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Broth Dilution Minimum Inhibitory Concentrations: Rationale for Use of Selected Antimicrobial Concentrations

FRANK G. WITEBSKY,* JAMES D. MACLOWRY, AND SALLY S. FRENCH

Microbiology Service, Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Maryland 20014

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A microdilution susceptibility testing procedure utilizing selected, clinically relevant concentrations of a large number of antimicrobial agents is described. A qualitative code designed to facilitate interpretation of quantitative results is coupled with each antimicrobial concentration. Both the antimicrobial concentrations selected for testing and the assigned codes are based on published data regarding attainable antimicrobial levels in serum and urine.

Although agar disk diffusion procedures continue to be the most widely employed means for determining susceptibilities to antimicrobial agents in the United States, there is increasing interest in methodologies which provide a quantitative measure of susceptibility to antimicrobial agents for at least certain critical clinical isolates. This increasing interest is based both on growing sophistication in the use of antimicrobial agents and on advances in the design of equipment and the preparation of products for such quantitative studies. Agar disk diffusion procedures are restricted to interpretive systems comprised of two or three categories, such as susceptible, intermediate, and resistant. Broth and agar dilution testing for determination of minimum inhibitory concentrations (MICs) has tended to be done by employing serial twofold dilutions between selected maximum and minimum concentrations. Most laboratories employing quantitative susceptibility testing procedures report susceptibility results on the basis of such a twofold dilution scheme. This twofold dilution procedure has become widely employed because such dilutions are easy to prepare; in fact, by some techniques, such as semiautomated susceptibility testing with a microdiluter, the use of a twofold dilution system is unavoidable. However, the clinical need for a specifically twofold dilution system has had little evaluation. Although in many cases there is considerable relevance to the maximum and/or minimum concentrations selected for testing a given antimicrobial agent, we feel that many of the intermediate concentrations tested provide no clinically useful information.

For 10 years our laboratory has been performing antimicrobial agent susceptibility testing by using a serial twofold microdilution system. However, there have been persistent problems in the use of MIC data by clinicians. Our experience indicates that in many medical schools and residency training programs, there is little instruction in the proper interpretation of MIC data. In fact, it requires considerable knowledge of antimicrobial pharmacology to be able to use MIC data appropriately. Most physicians are accustomed to having isolates reported as being susceptible, intermediate, or resistant. Over the years many physicians have called us because of their inability to understand the MIC data we have reported. Although we have attempted to give them brief instruction in the interpretation of MIC data, it is obvious that all of the complexities involved cannot be adequately handled in such a fashion. More formalized, didactic instruction to groups of physicians may be a more rational approach but has not proved practicable either. Suggestions to obtain infectious disease consultation were frequently unheeded by the primary physician, especially in cases of infections generally considered easy to treat, such as initial urinary tract infections. Attempts have been made to circumvent these problems by providing susceptibility reports containing extensive information on the antimicrobial agent levels which can be expected in different body fluids with varying doses and routes of administration. It seems doubtful that a busy clinician will make adequate use of such information unless he already has considerable expertise in the use of MIC data. In our institution we provided each patient-care physician with a table to assist in the interpretation of MIC data. Nevertheless, exasperation with MIC data has occasionally reached such levels that some physicians have ignored our susceptibility reports and have treated patients on the basis of their assessment of a given pathogen's most probable antimicrobial agent susceptibility.

In view of these considerations, we felt it necessary to provide clinicians with a clinically relevant interpretation of the MIC data, but at the same time provide the information inherent in quantitative data. The Kirby-Bauer reporting system of three categories does not provide an optimum amount of information and, additionally, for most antimicrobial agents is restricted to serum levels.

Consequently, we have devised an interpretive scheme which is more clinically useful. The categories, their abbreviations, and meanings are as follows:

(i) Sensitive (S). This designation is used when an organism has an MIC such that adequate serum levels (at least two to four times the MIC) can easily be attained by ordinary doses of that antimicrobial agent administered by its usual route.

(ii) Resistant (R). This designation is used when an organism has an MIC such that adequate serum levels (maximum serum level is less than the MIC) cannot be attained for reasons of antimicrobial pharmacology or toxicity, regardless of how the antimicrobial agent is administered.

