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Antibiotic Susceptibility of the Subspecies of *Bacteroides fragilis*

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Strains (115) of *Bacteroides fragilis* were identified at the subspecific level and were tested for susceptibility to ampicillin, cephalothin, chloramphenicol, clindamycin, penicillin, and tetracycline using an agar dilution technique. We tested the following strains: *B. fragilis* subsp. *distasonis*, 12; *B. fragilis* subsp. *fragilis*, 39; *B. fragilis* subsp. *ovatus*, 10; *B. fragilis* subsp. *thetaiotaomicron*, 32; *B. fragilis* subsp. *vulgatus*, 10; and *B. fragilis* subsp. "other," 12. There were no marked differences in susceptibility between the subspecies. One strain of *B. fragilis* subsp. *thetaiotaomicron* had a minimal inhibitory concentration of 12.5 μg/ml for clindamycin, but all other strains were susceptible. All the strains were susceptible to chloramphenicol. Susceptibility to tetracycline was variable. Only a few strains were susceptible to the penicillins and cephalothin.

Bacteroides fragilis is the single most common anaerobic organism found in clinical specimens, accounting for 26.8% of the anaerobes isolated in our institution in 1974. Of the nonsporeforming, gram-negative anaerobic rods encountered, *B. fragilis* comprised 69.9%.

B. fragilis has been divided into five known subspecies, as well as a group called "other" by Holdeman and Moore (3). More recently, newer subspecies of *B. fragilis* have been described by the same authors (6).

The relative resistance of *B. fragilis* to antibiotics, as compared with other anaerobes, has been well documented. However, the susceptibility patterns of the various subspecies have not been well studied, except for one report by Chow and Guze (1) in which they examined 36 strains isolated from blood. The purpose of the present study was to determine whether any differences occur in susceptibility of the subspecies of *B. fragilis* to antibiotics.

MATERIALS AND METHODS

All organisms included in this study were isolated from clinical specimens in the Diagnostic Microbiology Laboratory of the University of Minnesota Hospitals.

Gram-negative, nonsporeforming anaerobic rods were identified as *B. fragilis* following the criteria outlined by Holdeman and Moore (3). Tests used were Gram stain, gas liquid chromatography, and biochemical reactions. All biochemical reactions were determined in prereduced media from Scott Laboratories, Fiskeville, R.I. Identification of subspecies of *B. fragilis* was determined mainly by considering acid production from mannitol, rhamnose, and trehalose and indole production. Carbohydrate fermentation was considered positive only if a pH less than 5.75 was achieved in the broth tubes (L. V. Holdeman, personal communication) after 48 h at 35 C.

Antibiotic susceptibility testing was carried out using an agar dilution technique. The antibiotic plates were prepared by incorporating appropriate serial twofold dilutions of antibiotic into brain heart infusion agar containing 1 ml of hemin-vitamin K (Scott Laboratories) per 100 ml. The plates were kept at room temperature and were used the day after preparation.

All strains were checked for purity by subculturing to an anaerobic sheep blood agar plate. One colony was picked to prereduced chopped meat glucose. After overnight incubation at 35 C, a 1:100 dilution of the chopped meat glucose culture was made in prereduced brain heart infusion broth; the dilutions of each organism were inoculated onto the previously prepared agar plates containing antibiotic by using a replicator (7), resulting in an inoculum of about 4×10^4 organisms. An agar plate without antibiotic was inoculated for a growth control, as was a sheep blood agar plate for an aerobic growth control.

All plates were incubated in a GasPak (BBL) jar at 35 C for 48 h, except for the sheep blood agar plate which was incubated aerobically.

After incubation, the minimal inhibitory concentration (MIC) was determined as the lowest concentration of antibiotic showing no growth or only a fine haze of growth. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were included on each lot of plates as controls.

RESULTS

The sources of the 115 strains tested are shown in Table 1.

