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Fastidious Anaerobe Agar Compared with Wilkins-Chalgren Agar, Brain Heart Infusion Agar, and Brucella Agar for Susceptibility Testing of *Fusobacterium* Species

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Fastidious anaerobe agar supported the growth of 82 strains of fusobacteria better than brain heart infusion agar, brucella agar, and Wilkins-Chalgren agar. Fastidious anaerobe agar showed less hazing and fewer tailing endpoints with beta-lactam antibiotics. Whole-blood supplementation improved the performance of all media. Wilkins-Chalgren agar without blood failed to support the growth of 17% of the strains. All *Fusobacterium ulcerans* strains were resistant to clindamycin.

The antimicrobial susceptibility of *Fusobacterium* species has been reported to be quite variable (5, 8). Poor reproducibility of MICs may result from poor growth of fusobacteria on agar media, from small inoculum size (10), or from "difficulty in determining the endpoint of growth" with beta-lactam antibiotics due to a visible haze composed of cell wall-defective variants (8).

The present study determined (i) the adequacy of strain growth on different media, (ii) the effects of different agar bases on the hazing problem, and (iii) the effect of the addition of laked versus whole blood on hazing. We studied media previously reported for agar dilution susceptibility studies (3–6, 8, 11) and also fastidious anaerobe agar (FAA), a new basal medium recommended for the growth of fusobacteria (2). Furthermore, the susceptibility of *Fusobacterium ulcerans*, a newly described species (1), is reported.

Seventy-eight *Fusobacterium* species isolated from human clinical specimens and four animal isolates (three *F. russii* isolates and one *F. gonidiaformans* isolate) were included in this study. They were identified according to standard criteria (7, 11). *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124 were used as controls.

Standard laboratory powders were supplied as follows: ampicillin-sulbactam from Pfizer Inc. (New York, N.Y.), clindamycin from the Upjohn Co. (Kalamazoo, Mich.), cefoxitin from Merck Sharp & Dohme (West Point, Pa.), and metronidazole and penicillin G from Sigma Chemicals (St. Louis, Mo.). The antimicrobial solutions were prepared on the day of study according to the manufacturers' instructions.

MICs were determined on brucella agar (BA), brain heart infusion agar (BHIA), and Wilkins-Chalgren agar (WCA) (Difco Laboratories, Detroit, Mich.), all supplemented with laked sheep blood, and on FAA (Lab M Ltd., Bury, England), which was supplemented with whole horse blood. The BA and BHIA were also supplemented with hemin and vitamin K_1. The BHIA was further supplemented with 1% yeast extract (Difco Laboratories). To determine the effect of blood constituents on hazing in MIC tests, media with laked sheep blood, whole defibrinated sheep blood, or whole defibrinated horse blood were tested with ampicillin-sulbactam, as an example of a cell wall-active antibiotic. To determine the adequacy of strain growth on the different media, the four basal media without blood or antibiotic supplementation were tested.

An agar dilution method was utilized with antibiotic concentrations of 32 to 0.03 μg/ml (9). Inocula were prepared by suspending colonies from 48-h plates into brucella broth (Difco Laboratories) to achieve a 0.5 McFarland standard. Colony counts were done on selected strains to verify inoculum density. The plates were inoculated by using a Steers replicator (Craft Machine Inc., Chester, Pa.), with a final inoculum of approximately 10^5 CFU per spot. All plates were incubated in an anaerobic chamber (Anaerobe Systems, San Jose, Calif.) at 37°C for 48 h and were then examined.

Hazing was seen only with the beta-lactam antibiotics tested (penicillin G, cefoxitin, and ampicillin-sulbactam). For these agents, FAA yielded clearer endpoints with less hazing. Hazing at ≥3 dilutions beyond the MIC was observed as depicted in Table 1. Hazing was seen among all the species tested, although only *F. nucleatum* produced hazing on all media studied. This was most evident on BA, on which all 17 strains produced hazing. *F. ulcerans* and *F. varium* isolates did not haze on FAA but did so on the other three media.

In the blood supplementation part of the study, hazing was

TABLE 1. Percentage of *Fusobacterium* strains exhibiting hazing on four different agar bases with three beta-lactam antimicrobial agents[a]

Antibiotic	% of *Fusobacterium* strains exhibiting hazing on:			
	FAA	BHIA	BA	WCA
Ampicillin-sulbactam	12	31	54	57
Cefoxitin	10	42	29	31
Penicillin G	3	22	32	22

[a] Hazing at ≥3 dilutions above the MIC. FAA was supplemented with whole horse blood; other media were supplemented with laked sheep blood.

