 85 to 95% cure make up the moderately susceptible range, and the concentrations that predict <85% cure are in the resistant range. This idea, more fully articulated in the 1987 publication, "Antibiotic-Resistant Strains of *Neisseria gonorrhoeae*: Policy Guidelines for Detection, Management, and Control" (18), has continued to be useful and is applied to new drugs that have undergone both clinical and in vitro evaluations. These guidelines also suggest estimating the percentage of penicillinase-producing *N. gonorrhoeae* (PPNG) strains in a region and basing the use of penicillin versus alternative therapy on whether an area has nonendemic (<1%), endemic (1 to 3%), or hyperendemic (>3%) PPNG. While policy decisions adapting treatment schedules to local patterns of in vitro susceptibility seem logical, objections to the unnecessary complexity of this approach have been raised (60). Thus, current recommendations simply suggest a single dose of 250 mg of ceftriaxone for localized gonorrhea, irrespective of site of infection or local susceptibility patterns of the organism to therapeutic antimicrobial agents (19).

SPECIFIC ANTIMICROBIAL AGENTS

Sulfonamides

Although the principle of sulfonamide therapy for bacterial infections was conceived shortly after the turn of the century, the actual production of effective agents was delayed until the mid-1930s. Sulfanilamide, under the name Prontosil, was tested as a treatment of gonorrhea. Many studies were published between 1937 and 1940, and the outcomes were quite varied. In some series (22, 24), there was a >90% improvement or cure rate, while in others, there was some benefit to treatment in about half the patients but a fair number of relapses (74). In no case was there any comment about in vitro susceptibility testing, but these studies differ from modern clinical trials in many other ways, including poor standardization of drug purity, lack of appropriate dose-ranging studies, and lack of search for nongonococcal urethritis as a reason for clinical failure. Sulfonamide resistance occurs spontaneously and frequently in *Neisseria* spp., and this could have been responsible for a number of treatment failures. A few authors blamed concomitant irrigation therapy for some of the treatment failures as well as for causing occasional cases of ascending infection. Many clinicians were reluctant to abandon mechanical treatment even after sulfonamides came into wide use because of a perception of poor efficacy of drug treatment alone (74).

A 1955 paper by Del Love and Finland described the susceptibility of *N. gonorrhoeae* to 12 antimicrobial agents, including sulfadiazine (25). They compared the susceptibility of *N. gonorrhoeae* obtained in 1949 with that of strains collected in 1953 to 1954. Although sulfadiazine was not a very potent antigonococcal agent in either period, the earlier strains had a median susceptibility more than fourfold lower than that of later strains. It is tempting to speculate that after the widespread use of penicillin for the treatment of gonorrhea there was less selective pressure for sulfa-resistant strains to persist.

Penicillin

The development of penicillin had an enormous impact on the management of patients with gonorrhea, and the rapid curative properties of this drug were quickly appreciated (82). The study of antimicrobial resistance also advanced rapidly with the discovery of penicillin. Appreciation of enzyme-mediated resistance and reproducible, quantitative measurements of susceptibility were in the published literature when penicillin was introduced into clinical practice at the end of World War II. The earliest susceptibility tests were performed on common organisms, including enteric gram-negative rods, *Staphylococcus aureus*, and *N. gonorrhoeae*, but the fastidious growth requirements of *N. gonorrhoeae* limited the practical availability of routine testing, and the initial uniformly successful clinical experience with penicillin rendered such testing superfluous.

Curiously, there was an intense interest in the prospect of the development of resistance to penicillin by a variety of bacteria. By 1945, experiments to search for the evolution of gonococcal penicillin resistance by serial passage of gonococci on subinhibitory concentrations of antibiotics had been done. In one such paper (2), 32 weeks of exposure to penicillin resulted in an 8- to 400-fold increase in the MIC of the drug for *N. gonorrhoeae*. The highest MIC measured in this study was 2 U (approximately 1 μg) of penicillin per ml. This strain was able to sustain this level of resistance to penicillin and presumably represents the end result of multiple mutations to diminish the susceptibility of the gonococcal penicillin-binding proteins and/or to reduce outer membrane permeability to penicillin. Even allowing for differences in MIC testing, this order of magnitude of the MIC is consistent with the chromosomally mediated resistance (CMRNG) to penicillin commonly seen today.

TABLE 1. Percentage of patients experiencing treatment failure after receiving aqueous procaine penicillin G (4.8×10^6 U intramuscularly) and probenecid (1 g orally) correlated with MICs of penicillin (43, 51)

MIC (μg/ml)	No. of isolates	% Treatment failures
≤0.008	75	4.0
0.015	180	2.8
0.03	73	1.4
0.06	96	1.0
0.125	183	2.7
0.25	191	4.1
0.5	128	6.3
≥1.0	52	13.5