The results of the antibiotic susceptibility

tests are shown in Tables 2 to 6. With ampicillin (Table 2), the *B. fragilis* subsp. *distasonis* and "other" appeared to be slightly more susceptible than the other subspecies. All subspecies were relatively resistant to cephalothin (Table 3), although 25% of the "other" strains were inhibited by 12.5 μg/ml. All strains of *B. fragilis* were inhibited by 6.3 μg or less of chloramphenicol per ml (Table 4). Differences in relative susceptibility were difficult to ascertain due to the narrow range of MICs obtained. *B. fragilis* subsp. *fragilis* appeared to be the most susceptible to clindamycin (Table 5), with all strains having MICs of 0.4 μg or less per ml. Two strains of *B. fragilis* subsp. *thetaiotaomicron* required 6.3 and 12.5 μg of clindamycin per ml, respectively, for inhibition; all other subspecies were inhibited by 3.1 μg or less per ml. With penicillin (Table 6), strains of *B. fragilis* subsp. *distasonis* and "other" were more susceptible than the other subspecies, although only one strain of "other" had an MIC as low as 1.6 μg/ml. This particular strain was confirmed as the newly described *B. fragilis* subsp. "a" by the VPI Anaerobe Laboratory. With tetracycline (Table 7), there appeared to be a bimodal distribution of all the subspecies, with 42% of the strains being susceptible at 3.1 μg or less per ml. There was a wide range of MICs seen with all of the subspecies.

DISCUSSION

Although the numbers of isolates of the subspecies other than *B. fragilis* subsp. *fragilis* and *B. fragilis* subsp. *thetaiotaomicron* are not large, the data seem sufficient to show that there are no marked differences in the pattern of susceptibility to antibiotics. Since *B. fragilis* subsp. *fragilis* is the most common isolate, one might have postulated that its frequency was due to increased resistance to antibiotics but, in some cases, such as with clindamycin, these strains were slightly more sensitive than the others.

The susceptibility of all the *B. fragilis* strains to the six antibiotics tested is similar to that

TABLE 1. *Source of B. fragilis strains tested for susceptibility to antibiotics*

Species	No. of strains tested	Source					
		Blood	Abscess	Peritoneal fluid	Wound	Genitourinary	Miscellaneous
B. fragilis subsp. *distasonis*	12	2	0	3	4	3	0
B. fragilis subsp. *fragilis*	39	4	5	7	11	3	9
B. fragilis subsp. *ovatus*	10	0	0	4	2	0	4
B. fragilis subsp. *thetaiotaomicron*	32	6	5	3	9	2	7
B. fragilis subsp. *vulgatus*	10	2	1	2	3	0	2
B. fragilis subsp. "other"	12	1	0	1	3	2	5

TABLE 2. *MIC of ampicillin against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml						
		1.6	3.1	6.3	12.5	25	50	≥100
B. fragilis subsp. *distasonis*	12	17	25		50	75	83	100
B. fragilis subsp. *fragilis*	39			3	38	79	82	100
B. fragilis subsp. *ovatus*	10				20	90		100
B. fragilis subsp. *thetaiotaomicron*	32	3			31	91		100
B. fragilis subsp. *vulgatus*	10				50	100		
B. fragilis subsp. "other"	12	25			67	83		100

TABLE 3. *MIC of cephalothin against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml						
		1.6	3.1	6.3	12.5	25	50	≥100
B. fragilis subsp. *distasonis*	12	8				17	58	100
B. fragilis subsp. *fragilis*	39					8	49	100
B. fragilis subsp. *ovatus*	10						70	100
B. fragilis subsp. *thetaiotaomicron*	32			3		6	56	100
B. fragilis subsp. *vulgatus*	10					4	70	100
B. fragilis subsp. "other"	12			17	25		75	100

reported by Martin et al. (5) in 1972, even though the agar dilution method used was slightly different. A few more of our strains were susceptible to cephalothin, and one strain of *B. fragilis* subsp. *thetaiotaomicron* had a higher MIC (12.5 μg/ml) with clindamycin than any of those tested by Martin et al. The results are also similar to those of Kislak (4), with the exception of the one clindamycin-resistant strain. Since the time of these studies, there has been at least one report of some strains of *B. fragilis* being relatively resistant to clindamycin (2).

