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Variation in Susceptibility Patterns of Species Within the *Bacteroides fragilis* Group

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Minimal inhibitory concentrations of eight antibiotics were determined for 159 clinical isolates of members of the *Bacteroides fragilis* group using the National Committee for Clinical Laboratory Standards proposed agar dilution reference method. Isolates were identified by standard techniques with deoxyribonucleic acid homolog confirmation. These closely related species demonstrated differences in susceptibility to commonly used drugs.

Currently there is no standardized procedure for antimicrobial susceptibility testing for anaerobes. The National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antimicrobial Susceptibility Testing sponsored a study to evaluate a proposed reference method. Sutter et al. have recommended that this method be accepted as the reference method (22).

The *Bacteroides fragilis* group of organisms are separated from other gram-negative anaerobic bacilli by their ability to grow in media containing 20% bile. Work by Johnson, who determined deoxyribonucleic acid (DNA) homologies, and by Johnson and Ault, who correlated phenotypic characteristics with DNA homology, identified seven distinct species within this group of organisms (9, 10). Phenotypic characteristics can reliably be used to separate *B. fragilis*, *Bacteroides vulgatus*, *Bacteroides distasonis*, *Bacteroides uniformis*, and *Bacteroides eggerthii*. *Bacteroides ovatus* are difficult to separate from *Bacteroides thetaiotaomicron* by using phenotypic reactions, because both have the same likelihood values for commonly used biochemicals (10).

Whether antibiotic susceptibility profiles can be used to differentiate within the *B. fragilis* group is controversial. Several investigators found no differences in susceptibility profiles (1, 5, 13, 17, 23). Others found minimal variation among the *B. fragilis* group (2, 4, 11).

The NCCLS reference method (22) with DNA confirmation of isolates was used to determine whether species differ in their susceptibility patterns and whether these patterns can be used to separate organisms within the *B. fragilis group*.

MATERIALS AND METHODS

Bacterial strains and identification procedures. A total of 159 organisms were isolated from clinical material submitted to the microbiology laboratory at the Veterans Administration Medical Center from the inpatient population. The clinical sources of the strains included blood (14 isolates), abscess (45 isolates), wound (59 isolates), peritoneal fluid (8 isolates), genitourinary (8 isolates), and miscellaneous sites (25 isolates).

Organisms were identified by Gram stain, the Minitek anaerobe system, and gas-liquid chromatography. Incubation was at 35°C with the GasPak system (BBL Microbiology Systems). Species identification was determined by acid production from mannitol, rhamnose, trehalose, salicin, and indole production. Deoxyribonucleic acid homologs were determined for each isolate by John Johnson at the Virginia Polytechnic Institute.

Fermentation of xylan was determined on indole-positive organisms, *B. ovatus* and *B. thetaiotaomicron*. The medium was prepared by adding 14 g of dehydrated Lombard Dowell broth (BBL Microbiology Systems) to 900 ml of distilled water, 100 ml of a 6% (wt/vol) solution of xylan (Sigma Chemicals) in distilled water, and 0.018 g of phenol red. The pH was adjusted to 7.3 with 1 N NaOH. Amounts (6 ml) were dispensed in screw-capped tubes and autoclaved at 12 lb for 15 min. Tubes were inoculated with a loopful of 24-h cultures of the organisms, incubated with the GasPak System, and read at 24 and 48 h by transferring a drop of culture to a spot plate and adding one drop of 0.025% phenol red (BBL). A yellow reaction was interpreted as positive; orange-red or red were considered negative.

Quality control organisms *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were included with each testing as recommended in the NCCLS method.

