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MINIREVIEW

Antimicrobial Susceptibility Testing of *Haemophilus influenzae*, *Branhamella catarrhalis*, and *Neisseria gonorrhoeae*

GARY V. DOERN[1]* AND RONALD N. JONES[2]

University of Massachusetts Medical Center, Worcester, Massachusetts 01655,[1] and The Clinical Microbiology Institute, Tualatin, Oregon 97062[2]

INTRODUCTION

During the last two decades, resistance to selected antimicrobial agents commonly used to treat infections caused by *Haemophilus influenzae* and *Neisseria gonorrhoeae* has become increasingly prevalent. In addition, during the last 10 years, information has accumulated which supports the role of *Branhamella catarrhalis* as the etiologic agent of a variety of human infections. It is now clear that most clinical isolates of *B. catarrhalis* produce β-lactamase and are, therefore, possibly not susceptible to beta-lactam antimicrobial agents such as penicillin, ampicillin, and amoxicillin, agents often considered the drugs of choice for treating infections such as those with which *B. catarrhalis* is often associated. The recognition of antimicrobial resistance among these three organisms has led to a need for in vitro susceptibility test procedures and interpretive criteria for the results which reliably predict therapeutic outcome. Because of the fastidious nature of these three organisms, susceptibility testing procedures which effectively assess the in vitro activity of antimicrobial agents for rapidly growing bacteria are of questionable value. The intent of this review is to provide specific recommendations for in vitro susceptibility testing of *H. influenzae*, *B. catarrhalis*, and *N. gonorrhoeae*.

H. INFLUENZAE

Susceptibility test methods. The results of qualitative and quantitative susceptibility test methods with *H. influenzae* are clearly influenced by the composition of the test medium (5, 44, 68). Numerous different media have been advocated for use when testing this organism (52). Until recently, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended that chocolate Mueller-Hinton agar (i.e., Mueller-Hinton agar supplemented with 1% bovine hemoglobin and a source of V-factor) be used to perform disk diffusion susceptibility tests with *H. influenzae* (50). Zone diameter interpretive criteria have been defined for ampicillin, chloramphenicol, amoxicillin-clavulanate, and ampicillin-sulbactam (50). The medium recommended for broth dilution tests consists of cation-adjusted Mueller-Hinton broth supplemented with 3 to 5% lysed horse blood and a source of V-factor (51). MIC interpretive criteria have been developed for the same four antimicrobial agents mentioned above (51).

Recently, extensive studies performed by Jorgensen and colleagues have led to the development of an alternative medium for performing both disk diffusion and broth dilution susceptibility tests with *H. influenzae* (37, 38). This medium has been referred to as haemophilus test medium (HTM). In its agar form, HTM consists of Mueller-Hinton agar, 15 μg of purified bovine hematin per ml, 15 μg of NAD per ml, and 5 mg of yeast extract per μl. The broth version is essentially the same, except that cation-adjusted Mueller-Hinton broth is used instead of Mueller-Hinton agar and the medium is supplemented with thymidine phosphorylase (0.2 IU/ml) in addition to hematin, NAD, and yeast extract.

The advantages of HTM in comparison with other media advocated for susceptibility tests with *Haemophilus* species include its optical clarity, its stability, its utility for testing trimethoprim and sulfonamide antimicrobial agents, the reproducibility of test results, and the fact that it soon will be commercially available in both its broth and agar forms (J. H. Jorgensen, personal communication).

A recent three-center collaborative study (G. V. Doern, J. H. Jorgensen, and C. Thornsberry, manuscript in preparation) was conducted to define the optimum conditions for performing susceptibility tests with *H. influenzae* using HTM, as well as to develop both zone diameter and MIC interpretive criteria. The results of this study serve as the basis for the following recommendations regarding susceptibility tests with *Haemophilus* species.

