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Medium-Dependent Zone Size Discrepancies Associated with Susceptibility Testing of Group D Streptococci Against Various Cephalosporins

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Mueller-Hinton (MH) agar media from various commercial sources, either supplemented or not supplemented with 5% sheep blood, were studied to determine their effect on disk diffusion susceptibility testing results obtained with 90 strains of group D streptococci and four cephalosporins. The cephalosporins investigated included cephalothin, cefamandole, moxalactam, and cefotaxime. Results showed that a number of *Streptococcus faecalis* and *Streptococcus faecium* strains were susceptible to cephalothin, cefamandole, and cefotaxime, but the number varied with both the commercial source and blood content of the MH medium used. Regardless of the MH medium used, none of the *S. faecalis* or *S. faecium* strains were found to be susceptible to moxalactam. The apparently medium-associated variations in the number of strains susceptible to cephalothin, cefamandole, and cefotaxime were largely due to minor discrepancies (one result being intermediate) among the various types of MH media used. However, major discrepancies (one result being resistant and the other susceptible or vice versa) were observed when *S. faecalis* strains were tested against cefotaxime. These major discrepancies were associated with both the commercial source of the MH medium and the blood content of the medium.

In studies on the in vitro effectiveness of second- and third-generation cephalosporins against numerous bacterial species, results have demonstrated that enterococci are generally resistant to these antibiotics (1, 2, 5–8). Although these studies were similar in that they commonly employed either agar or broth dilution methods, they differed markedly with respect to the blood content and the type of commercial media used for the susceptibility testing methods. On the basis of experiences in our laboratory, we believe that differences in the type of media used for susceptibility testing of enterococci to second- and third-generation cephalosporins may profoundly affect the results.

While performing disk diffusion susceptibility tests with cefotaxime, we noted that *Streptococcus faecalis* isolates frequently exhibited zone sizes that indicated resistance when unsupplemented Mueller-Hinton (MH) agar was used but would appear susceptible, on the basis of zone size, when MH agar supplemented with 5% sheep blood was used. The implications of this finding are of obvious clinical importance. Because cefotaxime and other second- and third-generation cephalosporins have received extensive attention in the medical community, their use for the treatment of various bacterial infections will undoubtedly become more widespread. As a result, workers at clinical microbiology laboratories, many of whom perform susceptibility testing by the disk diffusion method, may be called upon to test enterococci against cefotaxime and other newer cephalosporins. If major discrepancies, such as those we observed with cefotaxime, occur at other laboratories, grossly inaccurate susceptibility information may be reported to the physician. In addition to the possible clinical implications, the overall standardization and consistency of in vitro susceptibility testing results with cephalosporins are also of major interest. Information that would aid in identifying the sources of the discrepancy in susceptibility testing with cefotaxime described above may facilitate the establishment of more accurate and reliable in vitro methods for testing these newer cephalosporins. Therefore, studies were needed which characterized and more accurately delineated the situations in which the discrepancy is observed.

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To investigate the major factors (group D streptococci, media, cephalosporins) and combinations of factors that contributed to this discrepancy, various commercial brands of MH agar with and without 5% sheep blood were used to test the susceptibility of three species of group D streptococci to certain first-, second-, and third-generation cephalosporins.

(This study was presented in part previously [D. F. Sahm, C. N. Baker, and C. Thornsberry, Annu. Meet. Am. Soc. Microbiol. 1983, C309, p. 136].)

MATERIALS AND METHODS

Antibiotics. The following antibiotic disks (each at 30 μg) were supplied by BBL Microbiology Systems, Cockeysville, Md.: cephalothin, cefamandole, moxalactam, and cefotaxime.

Organisms. The group D streptococci used for this investigation included 30 strains each of *S. faecalis*, *Streptococcus faecium*, and *Streptococcus bovis*. All strains were kindly supplied by Richard Facklam, Reference Bacteriology Section, Respiratory and Special Pathogens Branch, Centers for Disease Control, Atlanta, Ga. Control bacterial strains for susceptibility testing included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains tested grew sufficiently on both blood-supplemented and unsupplemented media to allow for the interpretation of susceptibility testing results.

