Antibiotic Susceptibility of *Streptococcus bovis* and Other Group D Streptococci Causing Endocarditis

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Seventy-four strains of *Streptococcus bovis* and 35 strains of enterococci (*Streptococcus faecalis* and its varieties, *Streptococcus faecium* and *Streptococcus durans*), most of which were isolated from patients with endocarditis, were tested for their susceptibility to penicillin, ampicillin, erythromycin, cephalothin, vancomycin, methicillin, tetracycline, chloramphenicol, kanamycin, streptomycin, and gentamicin. Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined by a microtiter broth dilution technique. All of these organisms are group D streptococci, but the *S. bovis* strains are not enterococci. On the basis of both MIC and MBC, the *S. bovis* strains were much more susceptible in general to antibiotics than were the enterococcal strains. For the *S. bovis* strains, the lowest MICs were obtained with penicillin, ampicillin, and erythromycin, and the lowest MBCs with penicillin and ampicillin. Although these antibiotics were also the most active against the enterococci, the MICs and MBCs were much higher than obtained with the *S. bovis* strains. Gentamicin was the most active aminoglycoside. On the basis of in vitro susceptibility results, the *S. bovis* strains resemble the viridans streptococci rather than enterococci.

Speciation of the serological group D streptococci has been a point of confusion for many years. Although Sherman (9) brought a great deal of order to this chaos, recent studies indicate continuing disagreement about exact speciation (2, 4, 6). However, the group D streptococci can be divided into enterococcal and nonenterococcal groups by properly selected tests (2, 3, 4). The importance of this separation of the clinician has been pointed out by Duma et al. (3) and Wilkowske et al. (15).

In this report we present data on the antimicrobial susceptibility of *Streptococcus bovis* and other group D streptococcal strains, most of which were isolated from the blood of patients with bacterial endocarditis. These data show that *S. bovis* strains have susceptibility patterns that are different from those of the other group D streptococci tested and should be identified as a nonenterococcal species.

MATERIALS AND METHODS

Cultures. Most of the cultures used in this study were isolated from the blood of patients with endocarditis and were submitted to the Center for Disease Control for identification or for confirmation of identification. A few of the cultures were from the laboratory's stock collection. These cultures were speciated by previously described methods (4).

Seventy-four nonenterococcal group D streptococci

were used in this study. Sixty-eight of these strains were *S. bovis* and six were *S. bovis* variants. Twelve of the *S. bovis* strains were stock cultures, but the remaining strains were isolates from the blood of patients with endocarditis.

Thirty-five enterococcal species were studied. Twenty-four of these strains were *Streptococcus faecalis* and its varieties (nine *S. faecalis*, eight *S. faecalis* var. *liquefaciens*, and seven *S. faecalis* var. *zymogenes*). Eight strains were classified as *Streptococcus faecium* and three as *Streptococcus durans*.

Antibiotics. The antibiotics and their sources were ampicillin, methicillin, and kanamycin from Bristol Laboratories; erythromycin, cephalothin, and vancomycin from Eli Lilly & Co.; penicillin from Wyeth Laboratories; gentamicin from Schering Corp.; streptomycin and tetracycline from Pfizer Laboratories; and chloramphenicol from Parke, Davis & Co.

Stock solutions of the antibiotics were prepared with the appropriate diluent. Further dilutions of the stock solutions for use in the tests were made in Trypticase soy broth (TSB, BioQuest).

Susceptibility tests. The streptococci were inoculated onto plates of Trypticase soy-blood agar with 4% defibrinated rabbit blood (TSA) and incubated overnight at 35 C. Four to five similar colonies from each culture were touched with a sterile loop and inoculated into 5 ml of TSB. These cultures were incubated in a water bath at 35 C until they had produced a turbid broth. The cultures were then diluted to contain approximately 10^5 colony-forming units (CFU) per ml.