When penicillin was relatively scarce and expensive, there was an effort to use the smallest effective dose. Over time, some authors reported poor results with a very low (≤300,000 U) or a low (<1,000,000 U) dose of penicillin. At the same time, physicians noted the community-wide spread of strains of *N. gonorrhoeae* with increasing MICs. This increase in MIC was definitely associated with an increased failure rate following penicillin therapy. A Boston study from 1963 showed that men who were given 600,000 U of penicillin had a 1.4% failure rate when the penicillin MIC was <0.125 μg/ml but a 32% failure rate when the MICs were ≥0.125 μg/ml (63). Later studies showed a good correlation between MIC and treatment failure even with higher doses of penicillin (2.4×10^6 to 4.8×10^6 U) (47). Table 1 shows the results of treatment with high-dose penicillin and probenecid as correlated with penicillin MIC in a large multicenter study done from 1972 through 1974 (before the detection of PPNG) (52, 62). In an effort to forestall an increase in the MIC of penicillin, a strategy to use high-dose therapy was launched. In a study performed in Greenland, where the population is somewhat geographically isolated and island-wide data can be easily collected, antibiotic susceptibilities were analyzed year by year from 1964 through 1970 (86). An initial drop in MICs lasted for a few years after the adoption of a standard dose of 5,000,000 U of penicillin given intramuscularly. Within a few more years, however, the pattern had reversed, and the MICs were slightly higher than at the beginning of the study. This suggests that even consistent efforts to use high doses cannot prevent the emergence of resistant strains indefinitely.

N. gonorrhoeae in the United States showed a comparable decrease in susceptibility to penicillin. A study published in 1970 showed that, between 1955 and 1969, maximal and mode susceptibilities increased and susceptibilities were distributed more widely throughout the tested MIC range (78). Furthermore, in comparing 771 cultures collected from 1945 through 1954 with 1,124 cultures collected from 1955 through 1965, the percentage of strains for which penicillin MICs were >0.05 U/ml (approximately 0.03 μg/ml) increased greatly, from 0.6 to 42%. Another study published in 1968 compared MICs of penicillin and tetracycline for strains obtained in 1965, 1967, and 1968 in the Seattle, Wash., area

(92). The number of strains with relatively high MICs of both agents unmistakably increased in the last 2 years of this study, and the association between MICs of penicillin and tetracycline was strikingly clear.

It is now widely recognized that there are distinct resistance mechanisms to consider when clinical failure based on lowered susceptibility follows treatment with penicillin (29). Chromosomal resistance is the result of serial changes in the structure of penicillin-binding proteins and/or outer membrane permeability that culminate in penicillin MICs that exceed 1 μg/ml. In an epidemic in North Carolina, 199 patients were found to be infected with a strain of *N. gonorrhoeae* for which the penicillin MIC was 2 to 4 μg/ml (33). None of these isolates produced β-lactamase, and auxotyping, serotyping, and AST suggested that a single clone of *N. gonorrhoeae* was responsible for all cases. Of the 16 patients who were treated with the standard doses of penicillin, 4.8×10^6 U, accompanied by 1 g of probenecid, 15 failed therapy.

The other mechanism for stable, heritable penicillin resistance is the elaboration of β-lactamase enzyme. This was first reported in 1976, when two different plasmid-bearing strains were discovered to have very high penicillin MICs, the capacity to produce a TEM-like penicillinase, and an almost universal failure to respond to high-dose penicillin therapy (16). The initial epidemiology of these PPNG strains showed a spread from Asia and Africa throughout the world. The Asian and African varieties of PPNG showed a distinct difference in plasmid size, which suggested that the two plasmids evolved separately but probably from a common ancestor that resembled the larger Asian-type plasmid. The enzyme was identical to the one in *Haemophilus influenzae* and was thought to be acquired from that organism, which had only a few years earlier acquired this enzyme (presumably from an enteric gram-negative bacillus). Since 1976, the PPNG phenotype has been found in most countries, and it has been associated with many different serotypes and auxotypes. For example, in a 1987 Canadian survey (26), 138 PPNG phenotypes were distributed among 14 different serovars, several auxotypes, and several plasmid profiles. This reflects both strain geographic dispersion and sharing of plasmids among strains. A similar study in 1988 with strains collected in Africa showed nine different patterns of chromosomal DNA (using restriction endonuclease testing) among 27 strains of PPNG (32). These patterns correlated with auxotype, but further distinctions could be made with serotyping. Thus, these 27 strains had 17 unique combinations of serotype and chromosomal DNA restriction pattern.

A Japanese study showed that there can be an interaction between chromosomal and plasmid-mediated resistance that can influence the final MIC of a drug for a strain (49). Here, transformation of a penicillin-susceptible strain with a β-lactamase-coding plasmid resulted in a 32-fold increase in MIC, whereas the same plasmid caused a 128-fold increase in MIC when transformed into a CMRNG strain.

Many studies have explored chromosomally mediated penicillin resistance and its association with resistance to other antimicrobial agents (53). In several studies, other β-lactams such as cefmetazole (26), cefotetan (59), and cefuroxime (40) showed diminished in vitro potency for CMRNG compared with penicillin-susceptible strains. In some cases, there was a weaker association between the MIC of penicillin and those of spectinomycin and tetracycline (61). Some single mutations can give rise to decreased susceptibility to a variety of antimicrobial agents such as tetracycline, erythromycin, rifampin, and chloramphenicol, which are all restored in concert when the mutation is reversed (77). The last examples suggest some nonspecific alteration in the bacterial cell such as a permeability alteration, while the first supports a change in penicillin-binding protein (the target site for all β-lactams).

