JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2004, p. 493–496 Vol. 42, No. 2
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.2.493–496.2004

MINIREVIEW

Need for Susceptibility Testing Guidelines for Fastidious or Less-Frequently Isolated Bacteria

James H. Jorgensen*

Department of Pathology, The University of Texas Health Science Center, San Antonio, Texas 78229

In the United States, clinical microbiology laboratories rely upon the National Committee for Clinical Laboratory Standards (NCCLS) for written standards that guide the important elements of antimicrobial susceptibility testing. Whether a laboratory performs a direct version of an NCCLS standard method (e.g., disk diffusion) or uses a commercial device that has been "cleared" by the Food and Drug Administration because it provides results that are essentially equivalent to the NCCLS reference dilution susceptibility method, the NCCLS standards provide important, up-to-date guidance on the most relevant drugs to report on specific organisms, quality control ranges to assure reproducible results, and "breakpoints" to interpret disk diffusion zones or MICs.

DEVELOPMENT OF NCCLS TESTING CRITERIA

NCCLS documents M2, *Performance Standards for Antimicrobial Disk Susceptibility Tests* (26), and M7, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* (25), describe reference and standardized methods for antimicrobial susceptibility testing of common, rapidly growing aerobic bacteria, including staphylococci, enterococci, members of the *Enterobacteriaceae*, and *Pseudomonas* and *Acinetobacter* spp. (in addition to a few other non-glucose fermenters). During the past 15 years, the NCCLS Antimicrobial Susceptibility Testing (AST) subcommittee has expanded the list of standard susceptibility testing methods, quality control values, and interpretive breakpoints to include several fastidious bacterial species. Specialized media, modified incubation conditions, and specific breakpoints have been established for the testing of *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Streptococcus* spp. (including *Streptococcus pneumoniae*). In addition, there is a limited amount of information on the testing of *Helicobacter pylori*, *Listeria* spp., *N. meningitidis*, *Vibrio cholerae*, and *Bacillus anthracis* (25, 26).

In order to establish MIC interpretive breakpoints for new antimicrobial agents, to modify existing breakpoints, or to establish breakpoints for organisms for which breakpoints have not previously existed, the AST subcommittee has used a detailed analysis of four different types of data (24). This begins with an analysis of MIC ranges of a particular drug with isolates that lack known resistance mechanisms to determine the intrinsic degree of susceptibility of a species. Next, the susceptibility to the drug is determined with strains that contain known resistance mechanisms that affect the activity of the particular drug class in order to assess the impact of that resistance mechanism.

In recent years the AST subcommittee has found pharmacokinetic and pharmacodynamic determinations to be a very valuable third aspect of establishing breakpoints. The recognition of the importance of the time the drug levels in blood are maintained above the drug's proposed MIC breakpoint ($T >$ MIC; with beta-lactams, glycopeptides, macrolides) or the area under the drug concentration curve in blood divided by the proposed MIC breakpoint (with aminoglycosides or fluoroquinolones) have contributed to the ability to set appropriate breakpoints (5). Simulations such as the Monte Carlo analysis (22) have allowed an assessment of the likelihood of achieving sufficient drug levels at the site of the most common infections by using various dosing regimens of a drug. Lastly, the AST subcommittee reviews clinical and bacteriological response data collected during large clinical trials of a new agent when they establish breakpoints. However, the latter data are often limited by the design of clinical trials to systematically exclude patients whose isolates are thought to be "resistant" based upon the three other types of data described above. The process of integrating these four types of data has been outlined in detail in NCCLS document M23-A2, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters* (24). Notably, however, when the AST subcommittee establishes or reestablishes breakpoints for older drugs or for organisms that have previously lacked breakpoints, large prospectively collected clinical data are often not available.