Media. MH agar media were supplied by Oxoid Ltd., Basingstoke, Hampshire, England; GIBCO Diagnostics, Madison, Wis.; and Difco Laboratories, Detroit, Mich. Additionally, BBL Microbiology Systems supplied the following MH media: MH I, MH II (dry powder and prepoured plates), and prepoured MH agar plates supplemented with 5% sheep blood. All MH agar plates made in-house were prepared with or without the addition of 5% defibrinated sheep blood according to the directions of the manufacturer. In all, 11 types of MH agar media were used for this study. These media were Oxoid medium (OX) with and without blood, GIBCO medium (GB) with and without blood, Difco medium (DF) with and without blood, BBL MH I medium (MH I) and BBL MH II medium (MH II) both with and without blood, and prepoured BBL MH agar plates supplemented with 5% defibrinated sheep blood (PMH).

Susceptibility testing. Susceptibility testing of the group D streptococci and control strains with the various cephalosporins, using the different types of MH agar media, was performed by the standard disk diffusion method reported by the National Committee for Clinical Laboratory Standards (NCCLS [4]).

RESULTS

Zone sizes used as criteria for determining the susceptibility of group D streptococci to the various cephalosporins are given in Table 1. Susceptibility testing of each of the control strains (*S. aureus*, *E. coli*, and *P. aeruginosa*) with the various cephalosporins on different types of MH agar media resulted, for each case, in zone sizes which were within the limits previously set by the NCCLS (4).

TABLE 1. Interpretive zone sizes used to determine susceptibilities to four cephalosporins[a]

Antibiotic	Zone sizes (mm) used to determine following category:		
	Resistant	Intermediate	Susceptible
Cephalothin[b]	≤14	15–17	≥18
Cefamandole[c]	≤14	15–17	≥18
Moxalactam[d]	≤14	15–22	≥23
Cefotaxime[d]	≤14	15–22	≥23

[a] Zone sizes were determined according to NCCLS standard M2-A2S2 (see reference 4).
[b] First-generation cephalosporin.
[c] Second-generation cephalosporin.
[d] Third-generation cephalosporins.

To initially assess the interactions of MH media, cephalosporins, and group D streptococci, we compared the numbers of strains of each species that were susceptible to each cephalosporin with respect to both the commercial brand of MH media used and the presence or absence of blood in the media. Regardless of the commercial source or blood content of the MH medium used, none of the 30 *S. faecalis* strains tested against moxalactam were susceptible (Table 2). Some strains were found to be susceptible to cephalothin and cefamandole, but the number of strains susceptible to these cephalosporins varied slightly with the type of MH medium used. The largest number of *S. faecalis* strains susceptible to cephalothin occurred when OX with blood was used as the testing medium. For cefamandole, the highest number of susceptible strains occurred with the use of OX without blood. Although medium-associated variations in the number of strains susceptible to cephalothin and cefamandole occurred, these variations were not nearly as pronounced as those that occurred with cefotaxime. The number of *S. faecalis* strains susceptible to cefotaxime varied markedly with both the commercial source and the blood content of the medium used. Whereas 18 strains were found to be susceptible when tested on OX with blood and MH I with blood, only 2, 3, 4, and 5 strains were susceptible to cefotaxime when blood-supplemented MH II, GB, PMH, and DF, respectively, were used. Additionally, the number of strains (18) susceptible to cefotaxime when OX with blood and MH I with blood were used as the testing media far exceeded the number of strains susceptible on those two commercial brands of media without blood.

As with the *S. faecalis* strains, none of the *S. faecium* strains were found to be susceptible to moxalactam, regardless of the types of MH agar

TABLE 2. Susceptibility results obtained with 30 *S. faecalis* strains tested against four cephalosporins, using different MH agar media with and without 5% sheep blood

Antibiotic	Blood[b]	No. of strains susceptible on following medium[a]:					
		OX	MH I	MH II	GB	DF	PMH
Cephalothin	No	4	4	3	1	2	—[c]
	Yes	7	3	2	2	2	2
Cefamandole	No	4	3	2	0	2	—
	Yes	2	1	2	1	1	1
Moxalactam	No	0	0	0	0	0	—
	Yes	0	0	0	0	0	0
Cefotaxime	No	4	1	2	1	2	—
	Yes	18	18	2	3	5	4

[a] Remaining results were either resistant or intermediate for the 30 strains tested.

[b] Each medium used either did (yes) or did not (no) contain 5% sheep blood.

[c] —, Not tested.

employed (Table 3). For cephalothin, cefamandole, and cefotaxime, some of the *S. faecium* strains were susceptible, and the number of susceptible strains varied with the commercial brand of MH medium. The lowest number of strains susceptible to these three cephalosporins occurred with the use of PMH. In contrast, the greatest number of *S. faecium* strains susceptible to these cephalosporins occurred with the use of OX with blood. When a single commercial brand of MH medium was considered, the presence of blood in either OX or MH I was most notably associated with an increased number of strains being susceptible to cefotaxime, a pattern which was similar to that observed with the *S. faecalis* strains and cefotaxime (Table 2).