Clinical outcome is very strongly associated with susceptibility to penicillin. A study comparing the use of penicillin alone or in combination with probenecid showed very few failures in the probenecid-containing regimens (23 of 489, or 4.7%), and some of these failures may represent reinfection (47). But in the penicillin-alone arm, there were 70 failures in 651 cases or a 10.7% failure rate. When the failure rate was correlated with the MIC for the pretreatment culture, there was a very strong association for both men and women. A British study had suggested the same close correlation between MIC and treatment outcome in 1969 (72).

Tetracyclines

Ironically, the development of antimicrobial agents in other classes such as spectinomycin and tetracycline made susceptibility testing relevant even for bacteria that were uniformly responsive to penicillin. Adverse reactions to penicillin and reluctance to accept parenteral therapy were the most common contraindications to penicillin use. In addition, the occurrence of postgonococcal genital infection made the search for alternative therapies important. One of the biggest problems with the use of tetracyclines in the treatment of gonorrhea is that single-dose oral therapy (even with very high doses) is prone to failure. Thus, most papers recommended at least two doses, and often more, of tetracycline, doxycycline, minocycline, or some other tetracycline. Given the variations in dose and treatment schedules, it may be more difficult to associate response to treatment with the results of in vitro susceptibility tests. In many cases, short-course tetracycline regimens were compared with single-dose penicillin therapy. There was concern about the ability of short courses of tetracyclines to abort incubating syphilis as well as worry about the compliance of patients with a multidose, unsupervised treatment regimen. Still, studies comparing the outcome of treatment with the MIC of tetracycline showed a good correlation. A trial of methacycline versus doxycycline showed almost identical response curves for the two drugs when the percentage of patients who failed treatment was plotted against the MIC of the drug used (104).

As was the case with penicillin, there was an early appreciation of variability in MICs of tetracycline. For many strains, the MICs were sufficiently high to account for occasional treatment failures. However, in 1984 and 1985, a new, high-resistance (MIC, >16 μg/ml) phenotype was discovered in the United States (68). This was attributed to a new tetracycline resistance gene that was plasmid borne. This gene spread fairly quickly through *N. gonorrhoeae* strains in the United States and abroad. The first 99 such strains studied in the United States were divided into 19 serotype-auxotype categories. There was strong evidence that a conjugative plasmid (also found in many strains of PPNG) was responsible for disseminating this high-level tetracycline resistance.

Although no antimicrobial agent is recommended as routine post-sexual exposure prophylaxis for gonorrhea, a trial of minocycline showed an excellent dose-response curve for the correlation between the prevention of infection and the MICs for prevailing strains (46). A 200-mg dose of minocycline was 100% effective at preventing infections with *N.*

TABLE 2. Percentage of men experiencing treatment failure after receiving erythromycin (9 g over a 4-day period) correlated with MICs of erythromycin (8)

MIC (μg/ml)	No. of isolates	% Treatment failures
0.06	6	0
0.125	23	4.3
0.25	40	12.5
0.5	27	29.6
1.0	19	68.4
2.0	5	80

gonorrhoeae when the tetracycline MIC was <1.0 μg/ml and 0% effective when the MIC was >2.0 μg/ml. *N. gonorrhoeae* strains for which MICs were 1 or 2 μg/ml caused delayed infection in a variable percentage of patients.

Spectinomycin

Almost every antimicrobial agent developed has been looked at for potential activity and/or clinical utility with respect to gonorrhea. One antibiotic, spectinomycin, was developed and marketed with only one indication: treatment of infection caused by *N. gonorrhoeae*. While spectinomycin does not show the degree of potency associated with penicillin in terms of very low MICs, there is a consistently narrow range of MICs that indicates a high probability of clinical efficacy. Resistance to spectinomycin was first described in 1973 (90) and emerged in the early 1980s (107) as a widespread phenomenon, though it was relatively rare in some parts of the world. Nevertheless, clinical failures are closely associated with high-level in vitro resistance (8, 80).

Erythromycin

Erythromycin is a widely used oral antibiotic. It is thought to be safe even during pregnancy and is an important therapeutic agent in the treatment of nongonococcal urethritis and cervicitis. However, in vitro and clinical data show it to be of limited usefulness in the treatment of gonorrhea. In a study from 1977, the median MIC was 0.25 μg/ml, and the overall treatment failure rate was 24% irrespective of whether the estolate or a base preparation was used (9). There was a very strong association between increasing MIC of erythromycin and the risk of treatment failure. The correlation of failure rate with erythromycin MIC from this study is shown in Table 2. Somewhat better results were obtained when erythromycin was combined with rifampin (which itself has good antigonococcal activity) (6).