Antimicrobial agents. Laboratory standard powders were supplied as follows: sodium cefoxitin from Merck, Sharp and Dohme, Rahway, N.J.; clindamycin from The Upjohn Co., Kalamazoo, Mich.; chloramphenicol from Parke-Davis & Co., Detroit, Mich.; doxycycline from Pfizer Inc., Brooklyn, N.Y.; metronidazole from G. D. Searle & Co., Chicago, Ill.; penicillin from Eli Lilly & Co., Indianapolis, Ind.; rifampin from Ciba Pharmaceutical Co., Summit, N.J.; and ticarcillin from Beecham Labs, Bristol, Tenn.

Procedure. The NCCLS agar dilution proposed reference method was used to determine the minimum inhibitory concentration of eight antimicrobial agents. The following modifications were made. Antibiotic

dilutions were prepared and incorporated into Wilkens-Chalgren agar (Difco) the day before they were to be inoculated, and inocula were prepared from 18- to 24-h cultures grown on brucella agar (BBL Microbiology Systems) supplemented with hemin, vitamin K, and 5% sheep blood by suspending the organisms in freshly prepared brucella broth (BBL Microbiology Systems) to a turbidity of one-half a no. 1 McFarland standard.

Final concentrations of all antimicrobial agents were prepared from serial twofold dilutions in sterile water ranging from 256 to 0.015 μg/ml, except for rifampin which was in a concentration of 128 to 0.015 μg/ml diluted in Sorensons buffer.

RESULTS

Members of the *B. fragilis* group identified during this study are listed in Table 1. There were no isolates of *B. vulgatus* or *B. eggerthii*. Three of these strains showed no good DNA fit and were unable to be genotypically identified as belonging to homology group I or II (J. Johnson, personal communication).

Laboratory identification of isolates agreed with DNA homolog identification except with indole-positive, mannitol-positive, salicin-negative strains of *B. thetaiotaomicron*, which were called *B. ovatus*, and indole-positive, mannitol- and salicin-positive strains of *B. thetaiotaomicron*, which had been identified as *B. ovatus*. Xylan fermentation on these 50 organisms resulted in acid production with the 17 *B. ovatus* strains and no fermentation with the 33 *B. thetaiotaomicron* strains.

The comparative in vitro activities of the eight antibiotics against members of the *B. fragilis group* are shown in Table 1. There was variation among species in the concentration of several drugs required to inhibit 90% of the strains. Susceptibility to cefoxitin, from most susceptible to resistant, was *B. fragilis* = *B. uniformis* > *B. thetaiotaomicron* = NGF (no good fit) > *B. distasonis* > *B. ovatus*; that to clindamycin was NGF > *B. fragilis* = *B. uniformis* > *B. ovatus* > *B. thetaiotaomicron* > *B. distasonis*; that to doxycycline was *B. ovatus* = NGF > *B. thetaiotaomicron* > *B. fragilis* > *B. uniformis* > *B. distasonis*; and that to metronidazole was *B. uniformis* > *B. fragilis* = *B. distasonis* > *B. ovatus* > *B. thetaiotaomicron* = NGF. Chloramphenicol and rifampin showed minimal variation in their minimum inhibitory concentration for all strains tested regardless of species. *B. uniformis* and *B. thetaiotaomicron* were not as resistant to penicillin as were the other species; with ticarcillin, *B. uniformis* > *B. fragilis* = *B. thetaiotaomicron* > *B. ovatus* = *B. distasonis* = NGF.

The percentage of isolates inhibited at achievable serum levels are shown in Table 2. Species clearly vary in their susceptibility to antibiotics. Two serum levels of penicillin are listed because clinical use of ticarcillin is usually directed at achieving a serum concentration of 128 μg/ml. Penicillin would be as effective if this level were achieved.

Reproducibility of the proposed reference method with control strains was evident in this study. Deviation from the NCCLS method of inoculum preparation had no effect on expected minimum inhibitory concentration (in micrograms per milliliter) results for the control organisms as seen in Table 3. Four additional antimicrobial agents not used in the collaborative study are included in Table 3. Results for all antibiotics listed were ±1 dilution from the expected mode (NCCLS) and from the calculated mode for the additional antibiotics.