The following procedure is advocated for disk diffusion susceptibility tests. Colony growth from an overnight chocolate agar culture is suspended in Mueller-Hinton broth to a turbidity equivalent to a 0.5 McFarland standard by using a photometric device. This suspension is used to confluently inoculate the surface of an HTM agar plate. Antimicrobial disks are applied, and the plate is incubated for 16 to 18 h at 35°C in 5 to 7% CO_2 before zones of inhibition are measured. Zone diameter interpretive criteria and MIC correlates have been developed for 18 antimicrobial agents with HTM by using this method (Table 1). These interpretive criteria will serve as the basis for revised NCCLS recommendations regarding disk diffusion susceptibility tests with *Haemophilus* species (National Committee for Clinical Laboratory Standards, *Tentative Standard M2-T4*, in press).

Determinations of MICs with HTM are best accomplished by using a microdilution format with 100 μl as the final broth volume. A suspension of test organism equivalent to a 0.5 McFarland standard is prepared as described above for the disk diffusion procedure. Appropriate dilutions of this suspension are made, and the wells of a microdilution tray are inoculated so as to achieve a final organism concentration of 5×10^5 CFU/ml. Trays are incubated for 20 to 24 h at 35°C in ambient air before being examined. The MIC interpretive criteria for use with HTM broth and this microdilution method are the same as the MIC correlates for disk diffusion tests with HTM (Table 1).

* Corresponding author.

TABLE 1. Interpretive criteria for susceptibility tests performed with *Haemophilus* species[a]

Antimicrobial agent	Disk content (μg)	Zone diam (mm)			MIC correlate (μg/ml)	
		Resistant	Intermediate	Susceptible	Resistant	Susceptible
Amoxicillin-clavulanate	20/10	<19		>20	>8.0/4.0	<4.0/2.0
Ampicillin	10	<21	22–24	>25	>4.0	<1.0
Ampicillin-sulbactam	10/10	<19		>20	>4.0/2.0	<2.0/1.0
Aztreonam	30			>26		<2.0
Cefaclor	30	<18	19–23	>24	>32	<8.0
Cefamandole	30	<20	21–23	>24	>16	<4.0
Cefonicid	30	<20	21–23	>24	>16	<4.0
Cefotaxime	30			>26		<2.0
Ceftazidime	30			>26		<2.0
Ceftriaxone	30			>26		<2.0
Ceftizoxime	30			>26		<2.0
Cefuroxime	30	<20	21–23	>24	>16	<4.0
Chloramphenicol	30	<25	26–28	>29	>8.0	<2.0
Ciprofloxacin	5			>21		<1.0
Imipenem	10			>16		<4.0
Rifampin	5	<16	17–19	>20	>4.0	<1.0
Tetracycline	30	<25	26–28	>29	>8.0	<2.0
Trimethoprim-sulfamethoxazole	1.25/23.75	<10	11–15	>16	>4.0[b]	<0.5[b]

[a] These interpretive criteria are applicable only to strains of *Haemophilus* species when susceptibility tests are performed with HTM by the methods described in the text.
[b] Refers to the concentration of trimethoprim; the concentration of sulfamethoxazole is 19-fold higher.

It should be noted that inoculum concentrations lower than 5×10^5 CFU/ml have been advocated for broth dilution testing of *H. influenzae* (47). Use of lower inoculum concentrations usually results in decreased MICs. Furthermore, it is clear that MICs obtained with selected beta-lactam antimicrobial agents, such as cefaclor, cefamandole, and, to some extent, cefuroxime and cefonicid, increase considerably, particularly with TEM-1-type β-lactamase-producing organisms, when inoculum concentrations are increased to levels higher than 5×10^5 CFU/ml (8, 44, 66). Evidence which clearly demonstrates that the results of tests performed with either lower or higher inoculum concentrations are of greater clinical value is, however, lacking. Furthermore, inoculum sizes of 5×10^5 CFU/ml with broth dilution procedures are considered standard for quantitative susceptibility tests with other organisms (51).