Despite the major contribution to the standardization of susceptibility testing that NCCLS documents M2 and M7 provide, there are several genera of bacteria that are isolated periodically by clinical microbiology laboratories from human diagnostic specimens for which there are no current NCCLS standards. These include various coryneform bacteria, *Bacillus* spp. (other than *B. anthracis*), *Micrococcus* spp., *Abiotrophia* and *Granulacatella* spp., and several species of fastidious gram-negative bacteria (e.g., HACEK group organisms and *Pasteurella* spp.). The NCCLS has not yet specifically addressed appropriate test methods for these organisms and has not recommended any interpretive breakpoints. In addition, more

* Mailing address: Department of Pathology, The University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229. Phone: (210) 567-4088. Fax: (210) 567-2367. E-mail: jorgensen@uthscsa.edu.

detailed guidance for test performance and interpretation are needed, especially breakpoints for *N. meningitidis*, *Listeria* spp., and for *Campylobacter* spp. The lack of test methods or interpretive criteria has made it difficult to assess possible acquired resistance mechanisms that may be present in these less-frequently isolated organisms and has fostered the use of nonstandardized test methods in research or when clinical laboratories are pressured by clinicians to generate susceptibility data on individual patient isolates. Indeed, some laboratories may perform disk diffusion testing of less-common species and invoke the NCCLS breakpoints for some other organism group for test interpretation. Alternatively, broth microdilution or gradient diffusion tests has been used to generate MICs for reporting without specific interpretations or by "borrowing" breakpoints from other organism groups. Some of these findings are briefly summarized below.

GRAM-POSITIVE RODS

Among the *Corynebacterium* spp., *Corynebacterium jeikeium* and *C. urealyticum* have been reported to be multidrug resistant, including resistance to beta-lactams, macrolides, and aminoglycosides (10). In addition, *C. diphtheriae* may be macrolide and rifampin resistant, while *C. pseudodiphthericum* and *C. striatum* may possess *erm* genes and be resistant to macrolides and lincosamides (10). Indeed, some strains of *C. striatum* are said to be resistant to tetracyclines and quinolones (10). The related bacilli, *Arcanobacterium* and *Arthrobacter* spp., have been described as resistant to aminoglycosides and quinolones (10), whereas *Brevibacterium* spp. may have reduced beta-lactam susceptibility (12) and *Turicella* spp. may be macrolide and clindamycin resistant (12). Perhaps most notably, *Microbacterium resistans* has been reported to be intrinsically vancomycin resistant (11), and *Leifsonia aquatica* strains have diminished vancomycin susceptibility (10). Most of these reports of resistance have been based upon either agar dilution testing or the use of the E test (AB BIODISK, Solna, Sweden) on Mueller-Hinton sheep blood agar (10, 20), although one expert on these organisms advocates use of disk diffusion testing with interpretations based upon the NCCLS streptococcal breakpoints (10).

Bacillus cereus and *B. thuringiensis* have long been noted as producers of a potent broad-spectrum beta-lactamase that affects penicillins and cephalosporins and is not inhibited by the currently available beta-lactamase inhibitors (1). However, these related species are often susceptible to several other drug classes, including vancomycin, aminoglycosides, macrolides, and quinolones that might be used to treat ocular or wound infections.

GRAM-POSITIVE COCCI

Leuconostoc and *Pediococcus* spp. are infrequently encountered relatives of the streptococci that are intrinsically resistant to vancomycin but are usually susceptible to beta-lactams, chloramphenicol, tetracyclines, and aminoglycosides. However, a recent case report illustrated the possibility of resistance of *Leuconostoc* to carbapenems and cephalosporins (7). *Abiotrophia* and *Granulacatella* spp. (formerly nutritionally deficient streptococci) have long been said to demonstrate diminished susceptibility to penicillin, resulting in greater difficulty in the treatment of patients with endocarditis. One report described fluoroquinolone resistance in an *Abiotrophia* isolate from a neutropenic cancer patient (23). Testing of these two genera has usually been accomplished by addition of pyridoxal hydrochloride to blood-supplemented media for broth or agar dilution methods (23). *Aerococcus viridans*, *A. urinae*, and *Gemella haemolysans* isolates have been reported to have variable susceptibility to aminoglycosides, chloramphenicol, macrolides, tetracylines, trimethoprim, and sulfonamides but demonstrate susceptibility to penicillin (3, 4). Even less-common gram-positive cocci with variable susceptibility to commonly used antibiotics include *Aloiococcus* and *Rothia*. The former genus may demonstrate resistance to macrolides and trimethoprim-sulfamethoxazole (2).