In contrast to the *S. faecalis* and *S. faecium* strains, some of the *S. bovis* strains were found to be susceptible to moxalactam, and the number of susceptible strains varied only slightly with the type of MH medium used (Table 4). Additionally, most of the *S. bovis* strains tested were found to be susceptible to cephalothin, cefamandole, and cefotaxime, and the number of strains susceptible varied little, if at all, with the type of MH medium used for testing.

Although the data in Tables 2 and 3 demonstrate MH medium-associated variations in the number of *S. faecalis* and *S. faecium* strains susceptible to cephalothin, cefamandole, and cefotaxime, these data do not reveal the nature of these variations. Therefore, we analyzed these variations more closely by comparing the category results (susceptible, intermediate, and resistant) observed with these cephalosporins on the various MH media and subsequently determining the number of minor and major discrepancies that occurred. Minor discrepancies were defined as those in which a single bacterial strain was resistant or susceptible to a particular cephalosporin when tested on one type of MH medium but intermediate on another type of MH medium. Major discrepancies were defined as those in which a single bacterial strain was resistant to a particular cephalosporin when tested on one type of MH medium but susceptible on a different type of MH medium. The results obtained with cephalothin, cefamandole, and cefotaxime against 30 *S. faecalis* strains were compared as follows: results obtained with each brand of MH medium without blood were compared with each other, results obtained with each brand of MH with blood were compared with each other, and the results obtained with brands of MH with blood were compared with the results obtained with brands of MH media without blood. The data in Table 5 represent a summation of all of these two-way media comparisons. For these comparisons, moxalactam was not included because all *S. faecalis* and *S. faecium* strains were resistant to this cephalosporin on all media tested.

Comparisons made among susceptibility testing results obtained with the use of MH media without blood demonstrated that for each cephalosporin the majority of discrepancies were minor (Table 5). In addition, the number of minor and major discrepancies obtained when *S. faecalis* strains were tested against each cephalosporin was greater than the number of discrepancies obtained with the *S. faecium* strains. Comparisons of the *S. faecalis* susceptibility

TABLE 3. Susceptibility results obtained with 30 *S. faecium* strains tested against four cephalosporins, using different MH agar media with and without 5% sheep blood

Antibiotic	Blood[b]	No. of strains susceptible on following medium[a]:					
		OX	MH I	MH II	GB	DF	PMH
Cephalothin	No	7	6	7	4	6	—[c]
	Yes	11	2	9	5	7	1
Cefamandole	No	7	6	7	6	5	—
	Yes	9	3	8	8	7	1
Moxalactam	No	0	0	0	0	0	—
	Yes	0	0	0	0	0	0
Cefotaxime	No	2	1	2	2	1	—
	Yes	9	6	2	2	2	1

[a] Remaining results were either resistant or intermediate for the 30 strains tested.

[b] Each medium used either did (yes) or did not (no) contain 5% sheep blood.

[c] —, Not tested.

TABLE 4. Susceptibility results obtained with 30 *S. bovis* strains tested against four cephalosporins, using different MH agar media with and without 5% sheep blood

Antibiotic	Blood[b]	No. of strains susceptible on following medium[a]:					
		OX	MH I	MH II	GB	DF	PMH
Cephalothin	No	30	30	29	29	29	—[c]
	Yes	30	30	29	30	30	29
Cefamandole	No	30	30	30	29	30	—
	Yes	30	30	29	30	30	29
Moxalactam	No	6	3	2	6	6	—
	Yes	4	4	4	7	8	2
Cefotaxime	No	29	29	29	29	29	—
	Yes	29	29	29	29	29	29

[a] Remaining results were either resistant or intermediate for the 30 strains tested.

[b] Each medium used either did (yes) or did not (no) contain 5% sheep blood.