Recognizing the effect of various inoculum concentrations on the results of broth dilution susceptibility tests with *H. influenzae*, for purposes of standardization it is appropriate to ascertain at some interval that the final organism concentration in the wells of microdilution trays is indeed the desired 5×10^5 CFU/ml. Furthermore, it should be understood that the concentration of organism in the initial *Haemophilus* suspension, even when carefully adjusted to a 0.5 McFarland standard, may vary significantly based on a variety of factors. Of greatest consequence is the age of the chocolate agar culture from which the suspension is prepared. For example, suspensions equivalent to a 0.5 McFarland standard contain roughly 3×10^8 to 4×10^8 CFU/ml when prepared from a 16- to 18-h chocolate agar culture, whereas 24-h growth from chocolate agar plates yields suspensions of approximately 1×10^8 to 2×10^8 CFU/ml (Jorgensen, personal communication).

The TEM-1-type β-lactamase produced by *H. influenzae* is readily detected by use of any of a variety of rapid β-lactamase assays. These include acidimetric tests (67), iodometric methods (10), and chromogenic cephalosporin procedures which utilize either nitrocefin or pyridine-1-azo-dimethylaniline cephalosporin (PADAC) as a substrate (36, 54). Assuming care is taken when performing these assays, they all seem to work well (63), although higher false-negative rates have been observed with iodometric procedures. The TEM-1 enzyme is constitutively produced in large amounts, it is extracellular, and it has high substrate affinity for penicillin, ampicillin, and the chromogenic cephalosporins (30). β-Lactamase-producing strains should be considered resistant to ampicillin, since the ampicillin MICs for such strains are ≥4.0 μg/ml (21, 23). Indeed, for most β-lactamase-positive isolates, ampicillin MICs will be ≥8.0 μg/ml (21, 23).

Production of chloramphenicol acetyltransferase (CAT) can be ascertained by using an inexpensive and simple 70-min colorimetric tube procedure (3). Test reagents are easily prepared and may be stored in frozen aliquots for prolonged periods. A commercially available filter disk modification of this assay (Remel, Lenexa, Kans.) should be used with caution, since numerous equivocal and falsely negative results were obtained in a recent evaluation of this procedure versus stock strains of *H. influenzae* (20). The accuracy of the commercial disk CAT test was found to be greatly increased, however, when the test was applied to fresh clinical isolates (G. V. Doern, unpublished observations). CAT-producing strains of *H. influenzae* should be considered resistant to chloramphenicol, since chloramphenicol MICs for such strains will be ≥8.0 μg/ml (20, 23).

Laboratory strategies for susceptibility testing of *H. influenzae*. Because of the high prevalence of TEM-1-type β-lactamase-mediated ampicillin resistance (23, 38), all clinically significant isolates of *H. influenzae* should be tested immediately with one of the rapid β-lactamase assays. β-Lactamase-positive strains can be considered ampicillin resistant. The meaning of a negative β-lactamase result must be interpreted in light of recent reports (4, 43, 55, 60) which have described clinical isolates of *H. influenzae* which are resistant to ampicillin by mechanisms other than production of a TEM-1-type β-lactamase (46, 48, 56, 60). However, the prevalence of such strains appears to be very low. In a 1986 nationwide U.S. surveillance study of antimicrobial resis-

tance among clinical isolates of *H. influenzae*, only 2 of 2,250 (<0.1%) β-lactamase-negative strains demonstrated clear resistance to ampicillin (23). For this reason, a negative β-lactamase assay nearly always means that the organism is susceptible to ampicillin. To wit, there exists little need to routinely perform ampicillin susceptibility tests directly on β-lactamase-negative isolates. In those rare instances when it is determined to be necessary to perform an ampicillin susceptibility test on a β-lactamase-negative strain of *H. influenzae*, either the disk diffusion or broth microdilution procedure described above should be used.