The stock solutions of the antibiotics were diluted

with TSB to twice the highest concentration to be tested. Serial twofold dilutions were made in microtiter plates either manually or with an Autotiter (Canalco). The microtiter plates containing the diluted antibiotics were stacked on top of each other, with an empty plate on top, and sealed in plastic bags for storage at -65°C . The plates were removed when needed and allowed to reach room temperature before inoculation. All plates were used within 2 weeks.

The microtiter plates containing the antibiotics were inoculated by adding 0.05 ml of the diluted culture to each well, thereby making another twofold dilution to effect the desired final concentration. They were then covered with clear adhesive tape and incubated for approximately 24 h at 35°C . The minimal inhibitory concentration (MIC) was read as the least concentration of antibiotic that caused complete inhibition of growth as judged by the unaided eye. To determine the minimal bactericidal concentration (MBC), approximately 0.025 ml of the cultures from each of the next five wells past the MIC well (that showed no macroscopic growth) were subcultured to TSA blood agar plates. The plates were incubated at 35°C for 24 h. The MBC was read as the least concentration of antibiotic that prevented any growth of streptococci on the subcultures. The MIC and MBC were expressed as micrograms per milliliter.

RESULTS

MICs. In this study two series of serial twofold dilutions were used depending upon the antibiotic. In one series the concentrations of antibiotics ranged from 0.0005 to $5.0\text{ }\mu\text{g/ml}$, and the other ranged from 0.006 to $500\text{ }\mu\text{g/ml}$. The MICs for the 109 strains of streptococci in this study are shown in Tables 1 to 4.

Except for two strains which had penicillin MICs of $1.25\text{ }\mu\text{g/ml}$, all strains of *S. bovis* (Tables 1 and 2) had MICs of $0.16\text{ }\mu\text{g/ml}$ or less for penicillin and $0.63\text{ }\mu\text{g/ml}$ or less for ampicillin and erythromycin. Most of the *S. bovis* strains had MICs of $5.0\text{ }\mu\text{g/ml}$ or less for cephalothin, methicillin, chloramphenicol, and vancomycin. The susceptibility of the *S. bovis* strains to tetracycline showed great variation, with MICs ranging from 0.06 to $125\text{ }\mu\text{g/ml}$. Gentamicin was the most active aminoglycoside for *S. bovis* strains.

The enterococci (Tables 3 and 4) (*S. faecalis*,

including its varieties *liquefaciens* and *zymogenes*, *S. faecium*, and *S. durans*) were less susceptible to most antibiotics than the *S. bovis* strains. For the enterococci, the lowest MICs were obtained with penicillin, ampicillin, and erythromycin, but these values were much higher than the MICs obtained for the *S. bovis* strains for the same antibiotics. For example, 89% of the *S. bovis* strains had MICs of $0.08\text{ }\mu\text{g/ml}$ or less for penicillin, but none of the enterococci had a MIC that low. MICs of chloramphenicol and tetracycline varied greatly for the enterococci. As with the *S. bovis* strains, gentamicin was the most active aminoglycoside.

As mentioned previously, the 74 strains of *S. bovis* included six strains of *S. bovis* variant, and the strains of enterococci included 24 strains of *S. faecalis* and its varieties *liquefaciens* and *zymogenes*. When the results for these different groups were compared, the MICs for the *S. bovis* and *S. bovis* variant strains were quite similar, as were the MICs for the strains of *S. faecalis* and its varieties.

MBCs. The MBC data for the *S. bovis* and the enterococcal strains are shown in Tables 5 to 8. For the *S. bovis* strains, the lowest MBCs were obtained with penicillin and ampicillin. The lowest MBCs for the aminoglycosides were obtained with gentamicin. The MBCs for erythromycin, a bacteriostatic antibiotic, were included because some of the values obtained for the *S. bovis* strains were quite low. The MBCs for the enterococcal strains were much higher, in general, than those for the *S. bovis* strains. However, for the enterococci, the lowest MBCs were obtained with penicillin and ampicillin; the lowest MBCs in the aminoglycosides were with gentamicin.

