ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1979, p. 622-624 Vol. 16, No. 5
0066-4804/79/11-0622/03$02.00/0

Minimal Inhibitory Concentrations of 34 Antimicrobial Agents for Control Strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853

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Received for publication 2 August 1979

The use of control strains of bacteria is important to monitor the accuracy and precision of antimicrobial susceptibility testing. Knowledge of the minimal inhibitory concentrations of commonly used organisms would be useful to achieve a degree of inter- as well as intra-laboratory reproducibility. The minimal inhibitory concentrations of 34 antimicrobial agents for control strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, as determined by a microdilution method in cation-supplemented Mueller-Hinton broth, are reported.

Broth dilution susceptibility testing has become more frequently used with the popularization of efficient microdilution methods (1). While no standard method is available, general recommendations for media, antibiotic preparation and dilution, test performance, and use of control strains are available (3, 8). Although many laboratories use control strains to monitor accuracy and precision, there are few published data on the minimal inhibitory concentrations (MICs) of antimicrobial agents for those organisms. Because comparison of control MICs would be useful to achieve a degree of inter- as well as intra-laboratory reproducibility, we are reporting the results of our experiences with testing 34 antimicrobial agents against two control strains in the medium that is currently recommended (8) for such tests.

MATERIALS AND METHODS

Control strains. The control strains studied were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

Antimicrobial agents. The antimicrobial agents studied are listed in Table 1. Each was obtained as a laboratory standard powder or solution from its respective manufacturer and stored as recommended. Stock solutions were prepared in concentrations of 1,000 or 1,280 μg/ml and either used immediately or stored at −70°C for up to 1 month until used.

Medium. The medium used was Mueller-Hinton broth (MHB) (Difco Laboratories, Detroit, Mich.), which contained approximately 1 mg of calcium per dl and 0.5 mg of magnesium per dl. It was supplemented with $CaCl_2$ and $MgCl_2$ to final concentrations of 5 mg of calcium and 2.5 mg of magnesium per dl.

Susceptibility tests. Susceptibility tests were performed over a period of 2 years as quality control procedures during the course of several in vitro studies. All MIC determinations were performed in volumes of 0.1 ml contained in microdilution plates (Dynatech Laboratories, Alexandria, Va.). Antimicrobial agents were diluted and dispensed with the Dynatech MIC-2000 (Dynatech Laboratories) (6) and used immediately or stored at −70°C until used. The ranges of antibiotic concentrations varied but always consisted of $\log_2$ dilution steps between 128 and 0.06 μg/ml. Freshly made or thawed plates were inoculated with a multiple-inoculum replicator (Dynatech Laboratories) so that the final inoculum was 1×10^5 to 5×10^5 colony-forming units per ml. Inoculated plates were incubated in a room-air incubator at 37°C for 18 to 20 h and read with the aid of a magnifying mirror. The MIC was the lowest concentration of antimicrobial agent which inhibited visible growth.

RESULTS AND DISCUSSION

The MICs of the antimicrobial agents tested for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 are shown in Table 1. By agar dilution (Mueller-Hinton agar with approximately 5 mg of calcium and 2 mg of magnesium per dl), MICs of seven antimicrobial agents for *E. coli* ATCC 25922 were similar to those observed in supplemented MHB in the current study (9). Using unsupplemented MHB, MICs of six antimicrobial agents for *E. coli* ATCC 25922 and of carbenicillin for *P. aeruginosa* ATCC 27853 were also similar, but MICs of tetracycline for *E. coli* and of aminoglycosides for *P. aeruginosa* were lower (1). Those differences were expected in view of the antagonistic effect that cation supplementation of MHB has on tetracycline activity against both *E. coli* and *P. aeruginosa* and on aminoglycoside activity against *P. aeruginosa* (2, 4, 5, 7). In some in-

TABLE 1. *In vitro susceptibilities of control strains*

Antimicrobial agent	*E. coli* ATCC 25922			*P. aeruginosa* ATCC 27853		
	No. of tests	MIC		No. of tests	MIC	
		Mode	Range		Mode	Range
Penicillin G	12	32	16–64	10	>128	>128
Oxacillin	17	>128	>128	12	>128	>128
Nafcillin	17	>128	>128	12	>128	>128
Ampicillin	28	4	2–8	26	>128	>128
Carbenicillin	11	8	4–16	28	32	32–64
Ticarcillin	11	4	2–8	10	16	16–32
Azlocillin	11	16	16	10	8	4–8
Mezlocillin	11	4	4–8	10	16	8–16
Piperacillin	11	2	2	10	4	2–4
Mecillinam	11	0.12	0.06–0.25	10	>128	≥128
Cephalothin	32	8	4–8	22	>128	>128
Cefazolin	12	1–2	1–2	11	>128	>128
Cefamandole	15	0.5	0.25–0.5	17	>128	>128
Cefoxitin	18	2	1–4	15	>128	>128
Cefazaflur	12	0.25–0.5	0.25–0.5	11	>128	>128
Cefuroxime	12	4	2–8	11	>128	>128
LY127935	15	0.12	0.06–0.25	11	16	8–32
HR756	12	≤0.06	≤0.06	11	16	8–16
Streptomycin	10	4	2–8	17	32	16–32
Kanamycin	18	2	1–4	29	>64	>64
Gentamicin	28	0.5	0.12–1	26	2	2
Tobramycin	28	0.5	0.5–1	30	0.5	0.5–1
Amikacin	28	1–2	1–4	26	4	4
Sisomicin	11	0.25	0.25–0.5	12	0.5	0.5–1
Netilmicin	11	0.5	0.25–0.5	12	4	4
Colistin	11	1	0.5–2	12	4	2–4
Tetracycline	28	2	2	30	32	32–64
Minocycline	10	1	0.5–2	10	32	16–64
Doxycycline	10	1	0.5–2	10	64	32–128
Chloramphenicol	28	4	4–8	25	>64	>64
Erythromycin	10	64	64	10	>64	>64
Clindamycin	10	>64	>64	10	>64	>64
Vancomycin	10	>64	>64	10	>64	>64
TMP-SMZ[a]	10	1	1–2	24	>128	>128

[a] Total trimethoprim (TMP)-sulfamethoxazole (SMZ) in a ratio of 1:19.

stances, minor variations in the degree of supplementing MHB have had marked effects on MICs. For example, the addition to MHB of 5 mg of calcium and 2.5 mg of magnesium per dl without regard for the small amounts already present, as is considered acceptable (8), changed the modal MIC of gentamicin for *P. aeruginosa* ATCC 27853 from 2 μg/ml to 4 to 8 μg/ml. The percentage of clinical isolates in our hospital that were susceptible to 4 μg or less of gentamicin per ml had a corresponding change from 74% to 62% (R. J. Fass and J. Barnishan, unpublished data).

In addition to using control strains of *E. coli* and *P. aeruginosa*, it has been recommended that strains of *S. aureus* and *S. faecalis* also be used so that MIC endpoints are reached with all drugs tested (3, 8). Those organisms were not included in the present study, however, because some streptococci may not grow adequately in MHB (9) and many laboratories use a richer medium for testing facultative gram-positive cocci. MICs of control strains of *S. aureus* and *S. faecalis* may vary up to 16-fold in various media (Fass and Barnishan, unpublished data), and agreement on a standardized medium for antimicrobial susceptibility testing of those organisms would be necessary before control values could be established.

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