(iii) Resistant, penicillinase producer (RP). This designation is used when a penicillinase-susceptible antimicrobial agent cannot be used to treat a specific isolate which is a penicillinase producer. The reasons for adding this category are detailed below.

(iv) High intramuscular or intravenous dose needed (MR). This designation is used when an antimicrobial agent can only be used in higher than usual parenteral doses to treat a given isolate. (The maximum attainable serum level approximates the MIC.)

(v) Very high intravenous dose needed (IV). This designation, applied presently only to carbenicillin and ticarcillin, is used when an organism has an MIC such that only very high intravenous doses can be used for treatment.

(vi) Sensitive when used to treat a lower urinary tract infection (US). This designates an antimicrobial agent which, when administered in ordinary doses by its usual route, can only be used to treat a given isolate from a lower urinary tract infection.

(vii) A combined category (MR, US) is used when an antimicrobial agent can be used in ordinary doses against an isolate causing a lower urinary tract infection, but must be used in high parenteral doses to treat an infection caused by that isolate in other sites.

It was felt that if only relevant antimicrobial agent concentrations were selected for testing, all antimicrobial agents to be tested could be incorporated into one battery in one plate. A total of 19 different antimicrobial agents were selected for incorporation into the test battery because they satisfied one or more of the following criteria: (i) efficacious and frequently used against susceptible organisms, e.g. ampicillin; (ii) typical representative of a class of frequently used agents, e.g. oxacillin for the penicillinase-resistant penicillins; (iii) rarely used but highly efficacious under certain circumstances, e.g. vancomycin; (iv) rarely used clinically but provides useful information for the diagnostic laboratory, e.g. colistin and some concentrations of aminoglycosides; (v) efficacious and frequently used but potentially toxic, e.g. gentamicin; and (vi) susceptibilities frequently requested because of our particular patient population, e.g. ticarcillin and tobramycin.

After the antimicrobial agents to be incorporated into the battery were selected, the particular concentrations of each to be tested were determined, making use of the data summarized by Voss and MacLowry (8) and MacLowry et al. (4). The antimicrobial agents and concentrations selected are shown in Table 1.

A key feature of the new approach is the association of a specific interpretive code with each concentration of each antimicrobial agent. Thus, each susceptibility report on a given isolate now includes the MIC and the interpretive code for that MIC for each antimicrobial agent in the battery. The interpretive codes associated with each MIC are shown in Table 1.

The rationale for selecting most of these antimicrobial agent concentrations and their associated interpretive codes should be clear from a consideration of the pharmacology of the agent. However, certain specific antimicrobial agent concentrations deserve explanation. (i) Colistin at 50 μg/ml is used for laboratory diagnostic purposes. The majority of *Proteus*, *Providencia*, and *Serratia* isolates are resistant at this level. (ii) Gentamicin and tobramycin at 48 μg/ml and amikacin at 128 μg/ml are used for investigational purposes to gather data on the frequency of high-level resistance to these agents. (iii) Streptomycin at 100 μg/ml is used because this is the usual upper limit of streptomycin resistance for enterococci, whereas a level of 2,000 μg/ml is used to detect enterococci having high-level resistance to streptomycin. (iv) Penicillin G at 0.05 μg/ml is the upper limit of penicillin G for the determination of penicillin susceptibility of *Staphylococcus aureus*. Unpublished data (V. J. Gill) from our laboratory reveal that 97% of penicillinase-producing *S. aureus* isolates have an MIC for penicillin G of greater than 0.05 μg/ml. (v) Penicillin G at 3 μg/ml was selected as