TMP-SMX

Despite disappointing results with sulfa in the early days of antimicrobial chemotherapy, combination treatment of gonorrhea with trimethoprim and sulfamethoxazole (TMP-SMX) has been tried with generally good results. Two studies published in 1973 exemplify the overall experience with TMP-SMX. In the first study, three different treatment schedules were tried on a total of 2,687 patients (71). Large doses given for a short time were more successful than smaller doses given over a longer time (98% cure for four tablets twice daily for 2 days versus 82.5% cure for one tablet four times daily for 5 days). The patients who failed to respond usually were infected with strains of *N. gonorrhoeae* with MICs of TMP of ≥50 μg/ml and MICs of TMP-SMX (1:20 ratio) of ≥4 μg/ml. The other study also showed generally good outcomes of TMP-SMX treatment, i.e., comparable to 4.8×10^6 U of penicillin plus 1 g of probenecid or a total 9-g multiple-dose schedule of tetracycline (1). Again, the regimen of higher but fewer doses performed better than the regimen of lower but more frequent doses. The other observation, however, was of regional differences in both outcome and TMP-SMX susceptibility. Again, higher MICs of either or both of these agents (which correlated closely with one another) were predictive of likely clinical failure.

Cephalosporins

β-Lactamase-stable cephalosporins have acquired an important role in the treatment of gonorrhea in direct proportion to the reduced efficacy of penicillin. Like penicillin, cephalosporins provide the ease and security of single-dose therapy with high confidence in a good clinical outcome. This was made clear in a 1979 study, in which 2 g of intramuscular cefoxitin (plus 1 g of oral probenecid) was shown to have complete success in 54 consecutive patients (from 21 of whom PPNG had been isolated), whereas penicillin had a dismal success rate of 64% (5). In vitro testing showed that cefoxitin MICs for PPNG strains were slightly lower than those for non-PPNG strains. Other in vitro (59) and clinical studies with so-called expanded- and broad-spectrum cephalosporins also showed good activity.

Ceftriaxone has a significant contemporary role in the treatment of gonorrhea because of its very high intrinsic potency, lack of resistant strains, and long half-life. A single dose of 250 mg, which can be injected into a deltoid muscle, has a cure rate comparable or superior to that of any other therapy. Thus, ceftriaxone has become the drug of choice in current Centers for Disease Control recommendations (18). Recently, an oral cephalosporin, cefixime (58), showed clinical activity equivalent to that of ceftriaxone (96 to 98% cure) after a single 400- or 800-mg dose (44, 69, 81). The greater ease and patient acceptance of oral therapy may well favor such an agent. One of the concerns about the β-lactamase-stable cephalosporins is that, for non-PPNG strains, the MIC of penicillin is highly correlated with the MIC of the cephalosporin. In Philadelphia in 1989, we showed a strong log-linear correlation between penicillin and cefmetazole MICs for 122 non-PPNG strains (Pearson $r = 0.787$) (34). Several strains had cefmetazole MICs of ≥4 μg/ml, suggesting that they might not be eradicated with single-dose cefmetazole. To a lesser extent, this has been shown with other cephalosporins such as cefuroxime (75), cefpodoxime (35, 93), cefoxitin (75) and even ceftriaxone (50, 102). The very widespread use of cephalosporins could conceivably accelerate the process of selecting less and less β-lactam-susceptible strains in the way that CMRNG increased slowly in incidence over the decades during which penicillin was the drug of choice for gonorrhea. Some authors think that the very slow emergence of strains with increased ceftriaxone MICs augurs well for the oral cephalosporins, since levels in serum and tissue are well above the MIC for 100% of *N. gonorrhoeae* strains studied (44).

Quinolones

Since newer fluoroquinolones are generally very active against gram-negative bacteria, are quite safe when administered to adults, and have good absorption and tissue penetration, it would be logical to consider them for the

treatment of gonorrhea. Several recent in vitro studies confirm excellent antigonococcal activity for a variety of fluoroquinolones (3, 30, 31, 38, 39). Fuchs et al. propose a breakpoint of ≤0.06 μg/ml (and a zone diameter of ≥36 mm) for gonococcal susceptibility to ciprofloxacin, but it should be noted that all of their clinical isolates fell into this category (38). A large clinical study conducted in Africa showed that treatment of 83 adult men with ciprofloxacin (a single, oral 250-mg dose) was 100% effective (11). The success rate for ceftriaxone was 98.7%. This population had a high percentage of patients with human immunodeficiency virus infection (>30%) and concomitant syphilis (>10%). The MIC testing identified only one strain that had a ceftriaxone MIC of >0.06 μg/ml, and all strains had ciprofloxacin MICs of ≤0.03 μg/ml. An earlier study that used two doses of norfloxacin also had an excellent outcome (23). Regrettably, resistance to fluoroquinolones appears to emerge quickly. The ciprofloxacin MICs for all gonococci isolated at a London hospital in 1989 were determined after two patients with ciprofloxacin-resistant strains failed oral therapy (42). Of 959 strains tested, MICs for 23 (4%) strains were ≥0.015 μg/ml, and these were all from the second half of the year. These strains showed reduced susceptibilities to other quinolones (nalidixic acid and ofloxacin) as well. Auxotyping and serotyping showed that several different clones were represented among these 23 strains; some were thought to be imported, while others were thought to be domestically acquired. There was no evidence of direct sexual contact among attenders of this clinic who carried *N. gonorrhoeae* with elevated MICs of ciprofloxacin. The precise breakpoint for ciprofloxacin resistance is not universally agreed upon, but there is evidence that infection with strains with higher MICs is more likely to lead to failure following a single 250-mg dose.

