STABILITY OF ANTIMICROBIALS IN SCHAEDLER'S ANAEROBIC AND BRAIN HEART INFUSION BROTHS STORED AT −20°C

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Penicillin-like drugs are found to be unstable in SCHAEDLER's broth in frozen storage (−20°C). Chloramphenicol, clindamycin and tetracycline remained at original potency to 45 days. No detectable antimicrobial decay was found in two formulations of supplemented BHI broth. Antimicrobial potency was measured by quality control organism endpoints, bioassays and MIC changes in use at four clinical microbiology laboratories.

There is a well recognized need for method standardization of the antimicrobial susceptibility testing of anaerobic organisms. A conference in 1972 devoted significant time to discussion of current and diverse methodologies[1,2]. Since that conference STALONS and others have suggested the use of SCHAEDLER's broth medium for anaerobic susceptibility testing due to outstanding growth characteristics compared to other commercially available products[3,4].

This paper presents data from a four-laboratory evaluation of the broth microdilution method of anaerobic antimicrobial susceptibility testing including MIC comparisons and stability evaluation of antimicrobials.

Study Design and Methods

Four clinical microbiology laboratories participated in the protocol. Antimicrobial agents (carbenicillin, chloramphenicol, clindamycin, penicillin G and tetracycline) were obtained as standard laboratory powders from the manufacturers. Fourteen $\log_2$ dilutions of each drug were prepared in broth and dispensed in 100 μl volume in microdilution plastic trays (Micro-Media Systems, San Jose, California). Trays were immediately frozen at −20°C and distributed to the collaborating laboratories. SCHAEDLER's broth (BBL) and Brain Heart infusion broth (BHI) were prepared according to manufacturers recommendations. BHI broth was supplemented with 5 μg/ml hemin and 0.5 μg/ml vitamin K_1, and one lot with 0.5% glucose (BHIG).

Cultures of ten organisms (nine anaerobes and one facultative anaerobe) were distributed as unknowns to each laboratory. These bacteria included *Bacteroides fragilis* ATCC 25285 and C-2613-2, *B. thetaiotaomicron* KFL-B1 and KFL-438-1, *B. vulgatus* ATCC 29327, *Clostridium perfringens* ATCC 13124, *C. butyricum* C-4347-4, *C. ramosum* C-3578-4, *C. sordellii* C-4071 and *Streptococcus faecalis* ATCC 29212. MICs for each antimicrobic using three media bases were determined in triplicate

for three weekly trials. Several of the strains (ATCC 13124, 25285, 29327) were also recommended as reference organisms by the Working Group on Anaerobic Antimicrobial Susceptibility Testing, National Committee for Clinical Laboratory Standards.[5]

Inocula were prepared from overnight broth cultures grown in SCHAEDLER's broth and diluted to equal the turbidity of a No. 1 MACFARLANDS $BaSO_4$ standard. Five μl of the dilution was transferred by replicate inoculator (Micro-Media Systems, San Jose, Calif.) to each well. Final inoculum concentration was 5×10^4 CFU/well. MICs were defined as the lowest antimicrobial concentration totally inhibiting growth (clear well) after incubation in GasPak jars at 35°C for 48 hours.

Antimicrobial potency in broths were measured by using four quality control organisms (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853) having reproducible MIC results. MICs and bioassays were monitored after storage for 15, 21, 30 and 45 days. All trays were stored at −20°C in freezers without frost free cycles.

Results

Table 1 shows modal MIC values of the non-penicillin antimicrobials against *B. fragilis* ATCC 25285. All endpoints were within 1 $\log_2$ dilutions with clindamycin showing total agreement and tetracycline MICs in BHI broths generally lower than SCHAEDLER's values. Similar results were noted with the other nine organisms and quality control strains.

Fig. 1 demonstrates the combined results of MIC and bioassay determination of penicillin G potency in SCHAEDLER's and BHI broths. Similar results were found for carbenicillin. By 15 days the SCHAEDLER's penicillin potency had dropped to approximately 50% of original values and at 45 days had been reduced to 1: 8 and 11% by MIC and bioassay methods respectively. No significant reduction in penicillin or non-penicillin antimicrobial activity were found in the BHI media to 45 days of storage.

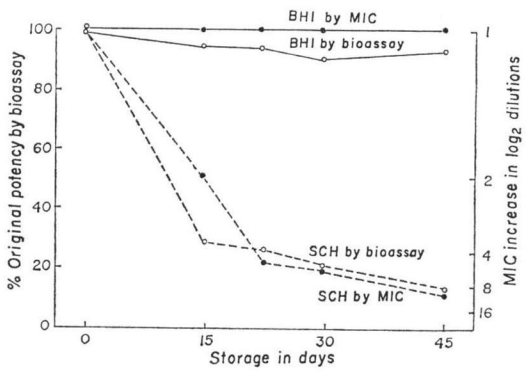
These quality control and bioassay data were further substantiated by the raising penicillin and carbenicillin MIC values from the collaborating laboratories as the study progressed. Table 2 shows strain ATCC 25285 penicillin G and carbenicillin MICs indexed by trial week and laboratory. Note the increasing MICs recorded for each protocol site indicating a 2~4 fold loss in potency during the study interval.