DISCUSSION

Sherman (9) first definitively classified the streptococci in 1937. He divided them into four divisions based on growth at 10 and 45°C . One of the divisions he called enterococcus, which included *S. faecalis* and *S. durans*, and excluded *S. bovis* and *S. equinus*. Diebel (2) in

TABLE 1. MICs of eight antibiotics for 74 strains of *S. bovis*

Antibiotic	Percentage of strains with MICs ($\mu\text{g/ml}$) of:								
	≤ 0.04	0.08	0.16	0.31	0.63	1.25	2.5	5.0	> 5.0
Penicillin	74	15	8	0	0	3	0	0	0
Ampicillin	43	28	22	4	3	0	0	0	0
Erythromycin	60	34	5	1	0	0	0	0	0
Cephalothin	1	10	26	54	7	0	0	1	1
Methicillin	0	1	1	3	15	27	46	3	4
Vancomycin	0	0	0	18	55	11	1	14	1

1964, reviewed the bacteriologic characteristics and taxonomy of the group D streptococci and recognized the species of group D streptococci as *S. faecalis*, *S. faecium* (including *S. durans* as a variety of this species), *S. bovis*, and *S. equinus*. The first two species are enterococci, the latter two are not. Hartman et al. (5) also considered *S. durans* to be a variety of *S. faecium* and considered *S. bovis* and *S. equinus* to be in the viridans group.

The classification of the streptococci has also been studied by numerical taxonomy through computer analysis. By this method, Raj and Colwell (8) classified the streptococci into two major taxonomic groups. The enterococci formed one large homogeneous group, and the pyogenic and viridans group formed a second group. They placed *S. faecalis*, *S. faecium*, *S. durans*, and *S. bovis* in the enterococcal group, but contrary to other recent schemes, placed *S. equinus* with the pyogenic and viridans streptococci. Colman (1), in 1968, concluded that the well-documented species of streptococci isolated from man should be designated *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, *S. salivarius*, and *S. faecalis* and its varieties. He found the *S. faecalis* and *S. faecium* strains to form a homogeneous group. Jones et al. (6) in their numerical taxonomic study of the group D streptococci concluded that they should be speciated as *S. faecalis*, *S. faecium* (included *S. durans*), *S.*

avium, *S. bovis*, and *S. equinus*. They did not find any evidence for different biotypes of *S. bovis*.

Facklam (4) classifies the group D streptococci into *S. faecalis*, *S. faecium*, *S. durans*, *S. bovis*, *S. bovis* variant, *S. equinus*, and *S. avium*. He has not seen isolates of the last two species from specimens from humans.

Obviously, the speciation of group D streptococci is unsettled. There are differences, however, in their susceptibility to antibiotics that may possibly have clinical implications. These susceptibility data show that the *S. bovis* (including the *S. bovis* variant) strains are much more susceptible to the penicillins, erythromycin, and cephalosporins. The other strains (i.e., the enterococci) were generally much more resistant to these antibiotics.

The susceptibility of different species of human isolates of group D streptococci to antibiotics has also been studied by Toala et al. (11) and by Wilkowske et al. (15). Toala et al. did not have any strains of *S. bovis* in their group, but they did have *S. faecalis* and its varieties (*liquefaciens* and *zymogenes*), *S. faecium* and *S. durans*. Wilkowske et al. (15) tested three strains of *S. bovis*, in addition to *S. faecalis* and its varieties and *S. faecium*, but they did not test *S. durans*. Where MICs obtained in these two studies and in our study could be compared, there was general agreement. Our data support the conclusion of Wilkowske et al. (15) that *S. bovis* is much more susceptible to antibiotics than the enterococci. Our data also support their conclusion that the susceptibility of the enterococci to antibiotics varies both between strains and within species, and for this reason speciation of the enterococci cannot be used to predict susceptibility. All three studies also showed that ampicillin and penicillin G were the most active antibiotics against the enterococci.