TECHNICAL PROBLEMS WITH GONOCOCCAL AST

Testing Media

Although laboratorians were aware of the fastidious growth requirements of *N. gonorrhoeae* from the beginning of the antimicrobial era, culture tests to isolate and propagate the organism from sterile sites or from those with a native bacterial flora were easy to develop. As is the case for other fastidious bacteria, special conditions are required to obtain consistent, reliable AST results for *N. gonorrhoeae* (28). For example, Mueller-Hinton agar, which is widely used in AST of enteric gram-negative rods and *Pseudomonas* spp., may contain inhibitors for the growth of *N. gonorrhoeae* (73). On many media that can support most gonococcal strains, growth is either light or inconsistent or additives that are difficult to standardize are required. Hemoglobin has been used frequently as an additive to enhance the growth of *N. gonorrhoeae*. In a carefully controlled study, the inclusion of 1% hemoglobin was responsible for consistent, though insignificantly lower MICs of penicillin, spectinomycin, and erythromycin but a more than twofold increase in the MIC of tetracycline (21). This variation in tetracycline MIC is potentially a serious limitation, because the distribution of MICs of tetracycline is such that moving the breakpoint for resistance by one dilution will substantially change the fraction of strains in the resistant category. Another large study found essentially no difference in the MICs of penicillin, tetracycline, spectinomycin, and ceftriaxone with or without hemoglobin supplementation for *N. gonorrhoeae* strains (70). However, 10 strains were unable to grow on the hemoglobin-free medium.

Inoculum Size

Inoculum can be a variable that influences the results of AST. A South African study showed that for PPNG an increase in inoculum from 10^4 to 10^7 resulted in a substantial rise in the MICs of penicillin, cefaclor, and cefamandole though not in those of a variety of other agents, including ampicillin, cefuroxime, and ceftriaxone (27).

Regional Differences

There has been awareness of regional differences in susceptibility of *N. gonorrhoeae* since ASTs were initially developed. Even before the appearance of PPNG in the United States, there were substantial differences in MICs of penicillin and tetracycline for gonococci (52). With the recognition of PPNG and high-level, plasmid-mediated tetracycline resistance, the Centers for Disease Control recommended that initial therapy, test of cure cultures, patient and provider education, and culture strategy be based on the prevalence of resistant strains found in the community (18). This is a refinement of earlier recommendations (17), which set forth the schedules but left the choice of medication to the local provider. To get a better understanding of the range of diversity in MICs from city to city, the Gonococcal Isolate Surveillance Project was implemented (94). This study established a national surveillance system to assess prospective regional susceptibility variations. In the latest report, 6,204 clinical isolates from men attending public clinics were submitted from 21 sites around the United States. MICs of penicillin, tetracycline, cefoxitin, spectinomycin, and ceftriaxone were determined. For example, in Cincinnati, Ohio, no PPNG strains were reported and only 7% of strains were CMRNG, while in Long Beach, Calif., more than 8% of strains were PPNG and 30% were CMRNG. No isolate was considered resistant to ceftriaxone, although MICs for a few strains were ≥0.06 μg/ml. In addition, some important clinical determinants for spread of new, antibiotic-resistant strains of *N. gonorrhoeae* were revealed. The total number of lifetime partners was significantly predictive of infection with tetracycline-resistant gonococci, and the number of lifetime partners, history of prior gonococcal infection, greater number of recent partners, and greater number of new partners all predicted CMRNG infection (48). These observations are a reminder that variations in human sexual activity may help predict populations at especially high risk of drug-resistant *N. gonorrhoeae* infection and that monitoring such populations may give early warning about new trends in antimicrobial refractory strains.

Test Quality Control

The accuracy of AST for *N. gonorrhoeae* is determined by quality assurance programs. In Australia, a long-standing program evaluated the error rate for MICs between 1980 and 1988, during a period of development of AST media (99). The overall error rate was 3.1% on >3,600 MIC determinations. Experience in MIC testing and the adoption of a more uniform test medium (Iso-Sensitest agar with 8% lysed horse blood) yielded more consistent results over the 9 years of the study. In the United Kingdom, a different scheme is used to examine the accuracy of clinical laboratories that do AST for *N. gonorrhoeae*. In one study, six strains were sent to 411

laboratories (320 responded) for testing of four antimicrobial agents (96). The laboratories rarely reported susceptible strains as being resistant (averaging 97% accuracy) but commonly reported resistant strains as being susceptible (7% for penicillin, 96% for cefuroxime, 76% for tetracycline, and 8% for spectinomycin). The testing was done mostly by disk diffusion, and the use of disks of different potencies was in part responsible for the errors in tetracycline and spectinomycin susceptibility results. A similar program in the United States, conducted from 1980 through 1987, showed that laboratories were able to identify *N. gonorrhoeae* and determine whether or not β-lactamase was being elaborated, but only a minority of laboratories did AST (54). The laboratories that used disk diffusion testing had an overall 98% accuracy rate for five tested antimicrobial agents in 1984. The results for MIC determinations showed an overall 92% accuracy, with cefoxitin MICs being the least accurately determined (78%).