[c] —, Not tested.

testing results obtained on all types of MH media without blood showed that the major discrepancies never exceeded 4%. When comparisons were made among the susceptibility results obtained with the use of MH media with blood, the number of minor discrepancies was greater than the number of major discrepancies. For cephalothin and cefamandole tested against *S. faecalis* and *S. faecium* strains, the percentages of major discrepancies were comparable but slightly greater than those observed when MH media without blood were compared. In contrast, when cefotaxime was tested against the *S. faecalis* strains, the percentage of major discrepancies was much greater among MH media with blood than that among MH media without blood (14% versus 3%). Similarly, when susceptibility testing results obtained with MH media with blood were compared with results obtained with MH media without blood, a noticeably higher percentage (18%) of major discrepancies was observed with *S. faecalis* strains tested against cefotaxime than that observed with any other organism-cephalosporin combination.

The data presented in Table 5 indicate that a disproportionate percentage of major discrepancies occurred when *S. faecalis* strains were tested against cefotaxime. Furthermore, this high percentage of major discrepancies was associated with comparisons made among MH media with blood and with comparisons made among MH media with and without blood. In view of these observations, further analysis of the data was necessary to determine the exact sources of these major discrepancies.

Comparisons made among all types of MH media with blood revealed that the most common sources of major discrepancies with cefotaxime involved the use of OX base and MH I base (Table 6). For both the *S. faecalis* and *S. faecium* strains, the use of OX base and MH I base most often resulted in the organisms being susceptible to cefotaxime, whereas the use of other blood-supplemented media (GB, DF, MH II, and PMH) resulted in the organisms being resistant. With the *S. faecalis* strains, a few major discrepancies were also evident with MH

TABLE 5. Number of minor and major discrepancies observed with 30 strains of *S. faecalis* and 30 strains of *S. faecium* tested against three cephalosporins on all types of MH agar media[a]

Antibiotic	Organism	No. (% total) of discrepancies observed when comparing all media					
		Without blood (300)[b]		With blood (450)[b]		With and without blood (900)[b]	
		Minor	Major	Minor	Major	Minor	Major
Cephalothin	*S. faecalis*	107 (36)	13 (4)	171 (38)	11 (4)	306 (34)	25 (3)
	S. faecium	37 (12)	8 (3)	109 (24)	26 (6)	179 (20)	55 (6)
Cefamandole	*S. faecalis*	66 (22)	10 (3)	111 (25)	5 (1)	217 (24)	17 (2)
	S. faecium	48 (16)	0 (0)	83 (18)	18 (4)	152 (17)	32 (4)
Cefotaxime	*S. faecalis*	50 (17)	8 (3)	106 (24)	61 (14)	208 (23)	160 (18)
	S. faecium	35 (12)	6 (2)	84 (19)	30 (7)	129 (14)	55 (6)

[a] Minor discrepancy is defined as a situation in which a strain is resistant or susceptible to a particular cephalosporin on one type of MH medium but intermediate on another type of MH medium. Major discrepancy is defined as a situation in which a strain is resistant to a particular cephalosporin on one type of MH medium but susceptible on another. MH media included OX, GB, DF, MH I, MH II (all with and without 5% sheep blood) and PMH.

[b] Total number of two-way media comparisons of category results (susceptible, intermediate, resistant) obtained with 30 *S. faecalis* or *S. faecium* strains tested against each cephalosporin on all commercial brands of MH agar media.

TABLE 6. Sources of major discrepancies observed with 30 strains of *S. faecalis* and 30 strains of *S. faecium* tested against cefotaxime on all MH agar media containing 5% sheep blood[a]

Result[b]		No. of strains	
Susceptible	Resistant	*S. faecalis*	*S. faecium*
OX	MH I	0	1
	MH II	8	4
	PMH	11	6
	GB	3	4
	DF	4	4
MH I	OX	0	0
	MH II	8	3
	PMH	11	3
	GB	3	2
	DF	4	3
MH II	MH I	0	0
	OX	0	0
	PMH	0	0
	GB	0	0
	DF	0	0
PMH	MH I	0	0
	MH II	1	0
	OX	0	0
	GB	1	0
	DF	1	0
GB	MH I	0	0
	MH II	0	0
	PMH	1	0
	OX	0	0
	DF	0	0
DF	MH I	0	0
	MH II	2	0
	PMH	3	0
	GB	0	0
	OX	0	0

[a] Major discrepancy defined as in Table 5, footnote *a*. MH media included OX, MH I, MH II, PMH, GB, and DF.

[b] Susceptible to cefotaxime on indicated medium and resistant to cefotaxime on other indicated media.

media other than OX and MH I. No major discrepancies occurred, however, with *S. faecium* tested on the other types of MH media with blood.