Several investigators (2, 4, 6) have suggested that there is little reason to separate *S. faecalis*

TABLE 2. MIC of five antibiotics for 74 strains of *S. bovis*

Antibiotic	Percentage of strains with MICs ($\mu\text{g/ml}$) of:					
	≤ 1.9	3.9	7.8	16	31	≥ 63
Gentamicin	1	9	15	34	27	14
Kanamycin	0	0	0	0	1	99
Streptomycin	0	0	1	4	19	76
Chloramphenicol	71	23	3	3	0	0
Tetracycline	52	1	6	4	21	16

TABLE 3. MICs of six antibiotics for enterococci (24 strains of *S. faecalis*, 8 strains of *S. faecium*, and 3 strains of *S. durans*)

Antibiotic	Percentage of strains with MICs of:							
	0.08 ^a	0.16	0.31	0.63	1.3	2.5	5.0	> 5.0
Penicillin	0	3	3	6	31	34	9	14
Ampicillin	0	0	11	30	34	14	11	0
Methicillin	0	0	0	0	0	3	3	94
Cephalothin	0	0	0	0	0	3	3	94
Vancomycin	0	0	0	3	26	37	11	23
Erythromycin	6	11	14	9	20	20	6	14

^a MIC reported as micrograms per milliliter.

into the varieties *liquefaciens* and *zymogenes*. On the basis of our data concerning in vitro susceptibilities, there is no reason to separate them. Deibel (2) considers *S. durans* to be a variety of *S. faecium*, and Jones et al. (6) did not find sufficient reason to separate the two. In this study, there was little difference in the MICs of these two groups. Facklam (4) recognizes a variant of *S. bovis* which can be easily distinguished from other *S. bovis* strains. However, there was little difference between the MICs we obtained for the two groups. Therefore, on the basis of antimicrobial susceptibilities, none of the varieties differed from the parent species.

It is difficult to compare minimum bactericidal concentrations from different laboratories because there is less standardization for this test than any other phase of antimicrobial susceptibility testing. Preliminary studies in our laboratory have shown that the MBC is very method dependent, particularly as to the definition of end point. In this study, 0.025 ml of a total culture volume of 0.1 ml was subcultured to TSA-blood agar plates and incubated for 48 h at 35 C. The end point was the concentration of antibiotic that caused total inhibition of growth of the organism on subculture. If, however, we had used a different end point, as, for example, 10 colonies or less, or 99.9% reduction in num-

TABLE 4. MICs of five antibiotics for enterococci (24 strains of *S. faecalis*, 8 strains of *S. faecium* and 3 strains of *S. durans*)

Antibiotic	Percentage of strains with MICs of:						
	≤1.9 ^a	3.9	7.8	16	31	63	≥125
Streptomycin	0	0	0	0	9	6	85
Kanamycin	0	0	0	3	9	9	79
Gentamicin	3	3	6	9	6	44	29
Chloramphenicol ..	36	6	3	0	15	20	20
Tetracycline	44	29	18	3	3	3	0

^a MIC reported as micrograms per milliliter.

TABLE 5. Distribution of MBCs of six antibiotics for *S. bovis* strains

Antibiotic	Percentage of strains with MBCs of:							
	≤0.08 ^a	0.16	0.31	0.63	1.3	2.5	5.0	>5.0
Penicillin	53	7	13	12	7	7	1	0
Ampicillin	34	17	17	3	15	7	5	2
Methicillin	0	0	0	6	13	14	24	43
Vancomycin	0	0	0	13	17	7	13	50
Cephalothin	0	8	21	17	7	8	7	32
Erythromycin	7	20	18	18	23	12	0	2

^a MBC reported in micrograms per milliliter.

TABLE 6. Distribution of MBCs of three aminoglycosides for *S. bovis* strains

Antibiotic	Percentage of strains with MBCs of:					
	3.9 ^a	7.8	16	31	62	≥125
Streptomycin	0	0	0	5	30	65
Kanamycin	0	0	0	0	4	96
Gentamicin	1	10	21	37	23	8

^a MBC reported in micrograms per milliliter.

bers of bacteria, many of the MBCs, particularly the higher ones, would have been lower.