FASTIDIOUS GRAM-NEGATIVE RODS

The HACEK (i.e., *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella* spp.) group of fastidious gram-negative bacilli have long been recognized as causative agents of infective endocarditis (6). All of these are normal oropharyngeal flora that are repeatedly exposed to antibiotics during therapy for various minor and serious infections. *Haemophilus aphrophilus* or *H. paraphrophilus* are the species most often associated with endocarditis or brain abscess. The NCCLS testing criteria (including the use of HTM broth or agar) have been validated primarily with *H. influenzae* and, to a lesser extent with *H. parainfluenzae*, and the even less frequently isolated species (16). In many ways, *H. aphrophilus* seems more closely related to *Actinobacillus actinomycetemcomitans*. The latter species may be resistant to penicillins, macrolides, and aminoglycosides (17). *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella* spp., *Capnocytophaga* spp., and *Pasteurella* spp. may also produce beta-lactamases that are inhibited by clavulanic acid (13, 18, 19, 28, 30). The even less common organism, *Dysgonomonas* spp. (formerly Centers for Disease Control and Prevention group DF-3) is said to be resistant to beta-lactams, macrolides, aminoglycosides, and quinolones (21). The testing conditions for *Campylobacter* spp. have been recently defined by the NCCLS (25), although breakpoints have yet to be derived. Certainly, fluoroquinolone and macrolide resistance occur in *Campylobacter jejuni*, *C. coli*, and *C. fetus* (8), and clinical laboratories will undoubtedly be called upon to test individual clinical isolates in the near future. NCCLS has defined the test conditions and established breakpoints for a single agent (clarithromycin) for *Helicobacter pylori* (25). Lastly, efforts are currently under way to develop specific NCCLS breakpoints for *N. meningitidis*.

OTHER GRAM-NEGATIVE RODS

Whereas not nutritionally fastidious, certain less-frequently isolated gram-negative rods lack NCCLS interpretive criteria even though they are capable of growing quite well in or on unsupplemented Mueller-Hinton medium. These include *Aeromonas* spp. and non-cholera *Vibrio* spp. Aeromonas species may contain as many as three different beta-lactamases, including a carbapenemase (29). Although it is recognized generally that *Aeromonas* spp. are resistant to ampicillin, they may have variable susceptibility to cephalosporins, though con-

troversy exists over the significance and method of testing for isolates that contain the carbapenemase (14). Indeed, isolates may test susceptible to imipenem at the standard NCCLS inoculum density (29). Most of the clinically significant *Vibrio* spp. grow well in standard Mueller-Hinton medium, and reported susceptibilities from isolates tested using that medium demonstrate some variability between species, particularly with regard to the older penicillins, cephalosporins, and sulfonamides (9). Lastly, relatively little validation of the standard NCCLS testing methods has been performed with the more arcane glucose-nonfermentative gram-negative bacilli. The NCCLS suggests that nonfermenters can be tested by the standard broth microdilution method (25), but most should not be tested by the NCCLS disk diffusion procedure (26). However, the NCCLS disk test has recently been validated for testing *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole, ceftazidime, and levofloxacin (27). Similarly, the disk test can now be recommended for testing *Burkholderia cepacia* with ceftazidime, meropenem, and minocycline (27).