The sources of major discrepancies that occurred between susceptibility results obtained with the use of MH media with and without blood are shown in Table 7. A vast majority of these major discrepancies were characterized by the bacterial strains being susceptible to cefotaxime when tested on OX with blood or MH I with blood but being resistant when tested on other MH media without blood. The use of other MH media (GB, DF, and PMH) with blood also resulted, but to a much lesser extent, in enterococcal strains being susceptible to cefotaxime but resistant on media without blood. Major discrepancies that were characterized by bacterial resistance to cefotaxime on a blood-supplemented medium and susceptibility on an unsupplemented medium were rarely observed.

DISCUSSION

Results of this investigation have demonstrated that numerous discrepancies are associated with the disk diffusion method of susceptibility testing of enterococci (*S. faecalis* and *S. faecium*) against various cephalosporins. The discrepancies that were observed were apparently associated with both the commercial source of the MH base used and the blood content of the medium. Although most of the discrepancies associated with testing enterococci against cephalothin, cefamandole, and cefotaxime on various MH media were minor, a substantial percentage of major discrepancies was also noted (Table 5). The greatest percentage of major discrepancies that was observed were notably associated with the testing of *S. faecalis* strains against cefotaxime on OX with blood and MH I with blood (Tables 6 and 7). These major discrepancies were characterized by *S. faecalis* strains being susceptible to cefotaxime when tested on OX with blood or MH I with blood and being resistant when tested on other MH media with blood (Table 6) or without blood (Table 7). A similar pattern was also noted with the testing of *S. faecium*, but the number of strains that demonstrated the major discrepancies was less than that observed with *S. faecalis*.

Neu et al. (5) reported that the type of growth medium used had little or no effect on the susceptibility testing results obtained with cefotaxime. Their results, however, were obtained by testing members of the family *Enterobacteriaceae* and *P. aeruginosa* and cannot be accurately correlated with our data obtained with the group D streptococci. Although Muytjens et al. (3) have made reference to a personal communication indicating that medium-dependent susceptibility discrepancies occur with cefotaxime and *S. faecalis*, no data were presented, and therefore a comparison with our results is not possible.

The occurrence of major discrepancies such as those presented in this report immediately raises questions pertaining to their possible sources. For the problem to be addressed, at least three major factors must be investigated: the cephalosporin(s) involved, the media and medium components involved, and the organisms or strains involved. Although the results of the study serve only to describe situations in

TABLE 7. Sources of major discrepancies observed with 30 strains of *S. faecalis* and 30 strains of *S. faecium* tested against cefotaxime on all MH agar media used with and without 5% sheep blood[a]

Result[b]		No. of strains		Result[b]		No. of strains	
With blood	Without blood	*S. faecalis*	*S. faecium*	With blood	Without blood	*S. faecalis*	*S. faecium*
S OX	R OX	5	5	S GB	R OX	0	0
	R MH I	14	4		R MH I	0	1
	R MH II	16	7		R MH II	1	0
	R GB	16	7		R GB	1	0
	R DF	16	8		R DF	1	1
R OX	S OX	0	0	R GB	S OX	1	0
	S MH I	0	0		S MH I	0	0
	S MH II	0	0		S MH II	0	0
	S GB	0	0		S GB	0	0
	S DF	0	0		S DF	0	0
S MH I	R OX	5	3	S DF	R OX	1	0
	R MH I	14	3		R MH I	2	1
	R MH II	16	4		R MH II	3	0
	R GB	16	4		R GB	3	0
	R DF	16	5		R DF	3	1
R MH I	S OX	0	0	R DF	S OX	1	0
	S MH I	0	0		S MH I	0	0
	S MH II	0	0		S MH II	0	0
	S GB	0	0		S GB	0	0
	S DF	0	0		S DF	0	0
S MH II	R OX	0	0	S PMH	R OX	0	0
	R MH I	0	1		R MH I	2	0
	R MH II	0	0		R MH II	2	0
	R GB	0	0		R GB	2	0
	R DF	0	1		R DF	2	0
R MH II	S OX	1	0	R PMH	S OX	1	0
	S MH I	0	0		S MH I	0	0
	S MH II	0	0		S MH II	0	0
	S GB	0	0		S GB	0	0
	S DF	0	0		S DF	0	0

[a] Major discrepancy defined as in Table 5, footnote *a*. PMH could not be compared against itself because it was not tested without blood.

[b] S, Susceptible to cefotaxime on indicated media; R, resistant to cefotaxime on indicated media.

which a major discrepancy was encountered, certain speculations based on these results can be made with respect to the potential sources of the discrepancy.