DISCUSSION

Members of the *B. fragilis* group are the commonest of the intestinal anaerobes isolated from bacteremia (4, 15). It is generally accepted that this group of organisms is also the most frequently isolated from other anaerobic infections (12, 18).

The nomenclature in recent literature is misleading, since the generic term *B. fragilis* is often used to include other species which in the past were considered to be subspecies of *B. fragilis* (6–8, 19, 21). From a clinical standpoint, it would be reasonable to report all of these organisms as *B. fragilis* if there were no differences in the susceptibility patterns.

This study demonstrates that clinical isolates of species within the *B. fragilis* group differ in antibiotic susceptibility. This finding has not been the experience of others (2, 11, 16). Long et al. (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., abstr. no. 381, 1975) reported that *B. thetaiotaomicron* was more resistant to clindamycin than other species. The results of this study agree with their finding, but in addition increased resistance to clindamycin with *B. distasonis* was noted.

However, if the 159 clinical isolates in this study were combined and reported as *B. fragilis*, the percent susceptible at a minimum inhibitory concentration of 4 μg/ml with clindamycin is 86%, that for cefoxitin at 16 μg/ml is 68%, that for metronidazole at 16 μg/ml is 90%, and that for ticarcillin at 128 μg/ml is 87%. These results would be misleading (Table 2).

Differences in susceptibility patterns between different species of anaerobes are to be expected. Whether the differences are the result of plasmid transfer as demonstrated by Tally et al. (24) or

by other mechanisms, the organisms have the ability to code for antibiotic resistance (3, 14).

Of the 89 *B. fragilis* strains tested, better than 90% were susceptible at achievable serum levels of the antimicrobial agents tested, with the exception of penicillin and ticarcillin where less than 35% were at a minimum inhibitory concentration of 16 μg/ml. Less than half of the *B. thetaiotaomicron*, *B. ovatus*, and *B. distasonis* strains were susceptible to cefoxitin at achieva-

TABLE 1. *Comparative in vitro activity of eight antibiotics against members of the B. fragilis group*

Organism (no. of strains)	Antibiotic	MIC[a] range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
B. fragilis (89)	Cefoxitin	0.12–64	16	16
	Clindamycin	0.03–8	0.5	1
	Chloramphenicol	2–32	8	16
	Doxycycline	0.03–8	0.5	4
	Metronidazole	0.25–64	1	4
	Penicillin	8–256	32	256
	Rifampin	0.03–1	0.25	0.5
	Ticarcillin	8–256	32	64
B. thetaiotaomicron (33)	Cefoxitin	0.25–64	32	32
	Clindamycin	0.25–64	4	8
	Chloramphenicol	2–16	4	8
	Doxycycline	0.03–16	1	2
	Metronidazole	0.5–64	16	64
	Penicillin	4–256	32	64
	Rifampin	0.12–1	0.5	1
	Ticarcillin	8–256	32	64
B. ovatus (17)	Cefoxitin	16–128	32	128
	Clindamycin	0.25–4	1	2
	Chloramphenicol	4–8	8	8
	Doxycycline	0.03–4	0.5	1
	Metronidazole	1–64	8	8
	Penicillin	16–256	16	>256
	Rifampin	0.03–1	0.25	0.5
	Ticarcillin	16–256	64	>256
B. distasonis (14)	Cefoxitin	8–64	32	64
	Clindamycin	0.12–16	8	16
	Chloramphenicol	4–8	8	8
	Doxycycline	0.12–16	2	16
	Metronidazole	0.5–64	0.5	4
	Penicillin	8–256	32	>256
	Rifampin	0.12–1	0.25	1
	Ticarcillin	4–256	32	>256
B. uniformis (3)	Cefoxitin	4–16	8	16
	Clindamycin	0.12–1	0.25	1
	Chloramphenicol	4–8	8	8
	Doxycycline	0.06–8	1	8
	Metronidazole	0.5–1	1	1
	Penicillin	8–32	32	32
	Rifampin	0.25–0.5	0.5	0.5
	Ticarcillin	8	8	8
B. fragilis NGF[b] (3)	Cefoxitin	4–32	8	32
	Clindamycin	0.5	0.5	0.5
	Chloramphenicol	2–4	4	4
	Doxycycline	0.03–1	0.25	1
	Metronidazole	1–32	2	64
	Penicillin	16–256	32	256
	Rifampin	0.12–0.5	0.5	0.5
	Ticarcillin	4–256	32	>256