TABLE 1. *Antimicrobial agents, concentrations tested, and codes*

Antimicrobial agent	Concn tested	Code	Antimicrobial agent	Concn tested	Code
Amikacin	≤4[a]	S	Kanamycin	≤6	S
	16	MR		24	MR
	32	MR		>24	R
	128	R	Nalidixic acid	≤10	US
	>128[b]	R		50	US
Ampicillin	≤2	S (RP)[c]		>50	R
	8	MR, US (RP)	Nitrofurantoin	≤50	US
	16	MR, US (RP)		200	US
	160	US (RP)		>200	R
	>160	R (RP)	Oxacillin	≤1	S
Carbenicillin	≤40	S (RP)		6	MR
	120	IV, US (RP)		>6	R
	240	IV, US (RP)	Penicillin G	≤0.05	S (RP)
	>240	R (RP)		1	MR, US (RP)
Cephalothin	≤1	S		3	MR, US (RP)
	12	S		60	US (RP)
	24	MR, US		>60	R (RP)
	240	US	Streptomycin	≤25	S
	>240	R		100	R
Chloramphenicol	≤4	S		2,000	R
	20	MR		>2,000	R
	>20	R	Tetracycline	≤2	S
Clindamycin	≤1	S		4	MR, US
	5	MR		8	MR, US
	>5	R		>8	R
Colistin	≤5	S	Ticarcillin	≤40	S (RP)
	50	R		120	IV, US (RP)
	>50	R		240	IV, US (RP)
Erythromycin	≤0.5	S		>240	R (RP)
	1	S	Tobramycin	≤3	S
	4	MR		6	MR
	>4	R		12	R
Gentamicin	≤3	S		48	R
	6	MR		>48	R
	12	R	Vancomycin	≤5	S
	48	R		25	MR
	>48	R		>25	R

[a] The symbol ≤ preceding the lowest concentration of each antimicrobial agent tested is included on the reports to indicate that the MIC (expressed as μg/ml) is either equal to or less than the number indicated.

[b] The symbol > is included on the reports if an organism grows at all concentrations of an antimicrobial agent tested.

[c] RP is an alternative interpretive code for the indicated concentrations of penicillinase-susceptible antimicrobial agents.

the upper limit of penicillin susceptibility; generally, one would not attempt to use penicillin, even in high doses, for the treatment of an infection other than in the lower urinary tract if the MIC against the causative organism was above 3 μg/ml.

MATERIALS AND METHODS

Antimicrobial agent solutions are dispensed in 50-μl volumes by using a Dynatech MIC 2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.) (6). Figure 1 shows the concentrations of each antimicrobial agent dispensed (twice the final concentration obtained after the addition of 50 μl of bacterial suspension) and the arrangement of the antimicrobial agents on the microdilution plate.

For most isolates, Mueller-Hinton purity check plates (5) are also used for disk susceptibility testing of sulfisoxazole, neomycin, and trimethoprim-sulfamethoxazole.

The characteristics of the zone edge around the penicillin G disk are used to confirm whether an *S. aureus* isolate is a penicillinase producer (3).

The Trypticase soy broth we use in the microdilution plates contains approximately 30 mg of calcium and 33 mg of magnesium per liter. We feel that this is sufficiently close to the recommended concentrations of these cations (7), especially since our *Pseudomonas aeruginosa* MIC results for gentamicin agree reasonably well with published data (10). Furthermore, the problem of the optimal cation concentrations to use in testing *P. aeruginosa* susceptibility to aminoglycosides remains unresolved (9).

Amikacin	Ampicillin	Carbenicillin	Cephalothin	Clindamycin	Erythromycin	Gentamicin	Nalidixic Acid	Penicillin G	Ticarcillin	Tobramycin	
8	4	80	2	2	1	6	20	0.1	80	6	B
32	16	240	24	10	2	12	100	2	240	12	B
64	32	480	48	B	8	24	B	6	480	24	B
256	320	B	480	B	B	96	Nitrofurantoin 100	120	B	96	B
B	B	B	B	B	B	B	400	B	Tetracycline 4	B	O
B	B	B	Chloramphenicol 8	Colistin 10	B	Kanamycin 12	B	Streptomycin 50	8	Vancomycin 10	O
B	B	B	40	100	B	48	Oxacillin 2	200	16	50	C
B	B	B	B	B	B	B	12	4000	B	B	C

FIG. 1. *Microdilution plate configuration. Each letter or number represents a well in the microdilution plate. Numbers are concentrations of antimicrobial agents (in micrograms per milliliter) before bacterial suspensions are added. B, Wells filled with 50 μl of a colored solution (used to separate antimicrobial agents and facilitate reading of the plates). C, Control wells, filled with 50 μl of Trypticase soy broth when the plate is prepared and used as the medium control. O, Control wells, filled with 50 μl of Trypticase soy broth when the plate is prepared; 50 μl of the test organism suspension is added to serve as the organism control.*

REPORTING PROCEDURES

For each organism tested, a report is produced for each antimicrobial agent in the battery. A typical final susceptibility report is shown in Fig. 2. In this example, trimethoprim-sulfamethoxazole disk testing indicated susceptibility and sulfisoxazole and neomycin disk testing were not done. An explanation of the susceptibility code accompanies each report.