Systemic isolates of *H. influenzae* should be promptly examined for the production of CAT as an indication of chloramphenicol activity by using the 70-min tube CAT assay (3). This recommendation is made despite the low prevalence of chloramphenicol resistance (i.e., 0.5%) among clinical isolates of *H. influenzae* in the United States (22, 23). The assay is timely, inexpensive, and simple to perform; and it provides definitive therapeutic information. Organisms yielding positive results with the tube CAT assay should be considered resistant to chloramphenicol. As with β-lactamase-negative ampicillin-resistant *H. influenzae*, chloramphenicol-resistant strains which apparently lack the CAT enzyme have been described (9). Strains of this type are, however, extremely uncommon (23), and as a result there is little need to routinely perform chloramphenicol susceptibility tests on CAT-negative strains. Such strains may be considered chloramphenicol susceptible. Some laboratories, however, may choose not to use the tube CAT assay since this procedure requires in-house preparation and storage of test reagents. In this case, when a chloramphenicol susceptibility test is determined to be necessary, and then only with systemic isolates of *H. influenzae*, a disk diffusion or broth microdilution test as described above may be performed.

Susceptibility tests with antimicrobial agents other than ampicillin and chloramphenicol should be performed with HTM and either the disk diffusion or broth microdilution method described above. In general, however, there is little justification or need to test other agents against *H. influenzae*. Routine susceptibility testing is of little or no value with antimicrobial agents that are uniformly active (i.e., third-generation cephalosporins, imipenem, aztreonam, the fluoroquinolones, and β-lactamase-inhibitor combinations such as amoxicillin-clavulanate and ampicillin-sulbactam), that are relatively inactive (i.e., erythromycin and the sulfonamides), that lack a clinical indication for management of *Haemophilus* infections (i.e., tetracycline and penicillins other than ampicillin and amoxicillin), or for which interpretive criteria for results do not exist (i.e., the combination erythromycin-sulfisoxazole). Examples of agents for which susceptibility tests may be of some potential utility include trimethoprim-sulfamethoxazole and selected cephalosporins such as cefaclor, cefamandole, and, perhaps, cefuroxime (19, 22, 23). Routine testing of these agents is probably not warranted; however, when in individual cases it is determined to be necessary, testing should be restricted to isolates of *H. influenzae* recovered from patients with an infectious disease for which that particular agent is of potential therapeutic value.

B. CATARRHALIS

Susceptibility test methods. Quantitative dilution susceptibility tests with *B. catarrhalis* can be performed with unsupplemented Mueller-Hinton medium (26). Inoculum densities for agar dilution tests should be 10^4 CFU per spot. For broth macrotube or microdilution procedures, 5×10^5 CFU/ml is the desired final concentration of test organism (27). Plates, tubes, or microdilution trays should be incubated in ambient air at 35°C for at least 20 to 24 h. With the exception of penicillin and ampicillin, the MIC interpretive criteria published by the NCCLS (51) for use with nonfastidious aerobic bacteria may also be applied to *B. catarrhalis*. Based on the results of a recent investigation in which a large number of clinical isolates of *B. catarrhalis* were examined, strains for which ampicillin MICs were ≥1.0 μg/ml were categorized as being resistant, strains for which MICs were 0.125 to 0.5 μg/ml were defined as moderately susceptible, and strains for which ampicillin MICs were ≤0.06 μg/ml were considered susceptible (26). The same criteria should probably be applied when interpreting penicillin MICs for *B. catarrhalis*.

The medium of choice for performing disk diffusion susceptibility tests with *B. catarrhalis* is also unsupplemented Mueller-Hinton agar (26). Inoculum suspensions are prepared and plates are inoculated by the method of the NCCLS (51). Plates are incubated in ambient air at 35°C for 20 to 24 h. The following zone diameter interpretive criteria have been advocated for use when testing *B. catarrhalis* with penicillin (10-U disks) and ampicillin (10-μg disks): ≥29 mm = susceptible and ≤28 mm = resistant (42, 55). These criteria are predicated on studies which demonstrated that the large majority of β-lactamase-producing strains of *B. catarrhalis* show zones of inhibition of ≥29 mm, whereas β-lactamase-negative strains show zone sizes of ≤28 mm (41, 53).