Endocarditis is usually treated with bactericidal antibiotics given in sufficient doses to attain bactericidal levels (7, 13). In this study, three bacteriostatic agents, tetracycline, chloramphenicol, and erythromycin, were studied for reference purposes only and not to suggest that these antibiotics could be used in the therapy of endocarditis. Penicillin is the preferred antibiotic and our data support this choice. In most cases, the therapeutic regimen will also include an aminoglycoside, usually streptomycin. The use of these two antibiotics together results in a synergistic action on the streptococci (14). Although we have not performed synergism studies on these organisms with any of these antibiotics, it is possible that gentamicin might be preferred to streptomycin with some organisms because the organisms are more susceptible to gentamicin. The clinical efficacy of penicillin and gentamicin therapy has been recently reported (14).

Because of the greater sensitivity of *S. bovis* to penicillin as compared with the enterococci, it is possible that *S. bovis* endocarditis could be treated with penicillin alone rather than in combination with an aminoglycoside. There are recent reports of clinical evidence to support this hypothesis. R. C. Moellering (personal communication) reported that there were no bacteriological failures in five patients with *S.*

TABLE 7. Distribution of MBCs of six antibiotics for enterococci (24 strains of *S. faecalis*, 8 strains of *S. faecium*, and 3 strains of *S. durans*)

Antibiotic	Percentage of strains with MBCs of:							
	≤0.08 ^a	0.16	0.31	0.63	1.3	2.5	5.0	>5.0
Penicillin	0	0	0	0	9	34	20	37
Ampicillin	0	0	3	9	34	11	3	40
Methicillin	0	0	0	0	0	3	0	97
Vancomycin	0	0	0	0	0	9	0	91
Cephalothin	0	0	0	0	0	0	0	100
Erythromycin	0	0	0	0	3	7	20	70

^a MBC reported in micrograms per milliliter.TABLE 8. Distribution of MBCs of three aminoglycosides for enterococci (24 strains of *S. faecalis*, 8 strains of *S. faecium* and 3 strains of *S. durans*)

Antibiotic	Percentage of strains with MBCs of:					
	3.9 ^a	7.8	16	31	62	≥125
Streptomycin	0	0	0	3	0	97
Kanamycin	0	0	0	3	0	97
Gentamicin	3	6	9	0	35	47

^a MBC reported as micrograms per milliliter.

bovis endocarditis who were treated with penicillin alone. Hoppes and Lerner (Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 13th, Washington, D.C., abstr. no. 211, 1973) reported that seven patients with *S. bovis* endocarditis who were treated with penicillin alone survived without relapse. Because of the potential toxicity of the aminoglycosides, more studies need to be done to confirm if penicillin alone is efficacious in the treatment of nonenterococcal endocarditis.

Duma et al. (3) have pointed out that the classification of group D streptococci has been a source of confusion to physicians. They and Wilkowske et al. (15) have recommended that dividing the group D streptococci into enterococci and nonenterococci would be useful to the clinician. Our in vitro data support this conclusion, since the *S. bovis* strains are much more susceptible to antibiotics than the strains of *S. faecalis*, *S. faecium*, and *S. durans*. If the recent reports of the successful use of penicillin alone to treat *S. bovis* endocarditis are confirmed and if the prognosis for *S. bovis* endocarditis is much better than for enterococcal endocarditis, then group D streptococci would need to be separated into enterococci and nonenterococci. The nonenterococcal group D species of streptococci would be *S. bovis* and *S.*

equinus, but we have not seen the latter species in human infections. However, it must be emphasized that speciation alone cannot be used as the sole basis for the determination of a therapy regimen because occasionally strains of *S. bovis* and viridans streptococci will be relatively resistant to penicillin (10, 12). Therefore, susceptibility tests should always be performed regardless of the identity of the organism. If a nonenterococcal strain from endocarditis proves to be relatively resistant to penicillin, then therapy should probably be the same as that for enterococci (7).

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