Despite some good results in correlating disk diffusion testing with MICs (98), no single protocol for AST of *N. gonorrhoeae* has been widely accepted. The combination of inconsistency in testing conditions, reluctance to do any kind of susceptibility testing in most laboratories, and variations in quality control strains gave impetus to a multicenter approach to simplifying and standardizing agar dilution and disk diffusion testing (57). This ambitious project was undertaken by six reference laboratories in the United States, and four antimicrobial agents were studied initially: ceftriaxone, penicillin, spectinomycin, and tetracycline. The medium used was slightly different from those described in previous studies. The base was GC agar to which a complex supplement was added. The supplement resembled IsoVitaleX in that it contained X-like and V-like factors but did not contain L-cysteine. This amino acid has been responsible for giving falsely elevated MICs of certain antibiotics, notably, carbapenems and clavulanate (56). Each of the participating laboratories determined MICs 25 times for each of three control strains (*S. aureus* ATCC 29213, *N. gonorrhoeae* CDC F-18 [ATCC 49226], and WHO V) on several lots of agar (including a lot common to all of the laboratories). This allowed the setting up of proposed quality control ranges into which 99 to 100% of the assays fell. A similar exercise was done for disk diffusion, in which eight candidate quality control strains were narrowed down to three (*S. aureus* ATCC 25923 and the same *N. gonorrhoeae* strains used for agar dilution). Each of these three strains was tested more than 3,000 times to determine the zone diameters around disks of the four antibiotics in the study, and again, a proposed zone diameter quality control range was established. The final part of this study was the application of these standard conditions to 100 clinical isolates of *N. gonorrhoeae* and the correlation of the $\log_2$ MIC with the zone diameters for penicillin, tetracycline, and ceftriaxone. This allowed the establishment of zone diameter interpretive criteria for susceptibility and resistance.

An underappreciated problem with disk diffusion testing for *N. gonorrhoeae* is the relatively poor correlation of disk diffusion zone diameters with $\log_2$ MICs for certain drugs. Although Pearson *r* values are often given for this correlation, the information used to validate the disk diffusion test has to do with the number of interpretive errors that use breakpoints between highly susceptible and moderately susceptible and between moderately susceptible and resistant. Since MICs of ceftriaxone are in the highly susceptible range for all strains, there are no interpretive errors despite low *r* values. In the large cooperative study designed to set up quality control criteria for disk diffusion and agar dilution AST, the *r* values were 0.97 for penicillin, 0.88 for tetracycline, and 0.65 for ceftriaxone (57). In our study in 1991, the *r* values were 0.96 for penicillin, 0.94 for tetracycline, 0.62 for ceftriaxone, and 0.62 for cefpodoxime (34). Similar findings were reported for two other very potent cephalosporins: cefotaxime had an *r* value of 0.79, and ceftazidime had a value of 0.63 (55). Of the less potent agents in this study, cefoxitin had an *r* value of 0.93 and cefuroxime had a value of 0.95. In a more recent study, we showed a similarly poor correlation between $\log_2$ MIC and disk diffusion zone diameter for temafloxacin ($r = 0.74$), a fluoroquinolone no longer available (36). We proposed that the problem may be related to a combination of a narrow range of MICs and a very large zone (which may be difficult to read with accuracy).

Methods for Strain Characterization That Predict Susceptibility

Several epidemiological tools have been developed to characterize individual strains of *N. gonorrhoeae* and to classify these strains into groups that are genetically related. Some of these phenotypic properties may also predict susceptibility to antimicrobial agents.

Auxotyping and serotyping. A casual glance at the current literature on the epidemiology of *N. gonorrhoeae* would show considerable data on the auxotypes (strain-dependent nutritional requirements) and serotypes of the strains under study. This approach dates to studies published by Catlin (13) and others. In developing a chemically defined medium for the growth of *Neisseria* species, it was noted that, while meningococci had relatively uniform growth requirements, gonococci could be divided into 13 distinct auxotypes depending on growth responses to seven organic chemicals: thiamine pyrophosphate, thiamine, proline, arginine, methionine, isoleucine, and hypoxanthine. These growth requirements were stable and could thus be used as markers for a strain. Two subsequent developments made this observation even more valuable. In 1973, strains of gonococci responsible for disseminated infections were noted to have substantially lower MICs of penicillin and tetracycline than strains that caused local infection (103). Then in 1975, Knapp and Holmes showed a very strong correlation between strains that cause disseminated infection and those that required arginine, hypoxanthine, and uracil and had the previously described decreased MIC of penicillin (65). This marker was thus able to predict the likelihood of disseminated gonococcal infection (given the right clinical setting) as well as the likely response to penicillin therapy. In 1977, Catlin and Pace showed that gonococci with variable growth requirements had a selective ability to colonize the oropharynx versus the anogenital mucosa (14). There also seems to be a propensity for various auxotypes to cause infections in homosexual men rather than in heterosexual men or women (41). Thus, auxotyping seemed to be a marker for the propensity for various strains to colonize and infect certain body sites, to be passed through different populations, to invade and cause disseminated infection, and to serve as predictors of susceptibility to antimicrobial agents.