A few points regarding reports and their interpretation merit special emphasis. First, any *Staphylococcus* found to be a penicillinase producer has an RP interpretive code attached, regardless of the MIC, for each penicillinase-susceptible antimicrobial agent in the battery (ampicillin, carbenicillin, penicillin G, ticarcillin). Second, since a twofold dilution series is not being used, the report of an MIC as X μg/ml means that the actual MIC is not somewhere between X/2 and X, but somewhere between X and the next lower concentration tested. Thus, for example, an organism with an MIC for amikacin reported as 128 R actually has an MIC that is greater than 32 μg/ml (the next lower concentration tested) but less than or equal to 128 μg/ml. Ideally, we would like to report this as 32 MR < MIC ≤ 128 R, but we cannot do so because of the constraints of our computer system. Third, the use of the symbol S does not necessarily mean that an agent can be administered orally with the achievement of effective serum levels. For gentamicin, a susceptibility report of ≤3 S means that ordinary parenteral doses of gentamicin will achieve an adequate serum level.

Some additional examples of susceptibility reports may further clarify this system.

(i) A penicillin-susceptible (non-penicillinase-producing) *S. aureus* strain would show no growth at any of the concentrations of penicillin G tested and would show a zone edge with the penicillin G disk which indicated no penicillinase production. The susceptibility report for penicillin G would read ≤0.05 S.

(ii) A penicillin-resistant (penicillinase-producing) *S. aureus* strain might be able to grow at penicillin G concentrations of 0.05 and 1 μg/ml, but not at 3 μg/ml. The susceptibility report for penicillin G would read 3 RP.

(iii) A rare penicillin-resistant (penicillinase-producing) *S. aureus* strain might show no growth at any of the concentrations tested, but would show a heaped-up zone edge with the penicillin G disk which indicated penicillinase production. The susceptibility report for penicillin G would read ≤0.05 RP.

(iv) A *P. aeruginosa* isolate might be able to grow at carbenicillin concentrations of 40 and 120 μg/ml but not 240 μg/ml. The susceptibility report for carbenicillin would read 240 IV.

(v) A *Streptococcus faecalis* isolate might be able to grow at a streptomycin concentration of 25 μg/ml but not at the other concentrations tested. The susceptibility report for streptomycin would read 100 R. This indicates that the isolate does not have a high level of resistance to streptomycin.

(vi) Another *S. faecalis* isolate might be able to grow at all concentrations of streptomycin tested. The susceptibility report for streptomycin would read >2000 R. This indicates that the organism has high-level resistance to streptomycin.

DISCUSSION

The susceptibility testing procedure described here has numerous advantages. (i) Antimicrobial agent solutions are made up in large volumes, so that concentration errors introduced by diluting small concentrations, as is done in a serial twofold microdilution system, are considerably reduced. (ii) Unnecessary susceptibility testing at

SENSITIVITY RESULTS CODE

S=SENS R=RESIST RP=RESIST, PENASE PRODUCER
MR=HIGH IM OR IV DOSE NEEDED IV=VERY HIGH IV DOSE NEEDED
US=SENS ONLY IF LOWER URINARY TRACT INFECTION

ORG#1 PROTEUS MIRABILIS

ANTIBIOTIC SENSITIVITIES (MCG/ML)