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TABLE 2. Comparative susceptibilities of *Fusobacterium* species to a selected panel of antimicrobial agents as determined by using FAA

Organism (no. tested)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
F. ulcerans (9)	Cefoxitin	2–4	2	4
	Metronidazole	0.25–0.5	0.5	0.5
	Penicillin G	0.06–4	0.125	4
	Clindamycin	16–>32	>32	>32
	Amp-sulbactam[b]	1–4	2	4
F. mortiferum (9)	Cefoxitin	0.125–4	2	4
	Metronidazole	0.125–0.5	0.25	0.5
	Penicillin G	0.06–0.5	0.25	0.5
	Clindamycin	0.06–8	0.25	8
	Amp-sulbactam	0.5–2	1	2
F. varium (11)	Cefoxitin	0.5–8	4	8
	Metronidazole	0.125–0.5	0.5	0.5
	Penicillin G	0.125–1	0.25	0.5
	Clindamycin	0.03–>32	8	32
	Amp-sulbactam	0.5–4	2	4
F. nucleatum (17)	Cefoxitin	0.06–0.5	0.25	0.5
	Metronidazole	0.03–0.5	0.25	0.5
	Penicillin G	0.03–0.03	0.03	0.03
	Clindamycin	0.03–0.125	0.06	0.125
	Amp-sulbactam	0.03–0.5	0.125	0.25
F. necrophorum (12)	Cefoxitin	0.06–1	0.25	0.5
	Metronidazole	0.03–0.25	0.125	0.25
	Penicillin G	0.03–0.125	0.03	0.03
	Clindamycin	0.03–0.125	0.03	0.06
	Amp-sulbactam	0.06–0.25	0.125	0.25
F. naviforme (9)	Cefoxitin	0.125–2	0.5	2
	Metronidazole	0.03–0.5	0.06	0.5
	Penicillin G	0.03–4	0.03	4
	Clindamycin	0.03–32	0.03	32
	Amp-sulbactam	0.06–4	0.5	4
F. russii (3)	Cefoxitin	0.25–0.5	0.5	
	Metronidazole	0.5–0.5	0.5	
	Penicillin G	0.06–0.125	0.06	
	Clindamycin	0.06–0.06	0.06	
	Amp-sulbactam	0.5–0.5	0.5	
F. gonidiaformans (3)	Cefoxitin	0.25–0.5	0.5	
	Metronidazole	0.25–0.25	0.25	
	Penicillin G	2–4	4	
	Clindamycin	0.03–0.06	0.06	
	Amp-sulbactam	4–4	4	
Fusobacterium sp. (9)	Cefoxitin	0.06–8	0.25	8
	Metronidazole	0.06–0.5	0.06	0.5
	Penicillin G	0.03–2	0.03	2
	Clindamycin	0.03–0.125	0.03	0.125
	Amp-sulbactam	0.125–4	0.25	4

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b] Amp-sulbactam, Ampicillin-sulbactam tested in a ratio of 2 parts ampicillin to 1 part sulbactam.

more apparent with laked sheep blood than with whole sheep or horse blood, which provided an opaque background that made the endpoints more discernible. The growth of fusobacteria on basal media without added blood or antibiotics showed variable results among the species tested. The basal WCA medium without blood supplementation failed to support growth of 17% of the fusobacteria tested. Some strains also failed to grow on BA and BHIA without blood supplementation. All species grew adequately without blood on FAA, although growth of *F. naviforme*, *F. necrophorum*, and *F. nucleatum* strains was optimal with blood supplementation. The addition of blood to the basal media resulted in better growth of the more fastidious fusobacteria (*F. naviforme*, *F. necrophorum*, and *F. nucleatum*). Even with the addition of blood to WCA, growth of fusobacteria was not as luxuriant as with the other media.

The results of the susceptibility study of *Fusobacterium* species on FAA supplemented with whole horse blood are summarized in Table 2. Among the various species of fusobacteria, differences in susceptibility to each agent were noticeable. All strains were susceptible to penicillin G, cefoxitin, metronidazole, and ampicillin-sulbactam. Forty-six percent of *F. varium* strains and 100% of *F. ulcerans* strains were resistant to clindamycin, with MICs of ≥16 μg/ml. For the strains that grew on WCA, MICs were within 1 dilution of those obtained on WCA (supplemented with laked sheep blood) as follows: 99% (FAA), 97% (BA), and 100% (BHIA) for clindamycin; 91% (FAA), 96% (BA), and 99% (BHIA) for metronidazole; 77% (FAA), 82% (BA), and 88% (BHIA), for cefoxitin; 87% (FAA), 95% (BA), and 90% (BHIA) for penicillin G; and 73% (FAA), 80% (BA), and 83% (BHIA), for ampicillin-sulbactam. There was a trend towards higher MICs on the other media compared with WCA which was in part due to poorer growth of strains on WCA.

Our study underlines the importance of selection of an agar basal medium that supports optimal growth and therefore enables more accurate determination of MICs. WCA without added blood, which is recommended by the National Committee for Clinical Laboratory Standards for anaerobic susceptibility testing (9), failed to support the growth of 17% of the fusobacteria tested, including 64% of the *F. necrophorum* strains. R. J. Zabransky (Clin. Microbiol. Newsl. **11:** 185–192, 1989) has previously noted that WCA "does not support the growth of all anaerobes and certain organism-drug combinations do not produce reproducible results." While BA with blood supported the growth of all strains, tailing endpoints were frequent. FAA supported the growth of all *Fusobacterium* species tested, and the hazing problem was reduced on FAA compared with the other media. This suggests that FAA may be a more suitable medium for susceptibility testing of fusobacteria than the currently recommended WCA or BA (9).

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