

ANAEROBIC SUSCEPTIBILITY TESTS

EVALUATION OF THE STABILITY OF ANTIMICROBIALS IN WILKENS-
CHALGREN BROTH AND THE EFFECT OF
MEDIA PREREDUCTION

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The stability of eight antimicrobial agents in WILKENS-CHALGREN broth was evaluated. The activities of only carbenicillin and benzyl penicillin were significantly decreased after storage for eight weeks at -20°C . Anaerobic reduction of the susceptibility testing broth prior to inoculation was found to be unnecessary.

The need for a standardized susceptibility testing method for anaerobes is well-recognized. Agar-dilution testing with WILKENS-CHALGREN agar has been recommended as the reference method by the National Committee for Clinical Laboratory Standards (NCCLS) Working Group on Anaerobic Susceptibility Testing^{1,2)}. Agar dilution testing is not, however, practical for most laboratories which would only test a few isolates each week. In a previous study, the results of microtiter broth dilution tests with WILKENS-CHALGREN broth (WCB) were reported to be comparable with the standardized reference method³⁾. The advantage of the broth-dilution test method is that a large number of the microtiter trays can be prepared at one time and then frozen until needed. In addition, a single tray can be used for each organism.

In this study the stability of eight antimicrobial agents in WCB was determined at different storage temperatures. In addition, the effect of anaerobic reduction of WCB prior to inoculation was examined.

Materials and Methods

The three organisms used in this study were *Bacteroides fragilis* (ATCC 25285), *Peptococcus variabilis* (ATCC 14956), and *Peptococcus asaccharolyticus* (WAL 3218). These were three of the reference organisms recommended by the NCCLS Working Group on Anaerobic Susceptibility testing, and were provided by Dr. JON ROSENBLATT (Mayo Clinic).

Eight antimicrobial agents were evaluated, namely carbenicillin, cefoxitin, chloramphenicol, clindamycin, erythromycin, metronidazole, benzylpenicillin and tetracycline. The susceptibility tests were performed with WCB, which was prepared as described by WILKENS and CHALGREN¹⁾ except the agar was omitted. Serial two-fold dilutions of the antimicrobial agents (0.125 ~ 128 $\mu\text{g}/\text{ml}$) were prepared in WCB and automatically delivered (Dynatech MIC 2000 Dispenser) into microtiter trays, with 100 μl dispensed into each well. The trays were then sealed in plastic bags and frozen at either -20 or -70°C .

On the day the susceptibility testing trays were prepared, and weekly thereafter for 8 weeks, each of the three reference organisms was tested in duplicate on reduced and nonreduced medium. Prior to use, the trays were warmed at ambient temperature, and then were stored for 4 hours at 35°C in an anaerobic glovebox chamber (reduced) or an air-incubator (nonreduced). The test inoculum was prepared with an overnight growth in WCB, which was adjusted to a turbidity equivalent to one-half a MCFAR-

LAND No. 1 standard, and $1 \mu\text{l}$ (10^5 bacteria) was inoculated into each microtiter well (Dynatech MIC 2000 Inoculator). All trays were then incubated at 35°C for 48 hours in an anaerobic glovebox, after which time the minimal inhibitory concentration (MIC) values were determined as the lowest concentration of antimicrobial agent which prevented visible growth of the organisms.

Results

The MIC values of the eight antimicrobial agents, which were determined on the day the trays were prepared, are summarized in Table 1. There was no change in antimicrobial activity for cefoxitin, chloramphenicol, clindamycin, erythromycin, metronidazole, or tetracycline after storage of the trays for eight weeks at either -20 or -70°C . The activity of carbenicillin and benzyl penicillin did, however, decrease during storage, particularly at -20°C . There was a 2~4 fold decrease in carbenicillin activity (Fig. 1) and a 2~8 fold decrease in benzyl penicillin activity (Fig. 2) after storage for eight weeks at -20°C . The MICs of carbenicillin and benzyl penicillin were within one two-fold dilution after 8 weeks of storage at -70°C for the test organisms, with the exception of *P. asaccharolyticus* with benzylpenicillin.

There were no differences in MIC values of the eight antimicrobial agents for the three organisms tested in anaerobically reduced medium compared with nonreduced medium.

Fig. 1. MICs of carbenicillin for the three reference organisms tested in susceptibility testing trays stored at -20°C (closed symbols) or -70°C (open symbols).

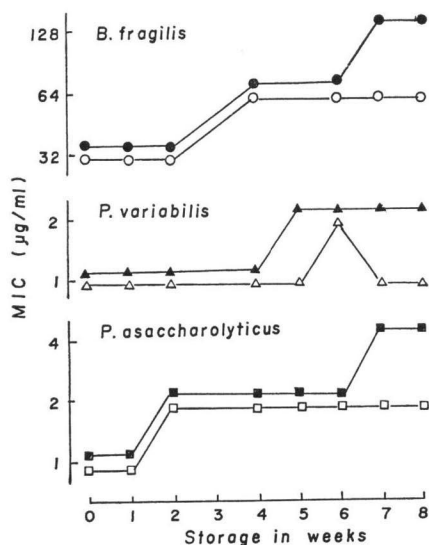
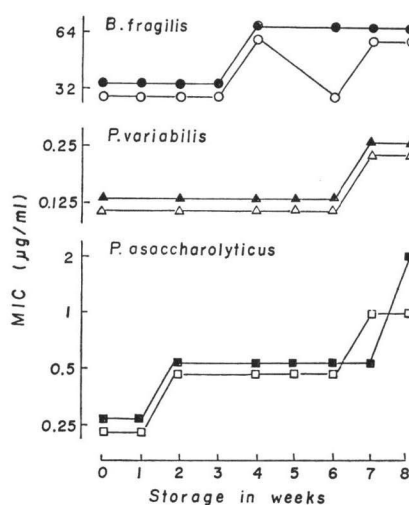


Table 1. MICs for the test organisms on freshly prepared susceptibility testing trays.

Antimicrobial agents	MIC ($\mu\text{g/ml}$) for:		
	<i>B. fragilis</i> (ATCC 25285)	<i>P. variabilis</i> (ATCC 14956)	<i>P. asaccharolyticus</i> (WAL 3218)
Carbenicillin	32	1	1
Cefoxitin	16	1	1
Chloramphenicol	8	2	2
Clindamycin	2	2	>128
Erythromycin	4	16	>128
Metronidazole	1	1	1
Benzyl penicillin	32	0.25	0.25
Tetracycline	2	4	128

Fig. 2. MICs of benzyl penicillin for the three reference organisms tested in susceptibility testing trays stored at -20°C (closed symbols) or -70°C (open symbols).



Discussion

ZABRANSKY and HAUSER demonstrated that antimicrobial agents were stable in WILKENS-CHALGREN agar for at least 2 weeks when stored at either room temperature or at 4°C⁴. Others have reported that antimicrobial agents were stable in Trypticase soy broth and MUELLER-HINTON broth when stored at -70°C^{5,6}. JONES and coworkers⁷ reported that, although all the antimicrobial agents which they tested were stable in brain heart infusion broth for 45 days, the potency of carbenicillin and benzylpenicillin declined in SCHAEGLER'S broth when stored at -20°C. In the present study, although carbenicillin and benzylpenicillin were unstable in WCB when stored at -20°C, there was no significant loss of antimicrobial activity in the trays stored at -70°C.

Reduction of the medium for 4 hours immediately prior to inoculation was found to be unnecessary. This is consistent with the previous report that the MIC values were essentially the same for organisms tested on plates stored either under anaerobiosis or an ambient atmosphere⁴.

References

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