A corollary use of auxotyping has been in the study of the evolution of *N. gonorrhoeae*. One study compared nutritional requirements and antimicrobial susceptibilities of strains saved between 1935 and 1948 (15). The strains (from Denmark and the United States) were uniformly susceptible to the usual antimicrobial agents studied except sulfadiazine.

However, some of the nutritional phenotypes, such as the arginine-hypoxanthine-uracil combination (AHU) associated with disseminated infection, were not found at all. A continuation of this study in Denmark showed the emergence and later dominance of AHU strains from the late 1940s to the 1980s (66). In the 1950s, a proline-arginine-uracil-methionine-requiring auxotype appeared, peaked at 30% of *N. gonorrhoeae* isolates in 1953, and disappeared totally in the early 1960s. A similar observation was made with strains auxotrophic for proline, citrulline, and uracil in Canada (10). This auxotype was similarly not found in the early days of penicillin therapy or in an analysis of 493 strains from the United States collected between 1972 and 1974, but strains auxotrophic for proline, citrulline, and uracil made up 18% of the isolates in the Canadian study and were associated with asymptomatic urethral carriage in men. Surveys in the United States and Sweden have corroborated this increase. In general, strains that cause minimal symptoms have an ecological advantage, since there is a lower likelihood of eradication and a higher likelihood of spread if the infected person is unaware of the infection.

Serotyping adds another dimension to the characterization of strains of *N. gonorrhoeae*, as it does for a variety of other bacteria. Various schemes divide gonococci into three serogroups (W-I, W-II, and W-III) on the basis of immunologic properties of the principal outer membrane protein (PI). In two outbreaks of PPNG infection in the United States, a combination of auxotyping and serogrouping demonstrated the spread of two distinct strains (45). Subdivisions in the serogroups allow for more precise discrimination of strain characteristics and permit more rational decisions regarding the assessment of local epidemics. The W-I serotype contains variants of protein IA, and the W-II and W-III serotypes possess variants of protein IB (67). Coagglutination tests can discriminate among these serovariants without much difficulty. In addition, serotyping correlates to some degree with antimicrobial susceptibility and site of infection (105). In a British study, the proportion of serovar IA-1/2 in cervical isolates was four times higher than the proportion in male rectal isolates, whereas the proportion of serovar IB-2 in male rectal isolates was more than three times higher than the proportion in cervical isolates. The authors speculate that this may be intimately related to the function of PI, the principle outer membrane protein whose epitope variations are determined by serotyping. The porin channel formed by this protein may confer a selective survival advantage in various environments (such as the hydrophobic rectum) as well as provide differing antimicrobial permeation into the cell. In a paper from Zimbabwe, the activities of penicillin, cefuroxime, tetracycline, ciprofloxacin, thiamphenicol, and erythromycin (but not kanamycin or spectinomycin) against serotype W-I strains were clearly superior to those against W-II and W-III strains (79).

Combinations of auxotyping, serotyping, and AST can give important information about the epidemiology of and clinical approach to gonorrhea (41, 64). Even with AST as the only tool for characterizing strains of *N. gonorrhoeae*, substantial regional variations clearly exist within the United States. In a nationwide survey of gonorrhea in 1974, strains with penicillin MICs of $\geq$0.5 μg/ml represented 7% of all strains in Des Moines, Iowa, and 27% in Denver, Colo., with almost identical percentages with MICs of tetracycline of $\geq$2.0 μg/ml in those two cities (52). A large study in Chicago that explored the characteristics of strains of *N. gonorrhoeae* causing disseminated infection versus those causing local infection showed that, in general, AHU phenotype, serum resistance, serogroup 1A, and low penicillin MICs were associated with disseminated infection (7). However, the very low MIC of penicillin was a characteristic of AHU strains; the penicillin MIC for non-AHU strains was not lower in the disseminated gonococcal infection group than in the strains associated with local infection. Thus, it is the presence of the AHU auxotype rather than the phenomenon of dissemination that predicted low penicillin MICs in cases of disseminated gonococcal infection in this population. This is compatible with the observation that disseminated gonococcal infection can be caused by PPNG (12) as well as CMRNG (97). In a large study done in Seattle, Wash., 1,433 strains of *N. gonorrhoeae* from around the world were typed, and the results showed 107 different auxotype-serotype combinations (67). This study reinforces the value of such measures in defining the distribution of strains in epidemics or across time and space.