Recently, alternative zone diameter interpretive criteria have been proposed for *B. catarrhalis* (26). Based on a comparison of zones of inhibition with MICs, the following categories were defined for disk diffusion tests which use 10-μg ampicillin disks: ≥38 mm = susceptible, 20 to 37 mm = moderately susceptible, and ≤19 mm = resistant. The MIC correlates for the susceptible and resistant categories were ≤0.06 and ≥1.0 μg/ml, respectively. The results of ampicillin disk tests could be extrapolated to predictions of penicillin and amoxicillin activity. The use of the latter zone diameter interpretive criteria means that some β-lactamase-positive strains of *B. catarrhalis* would be defined as moderately susceptible. This may be justified, however, since β-lactamase production by this organism does not necessarily correlate with either in vitro or in vivo resistance to antimicrobial agents such as penicillin, ampicillin, or amoxicillin (see below).

With respect to disk diffusion testing with other antimicrobial agents, it appears that NCCLS zone diameter interpretive criteria for amoxicillin-clavulanate, cephalothin, erythromycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole when tested against nonfastidious bacteria (51) may also be applied to *B. catarrhalis* (26). With cefaclor, a zone diameter of ≥21 mm, not ≥18 mm, was found to define the susceptible category (26). With these exceptions, zone diameter interpretive criteria of proven utility for *B. catarrhalis* do not exist.

The β-lactamase of *B. catarrhalis* is best detected by use of either the conventional tube nitrocefin assay or its disk modification (Cefinase disks; BBL Microbiology Systems) (28). Tube or disk acidimetric assays, iodometric tests, and procedures which utilize the chromogenic cephalosporin PADAC as a substrate should be used with caution, since false-negative results may be obtained when *B. catarrhalis* is tested (27, 35). This is probably the result of the specific type of β-lactamase produced by *B. catarrhalis*. The enzyme is produced constitutively in small amounts, it remains tightly

cell associated, and it has a high substrate affinity for nitrocefin (31, 39).

Laboratory approach to susceptibility testing. A high percentage of clinically significant isolates of *B. catarrhalis* produce β-lactamase (18). As a result, this organism when recovered in the laboratory should probably be examined for β-lactamase production as an indication of the activity of penicillin, ampicillin, and amoxicillin. It should be recognized, however, that penicillin, ampicillin, and amoxicillin have variable activity for β-lactamase-producing strains of *B. catarrhalis*. While MICs for most strains would be interpreted as indicating resistance, for at least some β-lactamase-producing strains MICs of these agents are 0.6 to 1.0 μg/ml (17, 27, 28, 64, 65). MICs of this low magnitude imply susceptibility. There have also been several reports of patients with infections presumably caused by β-lactamase-producing strains of *B. catarrhalis* who have responded when treated with penicillin, ampicillin, or amoxicillin (24, 34, 40). In other words, β-lactamase production may not always translate into either in vitro or in vivo resistance. The same concern applies to penicillin and ampicillin disk diffusion susceptibility testing with *B. catarrhalis* when a zone diameter of ≤28 mm is used to define resistance. Performance of a dilution susceptibility test or use of the alternative disk diffusion zone diameter interpretive criteria described above would eliminate this problem.

A large number of oral and parenteral antimicrobial agents are uniformly active against *B. catarrhalis* (1, 25, 29, 62, 65). These include erythromycin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, cephalosporins, extended-spectrum penicillins, imipenem, aztreonam, the fluoroquinolones, and the aminoglycosides. Resistance to these agents in the United States has not been described. In contrast, clindamycin, vancomycin, and trimethoprim (alone) are uniformly inactive. The isoxazoly-penicillins have variable activity.