[a] MIC, Minimum inhibitory concentration.
[b] No good fit based on DNA homology.

TABLE 2. *Variable susceptibilities of Bacteroides species to achievable blood levels of eight antibiotics*

Antibiotic	Achievable blood levels (μg/ml)	% Inhibited			
		B. fragilis	*B. thetaiotaomicron*	*B. ovatus*	*B. distasonis*
Cefoxitin	16	91	45	16	28
Clindamycin	4	97	67	100	43
Chloramphenicol	16	93	100	100	100
Doxycycline	4	91	97	100	86
Metronidazole	16	99	70	82	93
Penicillin	16	31	15	53	21
	128	89	97	88	64
Rifampin	1	100	100	100	100
Ticarcillin	16	34	27	6	43
	128	91	97	82	64

TABLE 3. *Results of eight antibiotics against quality control strains*

Organism	Antibiotic		No. of tests	Mode	On mode	Range
B. fragilis ATCC 25285	Cefoxitin	(8)[a]	7	8	4	4–8
	Clindamycin	(1)	7	1	4	1–2
	Chloramphenicol	(4)	7	4	4	4–8
	Doxycycline		6	0.5	5	0.25–0.5
	Metronidazole		6	8	5	4–8
	Penicillin	(32)	7	32	6	32–64
	Rifampin		7	0.25	6	0.25–5
	Ticarcillin		6	32	6	32
B. thetaiotaomicron ATCC 29741	Cefoxitin	(16)	7	8	4	8–16
	Clindamycin	(4)	7	4	7	4
	Chloramphenicol	(8)	7	8	7	8
	Doxycycline		6	2	3	1–4
	Metronidazole		6	8	3	4–16
	Penicillin	(32)	7	32	7	32
	Rifampin		7	0.5	6	0.5–1
	Ticarcillin		6	32	5	16–32

[a] Numbers in parentheses represent mode as determined by NCCLS collaborative study.

ble serum levels of 16 μg/ml. *B. thetaiotaomicron* and *B. distasonis* strains are more likely to be resistant to clindamycin than other species and *B. distasonis* strains, likewise for ticarcillin at serum levels of 128 μg/ml.

The use of DNA homology data for identification of organisms as to species is not practical. However, its use in this study demonstrates the difficulty of separating the indole-positive members of the *B. fragilis* group with recommended phenotypic characteristics. Fermentation results with xylan for *B. ovatus* (positive) and *B. thetaiotaomicron* (negative) agreed with the work of Salyers et al. (20). Xylan is not available as a commercially prepared substrate. Xylan and raffinose in addition to mannitol, rhamnose, trehalose, salicin, and indole would permit more accurate separation of the group to the species level (10, 20). Meticulous identification of species of *Bacteroides* may be clinically important because of differences in antimicrobial susceptibility, or put another way, susceptibility testing of *Bacteroides* isolates is clinically important because of variations in susceptibility patterns between species and between strains within species.

The NCCLS agar dilution reference method is not practical for daily use in clinical laboratories. Until a more practical method is developed which is comparable to the NCCLS method, it might be appropriate for laboratories to determine susceptibility profiles for anaerobes isolated in their institutions on a periodic basis by the NCCLS method. Such information could be helpful to clinicians when selecting appropriate antimicrobial drugs and in demonstrating the development of resistance in anaerobic bacteria.

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