Table 1. Comparison of chloramphenicol, clindamycin and tetracycline anaerobic MICs determined in SCHAEDLER's, BHIG and BHI broths for *Bacteroides fragilis* (ATCC 25285)

Antibiotics	Modal MICs of five laboratories		
	SCHAELDER's	BHIG*	BHI
Chloramphenicol	16	16	8
Clindamycin	0.25	0.25	0.25
Tetracycline	0.25	0.125	0.125

* BHIG=Brain-heart-infusion broth with 0.5% glucose.

Fig. 1. Penicillin G antimicrobial potency diluted in SCHAEDLER's (SCH) and brain-heart-infusion (BHI) broths stored at −20°C as determined by bioassay and MIC endpoint techniques.



Discussion

Comparable chloramphenicol, clindamycin and tetracycline MICs are found for ten study organisms and quality control strains in SCHAEDLER's and BHI broths. However, the carbenicillin and penicillin G MICs were only similar at the outset of the study due to antimicrobial instability at −20°C. Similar findings have been reported[6], but may be attributed to temperature fluctuations in storage from frost free freezers cycles, a variable controlled in this study. These experiences were in contrast to that of two other laboratories[7] where no detectable penicillin antimicrobial decay has been found. However, these microbiologists held trays at −60°~−70°C and/or generally utilized trays within two weeks of manufacture.

Table 2. Carbenicillin and penicillin G MICs by week of trial performed in SCHAEDLER's broth on *Bacteroides fragilis* (ATCC 25285)

Antibiotic	Trial week	Laboratory MIC (μg/ml)			
		A	B	C	D
Carbenicillin	1*	32	32	64	128
	2	64	64	128	256
	3	128	64	256	512
Penicillin G	1	32	32	32	64
	2	64	64	64	256
	3	64	64	128	256

* Not all laboratories initiated trials on same week of media lot storage.

The antimicrobial stability in BHI broths has been further substantiated in another protocol[8]. This later study also collaborates the work of ZABRANSKY and HAUSER who demonstrated excellent antimicrobial stability in WILKINS-CHALGREN medium[8,9,10]. The WILKINS-CHALGREN medium has been recommended by the Working Group on Anaerobic Susceptibility Testing, NCCLS for use in the reference agar dilution method. We would also favor the use of either WILKINS-CHALGREN medium or supplemented BHI broth for susceptibility testing of anaerobes because of penicillin stability in frozen storage.

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References

1) THORNSBERRY, C.: Anaerobic Bacteria: Role in Disease. Chapter 37, Factors Affecting Susceptibility Tests and the Need for Standardized Procedures. pp. 477~496, Charles C. Thomas Publisher, Springfield, I11., 1974
2) SHERRIS, J.: Anaerobic Bacteria: Role in Disease. Chapter 39. A Discussion of Susceptibility Testing. pp. 497~503, Charles C. Thomas Publisher, Springfield, I11., 1974
3) STALONS, D. R. & C. THORNSBERRY.: Broth-dilution method for determining the antibiotic susceptibility of anaerobic bacteria. Antimicr. Agents & Chemoth. 7: 15~21, 1975
4) STALONS, D. R.; C. THORNSBERRY & V. R. DOWELL, Jr.: Effect of culture medium and carbon dioxide concentration on growth of anaerobic bacteria commonly encountered in clinical specimens. Appl. Microbiol. 27: 1098~1104, 1974
5) SUTTER, V. L.; A. L. BARRY, W. J. MARTIN, J. E. ROSENBLATT, V. R. DOWELL, Jr., C. THORNSBERRY, T. D. WILKINS & R. J. ZABRANSKY: Preliminary trials of a reference method for antimicrobial susceptibility testing of anaerobic. Abstr. No. 453, 16th Intersci. Conf. on Antimicr. Agents & Chemoth. Chicago, I11., 1976
6) PRIOR, R. B. & R. J. FASS: Stability of penicillins frozen in SCHAEDLER broth for susceptibility testing of anaerobic bacteria. Abst. No. C60, 76th Amer. Soc. for Microbiol. Atlantic City, N. J., 1976
7) THORNSBERRY, C. & E. H. GERLACH: Personal communications, 1976
8) JONES, R. N.; P. C. FUCHS & C. THORNSBERRY: Antimicrobial susceptibility testing comparison of WILKINS-CHALGREN anaerobic agar and broth with brain-heart infusion broth including antimicrobial stability in frozen storage. Current Microbiol.; (in press) 1978
9) WILKINS, T. D. & S. CHALGREN: Medium for use in anaerobic susceptibility testing of anaerobic bacteria. Antimicr. Agents & Chemoth. 10: 926~928, 1976
10) ZABRANSKY, R. J. & K. J. HAUSER: Stability of antibiotics in WILKINS-CHALGREN anaerobic susceptibility testing medium after prolonged storage. Antimicr. Agents & Chemoth. 12: 440~441, 1977