Many minor discrepancies were observed with the testing of cephalothin, cefamandole, and cefotaxime on various types of MH media, but the use of cefotaxime was associated with the greatest number of major discrepancies (Table 5). That inherent characteristics of cefotaxime may be solely responsible for these major discrepancies seems highly unlikely for two reasons. First, no variations in the number of *S. bovis* strains susceptible to cefotaxime occurred (Table 4), whereas a considerable number of variations occurred when *S. faecalis* (Table 2) and *S. faecium* strains (Table 3) were tested against this drug. Second, there was not a substantial percentage of major discrepancies associated with the use of cefotaxime when different MH media without blood were compared (Table 5). Therefore, although certain characteristics of cefotaxime may contribute to the major discrepancies observed, properties associated with the organisms tested and the MH media used also seem to be of great importance.

The exact role that the media and blood components play in the major discrepancies observed can be discerned only by further, more extensive investigations, but from our results, some general patterns have been observed that may serve to direct these future investigations.

By comparisons made among all MH media with blood (Tables 5 and 6), it seems likely that constituents of the MH media may contribute to the major discrepancies. Significant numbers of *S. faecalis* and *S. faecium* strains were susceptible to cefotaxime when tested on either MH I with blood or OX with blood but were resistant when tested on other MH media (MH II, PMH, GB, and DF) that were supplemented with blood (Table 6). The only differences among these various media that gave discrepant results were their respective base constituents. Additionally, these data (Table 7) provide evidence that the constituents of the various MH bases were not uniquely responsible for the observed major discrepancies. Indeed, the presence of blood seems to be a major factor associated with the susceptibility of *S. faecalis* and *S. faecium* strains to cefotaxime. Although more enterococcal strains were susceptible to cefotaxime when OX with blood or MH I with blood was used, the use of blood-supplemented GB, DF, and PMH also resulted in susceptibility to cefotaxime. Therefore, both the MH base constituents (specifically those of OX and MH I bases) and the blood (or certain blood components) appear to be significant contributing factors to the major discrepancies observed in this study.

The results of this investigation suggest that, in addition to characteristics of cefotaxime and the MH media used, characteristics of the organisms tested may also contribute to the major discrepancies associated with susceptibility testing of cefotaxime. That particular species characteristics may be implicated was suggested by a number of observations. The greatest variation in the number of strains susceptible to cefotaxime was associated with *S. faecalis* (Table 2), whereas *S. faecium* strains were involved to a lesser extent (Table 3) and the *S. bovis* strains failed to demonstrate any variations in the number of strains susceptible to cefotaxime (Table 4). Additionally, the number of major discrepancies associated with cefotaxime and the various types of MH media was much greater for *S. faecalis* than for *S. faecium* (Tables 5, 6, and 7). Further evidence that the observed major discrepancies were in part due to characteristics of the organisms tested is given by the results obtained with the control strains of *S. aureus, E. coli*, and *P. aeruginosa*. When these organisms were tested against cefotaxime on all types of MH media investigated, the resultant interpretive zone sizes were consistently within the limits set forth by the NCCLS (4). In contrast, the *S. faecalis* control strain (ATCC 29212), which was one of the 30 strains included in the study, did demonstrate discrepant zone sizes with cefotaxime tested on the various types of MH media. That bacterial characteristics contributing to major discrepancies observed with cefotaxime may not only be species related but, more specifically, strain related is evidenced by the fact that not all strains of *S. faecalis* or *S. faecium* demonstrated these discrepant results.

Because of the large number of major discrepancies observed, the main question that arises is whether there are therapeutic implications; i.e., will enterococcal infections respond to treatment with cefotaxime? Because of the blood and blood products present in human tissue, some enterococci may be susceptible to cefotaxime in vivo. Although these organisms have usually been considered resistant to the third-generation cephalosporins, some clinical data suggest that patients with enterococcal infections may respond to some of these antibiotics (9). These clinical findings need to be confirmed with closely controlled studies. On the basis of the results obtained, decisions could be made about which MH media should be used for susceptibility testing and whether the media should be supplemented with blood.

Besides clinical studies, further in vitro studies should be performed to determined the specific components of media and blood that interact with cefotaxime and enterococci and result in the major discrepancies observed in this study. In addition, animal model studies might result in information that would help understand this in vitro phenomenon. Furthermore, it is important to know whether other cephalosporins exhibit the susceptibility testing characteristics observed with cefotaxime. Some of these studies are currently under way.

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