In vitro susceptibility tests can be performed with these agents by use of quantitative dilution tests or, in selected instances (see above), by using a disk diffusion procedure. Again, the appropriateness of routinely performing susceptibility tests with antimicrobial agents of known activity (or lack of activity) is questionable. In conclusion, routine in vitro susceptibility testing of *B.catarrhalis* may not be necessary. With this organism, laboratory efforts should be directed at providing accurate diagnostic information as expeditiously as possible. Correct therapeutic decisions do not require knowledge of the in vitro susceptibility test results obtained with an individual patient isolate.

N. GONORRHOEAE

Susceptibility test methods. Clinical isolates of *N. gonorrhoeae* have become increasingly resistant to the primary therapeutic drugs during the last 40 years (2, 6, 16, 33, 45, 49, 57–59). For instance, by the early 1970s, penicillin and tetracycline MICs for significant numbers of strains were as high as 0.25 to 1.0 μg/ml (33, 45, 57, 59). Similarly, elevated MICs and, in some cases, resistance to the macrolides, aminoglycosides, and sulfonamides were reported (2, 11, 49, 58). The susceptibility test method used for isolates of *N. gonorrhoeae* was primarily the agar dilution procedure, although several early studies indicated that the disk diffusion method could be reliably used to detect increased MICs of penicillins, tetracyclines, and other drugs with gonococci (42, 57, 59). In 1978, Biddle et al. described the results of MIC-disk diffusion correlation studies with penicillinase-producing and spectinomycin-resistant strains of *N. gonorrhoeae* (7). An acceptable correlation was achieved by using agar dilution MICs determined on supplemented Proteose Peptone agar no. 3 (Difco Laboratories) and disk diffusion zone diameters determined on GC agar base supplemented with 1% IsoVitaleX. The results of this investigation and several more-recent studies conducted at the Centers for Disease Control (6, 7) have led to the current NCCLS recommendations for testing gonococci (50, 51).

Agar dilution tests of *N. gonorrhoeae* with agents other than trimethoprim and the sulfonamides should be performed with Proteose Peptone agar no. 3 supplemented with 1% hemoglobin and 1% Kellogg's supplement (51; C. Thornsberry, J. M. Swenson, C. N. Baker, L. K. McDougal, S. A. Stocker, and B. C. Hill, Antimicrob. Newsl. **4**:47–55, 1987). A different medium, free of inhibitors of trimethoprim and the sulfonamides, must be used when testing these agents. This medium consists of Oxoid Diagnostic Sensitivity Test agar containing 5% lysed horse blood and 1% Kellogg's supplement (51; Thornsberry et al., Antimicrob. Newsl. **4**:47–55, 1987). The inoculum size for agar dilution tests with *N. gonorrhoeae* is 10^3 CFU/ml, 10-fold lower than that recommended for use when testing other organisms by this method (51). Agar dilution plates are incubated in 5% CO_2 for 20 to 24 h at 35°C.

Broth dilution procedures have been relatively unsuccessful as means for determining MICs with *N. gonorrhoeae* principally because of organism autolysis. However, broth media that avoid this problem have been formulated, thus enabling the use of broth-based procedures for manual and automated MIC determinations (32, 61; Y. A. Jeanlouis, R. J. Rice, and G. G. Goodwin, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 138, 1986). These broth microdilution tests, which employ Proteose Peptone broth no. 3 supplemented with 1% IsoVitaleX, produced results comparable to those obtained with the NCCLS agar dilution procedure for the penicillins, doxycycline, erythromycin, spectinomycin, and the quinolones (61). It should be noted, however, that some newer antimicrobial agents, such as imipenem, can be adversely affected by IsoVitaleX and other medium supplements (data on file, Merck & Co., Inc.).