TABLE 4. *MIC of chloramphenicol against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml			
		0.8	1.6	3.1	6.3
B. fragilis subsp. *distasonis*	12	17		67	100
B. fragilis subsp. *fragilis*	39	8	13	100	
B. fragilis subsp. *ovatus*	10			30	100
B. fragilis subsp. *thetaiotaomicron*	32		3	47	100
B. fragilis subsp. *vulgatus*	10			90	100
B. fragilis subsp. "other"	12	17	34	83	100

It is difficult to compare our results with those of Chow and Guze (1), even though they also identified their strains of *B. fragilis* at the subspecific level, because of their small number of subspecies other than *B. fragilis* subsp. *fragilis*. In addition, it is not clear whether they used a pH of <5.7 in determining the fermentation of the carbohydrates important for identification of subspecies. Of a total of 36 strains of *B. fragilis*, they listed 9 strains of *B. fragilis* subsp. *vulgatus;* this seems to be a relatively larger proportion of this subspecies than is usually seen from clinical specimens. If we had considered a pH between 5.7 and 6.0 to be positive for fermentation, many more of our strains would have been identified as *B. fragilis* subsp. *vulgatus*. However, their overall results with all the *B. fragilis* were fairly similar to ours.

TABLE 5. *MIC of clindamycin against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml						
		≤0.2	0.4	0.8	1.6	3.1	6.3	12.5
B. fragilis subsp. *distasonis*	12	33	50	92	100			
B. fragilis subsp. *fragilis*	39	92	100					
B. fragilis subsp. *ovatus*	10		30	50	80	100		
B. fragilis subsp. *thetaiotaomicron*	32	22	25	44	81	94	97	100
B. fragilis subsp. *vulgatus*	10	50	90		100			
B. fragilis subsp. "other"	12	75		92	100			

TABLE 6. *MIC of penicillin against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml						
		1.6	3.1	6.3	12.5	25	50	≥100
B. fragilis subsp. *distasonis*	12		17	25	42	83		100
B. fragilis subsp. *fragilis*	39				8	62	80	100
B. fragilis subsp. *ovatus*	10				10	60	90	100
B. fragilis subsp. *thetaiotaomicron*	32		3		19	78	91	100
B. fragilis subsp. *vulgatus*	10				40	90	100	
B. fragilis subsp. "other"	12	8	33		42	75	83	100

TABLE 7. *MIC of tetracycline against subspecies of B. fragilis*

Species	No. tested	Cumulative % at concn in μg/ml									
		≤0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	50	≥100
B. fragilis subsp. *distasonis*	12	8	42			58	67	75	92	100	
B. fragilis subsp. *fragilis*	39	10	36	41			44	49	80	100	
B. fragilis subsp. *ovatus*	10		20	30				60	80	90	100
B. fragilis subsp. *thetaiotaomicron*	32	3	41	44	50		53	72	84	100	
B. fragilis subsp. *vulgatus*	10		30					50	80	100	
B. fragilis subsp. "other"	12	8	17	25			50		92	100	

Differences in the identification of subspecies between our two studies would, therefore, not be significant, since our data show similar susceptibility patterns for all the subspecies.

This present study shows that one cannot differentiate between the subspecies of *B. fragilis* on the basis of antibiotic susceptibility patterns, nor can one predict susceptibility by knowing the identification of subspecies. Each pattern of susceptibility or resistance conforms, in general, to the pattern for all *B. fragilis*. Any slight variation of increased susceptibility or resistance to various agents can only be determined by testing the individual isolant.

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