A NEW NCCLS WORKING GROUP

Clinical microbiology laboratories would greatly benefit from an NCCLS consensus guideline that describes an approach to testing these organisms in a standardized manner, one that lists appropriate quality control measures, and suggests interpretive breakpoints. Because of the modest frequency of infections due to these organisms and the fact that most of the antibiotics of interest are agents that have been marketed for a number of years, it is not reasonable to expect that M23-specified studies will be conducted on this special group of organisms. It would be possible, however, to propose MIC interpretive criteria based upon a careful review of the extant literature, application of existing pharmacokinetic data on the drugs of interest, and application of microbiological data (distributions of MICs) by an expert working group. The NCCLS has, in fact, recently approved the formation of a new working group organized under the leadership of the AST subcommittee. The goal will be to generate a sufficient amount of information on the testing of these "orphan" organisms to justify publication of a new NCCLS document (designated M45). It is hoped that the new document can be developed through the consensus process and be ready for publication within 2 years. What should laboratories do in the interim, if pressed to provide susceptibility data on individual patient's isolates to assist in management of therapy? It has been suggested that citing successful therapeutic choices from previous literature is often sufficient to select appropriate therapy (15). If a clinical failure occurs or there are other strong indications that a test of an individual isolate is truly needed, the most reasonable approach would be to perform a standardized broth or agar dilution test with the drug(s) of choice and to report the MICs without interpretations borrowed from other organisms but with a qualifying statement that a nonvalidated test method was used (15).