AMIKACIN	16 MR	AMPICILLIN	≤2 S	CARBENICILLIN	≤40 S
CEPHALOTHIN	12 S	CHLORAMPHENICOL	20 MR	CLINDAMYCIN	>5 R
COLISTIN	>50 R	ERYTHROMYCIN	>4 R	GENTAMICIN	≤3 S
KANAMYCIN	≤6 S	NALIDIXIC ACID	≤10 US	NITROFURANTOIN	≤50 US
OXACILLIN	>6 R	PENICILLIN G	3 MR, US	STREPTOMYCIN	≤25 S
TICARCILLIN	≤40 S	TETRACYCLINE	>8 R	TOBRAMYCIN	≤3 S
VANCOMYCIN	>25 R	TRIMETH-SULFA	SEN	SULFISOXAZOLE	
NEOMYCIN					

FIG. 2. *Typical final antimicrobial susceptibility report.*

clinically irrelevant antimicrobial agent concentrations is eliminated. (iii) The laboratory need prepare only one antimicrobial agent battery for all organisms. Previously, our laboratory required different batteries for gram-positive organisms, gram-negative organisms, anaerobes, and urinary tract pathogens. This considerably simplifies susceptibility testing from the laboratory standpoint. (iv) Since each organism on which susceptibility testing is performed is tested against many different antimicrobial agents, a great deal of data can be accumulated regarding the susceptibility of different organisms to a variety of different agents. Studies are in progress to evaluate the usefulness of these data for organism identification. Previous studies (2) have suggested that this might be a useful adjunct to the traditional identification procedure. (v) The use of clinically relevant concentrations of antimicrobial agents, coupled with an interpretive code, considerably simplifies the clinician's task of interpreting susceptibility data, without eliminating quantitative data for those physicians best able to make use of such data. It should perhaps be pointed out here that we have retained in our laboratory the capability for serial twofold dilution MIC determinations. Since the introduction of our new system, such studies have very rarely been requested. (vi) The battery described should not be regarded as a final and unalterable version. Additional concentrations or antimicrobial agents can easily be added to or removed from the battery.

The interpretive code presented here bears some similarities to the four-category system discussed by Ericsson and Sherris (1). However, the code we have developed includes more categories (although not all of the categories are employed for each of the antimicrobial agents tested), the categories are defined somewhat differently and in some situations are combined, and the code is combined with quantitative MIC data. We have chosen to indicate specifically that, for certain MICs of selected antimicrobial agents (e.g., tetracycline at 4 μg/ml), urinary tract infections can be treated with ordinary doses, whereas soft tissue infections cannot in general be so treated. We have instituted this procedure for urinary tract infections because many clinicians are not sufficiently cognizant of antimicrobial agent pharmacology to know which agents are excreted in high concentrations in the urine. Additionally, they are not aware that the usual S-I-R interpretive system often does not apply to urinary tract infections, since it underestimates the efficacy of antimicrobial agents in body fluids which contain concentrations above the serum level.

In many instances more than one concentration of an antimicrobial agent is given the same interpretive code. The reasons for using such concentrations vary among the different antimicrobial agents. First, some concentrations are tested because the precise MIC data are frequently useful for confirming bacterial identification. Second, we are attempting to accumulate susceptibility data for other purposes, so each concentration tested is given an interpretive code. Third, in certain circumstances it is not possible to know what cutoff point to use for varying degrees of resistance, and we have attempted to use codes that minimize any possibility of confusion on the part of the clinicians receiving the results.

It should be borne in mind by any physician treating a patient with an infection that the in vivo susceptibility of an organism depends on many factors other than the in vitro susceptibility results, not the least of which is the ability of an antimicrobial agent to penetrate into the infected body space. Thus, an in vitro-susceptible organism isolated from the cerebrospinal fluid may not be susceptible clinically because of failure of an antimicrobial agent to penetrate the blood-brain barrier.

As already mentioned, the array of antimicrobial agents we employ was selected to meet the particular needs of our institution. Some would argue that certain isolates need not be tested against some of the antimicrobial agents in our battery, such as gram-negative rods against oxacillin and vancomycin. However, given the number of isolates we test daily and the research needs of our institution, the use of only one plate for susceptibility testing considerably facilitates laboratory operation.

Finally, it should be reemphasized that we are aware that in certain conditions, such as in patients with infective endocarditis, it may be useful to have more precise MIC data, as well as precise bactericidal data (minimal bactericidal concentrations). To meet this need, we have retained the capability for performing serial twofold microdilution MICs, and from this same system we can then obtain minimal bactericidal concentrations if necessary.

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