Disk diffusion tests with *N. gonorrhoeae* have been developed and standardized only for penicillin (50; Thornsberry et al., Antimicrob. Newsl. **4**:47–55, 1987) and spectinomycin (Thornsberry et al., Antimicrob. Newsl.). The preferred medium consists of GC agar base supplemented with 1% IsoVitaleX. The conditions of incubation are those described above for the agar dilution procedure. The NCCLS zone diameter interpretive criteria for penicillin (10-U disks) are ≥20 mm = susceptible and ≤19 mm = resistant; for spectinomycin (100-μg disks), the criteria are ≥18 mm = susceptible and ≤14 mm = resistant. Use of the aforementioned penicillin criteria effectively categorizes β-lactamase-producing strains of *N. gonorrhoeae* (PPNG) as being resistant since PPNG typically shows zone sizes of ≤19 mm. These criteria, however, may fail to identify strains with chromosomally mediated penicillin resistance (i.e., *N. gonorrhoeae* strains that are resistant to penicillin but lack a TEM-1-type β-lactamase). For these reasons, the Centers for Disease Control Sexually Transmitted Disease Laboratory recommends that zone sizes of ≤25 mm be considered resistant (12, 16). There exist no published data to corroborate this recommendation.

It should be noted that a collaborative study designed to standardize *N. gonorrhoeae* susceptibility test methods for

penicillin, tetracycline, spectinomycin, and ceftriaxone is now under way (R. N. Jones, personal observations). Quality assurance and medium performance guidelines will be developed for agar dilution and disk diffusion methods using GC agar base supplemented with 1% IsoVitaleX. A quality control strain(s) of *N. gonorrhoeae* will be selected, and medium performance limits will be established. The proposed inoculum will be consistent with other NCCLS standard procedures (50, 51). The incubation period will be 24 h in 5 to 7% CO_2 or in a candle jar. Regression-line studies with these four drugs are also forthcoming, thus widening the number of antibiotics with well-established interpretive guidelines.

β-Lactamase testing of all *N. gonorrhoeae* isolates has been advocated by the Centers for Disease Control and other agencies as the first level of recognition and control of PPNG (11, 13–16). Various β-lactamase test methods can be used to detect the TEM-type, plasmid-mediated enzyme produced by gonococcal strains. Nearly comparable levels of accuracy can be achieved by using the acidimetric, chromogenic cephalosporin, and iodometric techniques.

Laboratory testing of *N. gonorrhoeae*. A β-lactamase test should be performed on all *N. gonorrhoeae* isolates, even in PPNG-nonendemic geographic areas. This can be achieved most economically by using freshly prepared laboratory reagents (in acidimetric or iodometric tests), although several commercial products have proven to be highly reliable. A negative β-lactamase test result does not always assure a favorable clinical response to penicillin or ampicillin therapy because of the increasing prevalence of high-grade, chromosomal, non-enzyme-mediated resistance (12, 16, 58). Detection of penicillin or ampicillin resistance among chromosomally mediated penicillin-resistant strains requires the use of direct susceptibility tests with these agents. However, given the relatively low prevalence of such strains, there is probably no need to routinely examine primary isolates which are β-lactamase negative with a penicillin or ampicillin susceptibility test. Such testing should, however, be performed on β-lactamase-negative posttreatment isolates of *N. gonorrhoeae* from patients who failed to respond to penicillin or ampicillin therapy.

Similarly, routine agar dilution or disk diffusion susceptibility testing of *N. gonorrhoeae* with alternative therapeutic agents (i.e., tetracycline, spectinomycin, and cephalosporins such as cefoxitin, cefotaxime, and ceftriaxone) is not advocated, at least in general clinical microbiology laboratories. First of all, as stated above, with the exception of spectinomycin, susceptibility test procedures with these agents and *N. gonorrhoeae* have not been rigorously standardized. Secondly, resistance with these agents is uncommon. In general laboratories, testing of these antimicrobial agents should probably be restricted to isolates of *N. gonorrhoeae* obtained from patients known to have failed on therapy. Spectinomycin may be tested with either the disk diffusion or agar dilution procedure described above. The other agents should be tested with the agar dilution procedure.

In conclusion, routine antimicrobial susceptibility testing of *N. gonorrhoeae* other than performance of a β-lactamase assay should be restricted to public health laboratories, laboratories that service very active sexually transmitted disease clinics, and laboratories involved in epidemiologic investigations.

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