REFERENCES

1. **Andrews, J. M., and R. Wise.** 2002. Susceptibility testing of *Bacillus* species. J. Antimicrob. Chemother. **49:**1039–1046.
2. **Bosley, G. S., A. M. Whitney, J. M. Pruckler, C. W. Moss, M. Daneshvar, T. Sih, and D. F. Talkington.** 1995. Chracterization of ear fluid isolates of *Alloiococcus otitidis* from patients with recurrent otitis media. J. Clin. Microbiol. **33:**2876–2880.
3. **Buu-Hoi, A., C. LeBougunec, and T. Horaud.** 1989. Genetic basis of antibiotic resistance in *Aerococcus viridans.* Antimicrob. Agents Chemother. **33:** 529–534.
4. **Christensen, J., H. Vibits, J. Ursing, and B. Korner.** 1991. *Aerococcus*-like organism, a newly recognized potential urinary tract pathogen. J. Clin. Microbiol. **29:**1049–1053.
5. **Craig, W. A.** 1998. Pharmacokinetics/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. **26:**1–10.
6. **Das, M., A. D. Bradley, F. R. Cockerill, J. M. Steckelberg, and W. R. Wilson.** 1997. Infective endocarditis caused by HACEK microorganisms. Annu. Rev. Med. **48:**25–33.
7. **Deye, G., J. Lewis, J. Patterson, and J. Jorgensen.** 2003. A case of *Leuconostoc* ventriculitis with resistance to carbapenem antibiotics. Clin. Infect. Dis. **37:**869–870.
8. **Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Schmidt, and I. Nachamkin.** 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli* resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. **7:**24–34.
9. **Farmer, J. J., III, J. M. Janda, and K. Birkhead.** 2003. *Vibrio*, p. 706–718. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
10. **Funke, G., and K. A. Bernard.** 2003. Coryneform gram-positive rods, p. 472–501. InP. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
11. **Funke, G., P. A. Lawson, F. S. Nolte, N. Weiss, and M. D. Collins.** 1998. *Aureobacterium resistens* sp. nov. exhibiting vancomycin resistance and teicoplanin susceptibility. FEMS Microbiol. Lett. **158:**89–93.
12. **Funke, G., V. Pünter, and A. von Graevenitz.** 1996. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. Antimicrob. Agents Chemother. **40:**2874–2878.
13. **Gomez-Garces, J.-L., J.-I. Alos, J. Sanchez, and R. Cogollos.** 1994. Bacteremia by multidrug-resistant *Capnocytophaga sputigena.* J. Clin. Microbiol. **32:**1067–1069.
14. **Hayes, M. V., C. J. Thomson, and S. G. B. Amyes.** 1996. The "hidden" carbapenemase of *Aeromonas hydrophila*. J. Antimicrob. Chemother. **37:**33–44.
15. **Hindler, J. F., and J. M. Swenson.** 2003. Susceptibility test methods: fastidious bacteria, p. 1128–1140. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
16. **Jorgensen, J. H., A. W. Howell, and L. A. Maher.** 1990. Antimicrobial susceptibility testing of less commonly isolated *Haemophilus* species using *Haemophilus* test medium. J. Clin. Microbiol. **28:**985–988.
17. **Kugler, K. C., D. J. Biedenback, and R. N. Jones.** 1999. Determination of the antimicrobial activity of 29 clinically important compounds tested against fastidious HACEK group organisms. Diagn. Microbiol. Infect. Dis. **34:**73–76.
18. **Lion, C., A. Lozniewski, V. Rosner, and M. Weber.** 1999. Lung abscess due to β-lactamase-producing *Pasteurella multocida*. Clin. Infect. Dis. **29:**1345–1346.
19. **Lu, P. L., P.-R. Hsueh, C.-C. Hung, L.-J. Teng, T.-N. Jang, and K.-T. Luh.** 2000. Infective endocarditis complicated with progressive heart failure due to β-lactamase-producing *Cardiobacterium hominis*. J. Clin. Microbiol. **38:** 2015–2017.
20. **Martínez-Martínez, L., M. C. Ortega, and A. I. Suàrez.** 1995. Comparison of E-test with broth microdilution and disk diffusion for susceptibility testing of coryneform bacteria. J. Clin. Microbiol. **33:**1318–1321.
21. **Martínez-Sanchez, L., F. J. Vasallo, F. Garcia-Garrote, L. Alcala, M. Rodriguez-Créixems, and E. Bouza.** 1998. Clinical isolation of a DF-3 microorganism and review of the literature. Clin. Microbiol. Infect. **4:**344–346.
22. **Montgomery, M. J., P. M. Beringer, A. Aminimanizani, S. G. Louie, B. J. Shapiro, R. Jelliffe, and M. A. Gill.** 2001. Population pharmacokinetics and use of Monte Carlo simulation to evaluate currently recommended dosing regimens of ciprofloxacin in adult patients with cystic fibrosis. Antimicrob. Agents Chemother. **45:**3468–3473.
23. **Murray, C. K., E. A. Walter, S. Crawford, M. L. McElmeel, and J. H. Jorgensen.** 2001. *Abiotrophia* bacteremia in a patient with neutropenic fever and antimicrobial susceptibility testing of *Abiotrophia* isolates. Clin. Infect. Dis. **32:**e140–e142.
24. **National Committee for Clinical Laboratory Standards.** 2001. Development of in vitro susceptibility testing criteria and quality control parameters. NCCLS document M23-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
25. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
26. **National Committee for Clinical Laboratory Standards.** 2003. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A8. National Committee for Clinical Laboratory Standards, Wayne, Pa.

27. **National Committee for Clinical Laboratory Standards.** Performance standards for antimicrobial susceptibility testing. Supplement M100-S14, in press. National Committee for Clinical Laboratory Standards, Wayne, Pa.
28. **Paul, K., and S. S. Patel.** 2001. *Eikenella corrodens* infections in children and adolescents: case reports and review of the literature. Clin. Infect. Dis. **33:**54–61.
29. **Rossolini, G. M., T. Walsh, and G. Amicosante.** 1996. The *Aeromonas* metallo-β-lactamases: genetics, enzymology, and contribution to drug resistance. Microb. Drug Resist. **2:**245–251.
30. **Sordillo, E. M., M. Rendel, R. Sood, J. Belinfanti, O. Murray, and D. Brook.** 1993. Septicemia due to β-lactamase-positive *Kingella kingae*. Clin. Infect. Dis. **17:**818–819.