Plasmid analysis. Determining the sizes and restriction endonuclease fragment patterns of gonococcal plasmids has been another fruitful way of comparing and characterizing strains. Clearly, plasmids are responsible for carrying many of the genes that permit antimicrobial resistance, so their presence and gene contents can influence antibiotic susceptibility. In a study from Dade County, Fla., two different plasmids with genes coding for the β-lactamase enzyme were in circulation between 1983 and 1986 (108). The smaller (3.2-MDa) plasmid was associated with a lower penicillin MIC than the larger (4.4-MDa) plasmid. Continued use of penicillin and ampicillin led to the predominance of the larger plasmid in PPNG in Dade County until spectinomycin was offered as the standard initial therapy. At that point, the smaller plasmid again began to predominate in the PPNG strains.

Detailed plasmid analysis can shed light on the genesis and spread of strains of *N. gonorrhoeae*. In a Canadian study, a novel 4.9-kb plasmid was found in 20 isolates of PPNG from two provinces (106). All strains had the same auxotype and serotype, and analysis of the plasmid suggested that it was derived from the Asian-type strain which itself may be the parent of the African type or variety. While the Toronto plasmid is close in size to the African type, it includes material from the Asian-type plasmid that is lacking in the African-type plasmid and lacks material that is present in both the Asian- and the African-type plasmids.

Combinations of serotype and auxotype analyses with plasmid content are often predictive of the MICs of antimicrobial agents in PPNG. A Dutch study showed that the vast majority of strains that bore the Asian-type plasmid were proline requiring and that each of these phenotypes was independently associated with higher MICs of cefuroxime, cefotaxime, erythromycin, thiamphenicol, and tetracycline (100).

CONCLUSIONS

Despite many changes in the technology and interpretation of in vitro tests to determine gonococcal susceptibility to antimicrobial agents over five decades of chemotherapy, there has been surprising accuracy in correlating test results with clinical outcomes (91). In fact, this correlation has been more consistently shown for *N. gonorrhoeae* than for any other infectious agent. There are several reasons for this. First, gonococcal infections are very common and thus make possible large-scale studies by individual investigators or as part of cooperative studies. Second, the people who acquire gonorrhea are, in general, young and healthy, so there is less

variation in host factors than for infections that accompany a compromise in host defense. Third, there has been intense interest in curing gonorrhea quickly to minimize the risk of noncompliance with therapy and to abort the spread of the infection throughout the community. This simplifies study design, leads to single-dose regimens, and permits quick evaluation of efficacy. Fourth, while contemporaneous susceptibility testing of *N. gonorrhoeae* is somewhat cumbersome compared with that of enteric gram-negative bacilli or *S. aureus*, batch testing is only slightly more difficult for the gonococcus than for conventional pathogens. Fifth, the understanding of the epidemiology of gonorrhea as determined by techniques of auxotyping, serotyping, and plasmid analysis has given additional impetus to studying antibiograms to help decide when to apply the more specific but also more cumbersome grouping technologies. In this way, standardized AST (83–85) can be a screening test in public health much as it is in hospital infection control.

Sophisticated testing using organism nutritional requirements, presence of stable serologic markers, plasmid or chromosomal DNA analysis, and other tools has illuminated the complex ecology of *N. gonorrhoeae*. Patterns of human sexual behavior are entirely responsible for the continued existence and evolution of this species. Clearly, there are major regional variations in the prevalence of certain strains, and new strains are constantly being introduced (88). Thus, some factor(s) must be responsible for determining which strains are able to establish persistent residency. Ability to sustain colonization and ability to cause minimal symptoms are certainly important, but the ability to resist killing by antimicrobial agents or to develop increasing resistance after exposure has also been demonstrated as the most important clinical feature. Without a doubt, continued monitoring of *N. gonorrhoeae* strains will reveal new patterns of resistance and epidemiology, and some kind of a local ongoing monitoring of ASTs will be an important way of documenting these changes.

Current recommendations for treatment of gonorrhea in the United States rely heavily on cephalosporin therapy (19). Its continued high efficacy is crucial to keeping the recommendations for therapy simple, since resistance to all other classes of antimicrobial agents is well described. If the trend toward a slowly increasing top MIC for ceftriaxone continues, concurrent susceptibility testing at the time of species determination may become the reality for clinical laboratories. Fortunately, we now have techniques that are well adapted to large-scale use and that can give AST results in as timely a fashion as those for conventional bacterial pathogens (57). A long history of success in predicting gonorrhea treatment outcome on the basis of AST is very reassuring in justifying the expense and inconvenience of such testing, should it become necessary. Until we routinely need to use AST, some kind of ongoing testing (such as sentinel monitoring at public health clinics or among populations with higher-than-average numbers of sexual partners) should be implemented. These measures might pick up local variations that could influence treatment schedules, which may vary by geographic area.

So, while the case for immediate introduction of universal AST for *N. gonorrhoeae* cannot be strongly made, it is probably true that information on susceptibility is no more difficult to obtain for this pathogen than for many other fastidious bacteria that are routinely tested. There is ample evidence that gonococcal AST has been and continues to be consistent and reliable despite minor variations in recommended test conditions. With a commercial, prepared agar medium for disk diffusion testing available and with the careful determination of quality control parameters assembled by large multicenter studies (57, 58, 105), it will be interesting to see whether clinical laboratories will rise to the challenge and begin to determine more antimicrobial susceptibilities for their isolates of *N. gonorrhoeae*.

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