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Discriminant Analysis of Antibiotic Resistance Patterns in Fecal Streptococci, a Method To Differentiate Human and Animal Sources of Fecal Pollution in Natural Waters

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Discriminant analysis of patterns of antibiotic resistance in fecal streptococci was used to differentiate between human and animal sources of fecal pollution in natural waters. A total of 1,435 isolates from 17 samples of cattle, poultry, human, and wild-animal wastes were obtained, and their ability to grow in the presence of four concentrations of five antibiotics (chlortetracycline, halofuginone, oxytetracycline, salinomycin, and streptomycin) was measured. When the resulting antibiotic resistance patterns were analyzed, an average of 74% of the known isolates were correctly classified into one of six possible sources (beef, chicken, dairy, human, turkey, or wild). Ninety-two percent of human isolates were correctly classified. When the isolates were pooled into four possible categories (cattle, human, poultry, and wild), the average rate of correct classification (ARCC) increased to 84%. Human versus animal isolates were correctly classified at an average rate of 95%. Human versus wild isolates had an ARCC of 98%, and cattle versus poultry isolates had an ARCC of 92%. When fecal streptococci that were isolated from surface waters receiving fecal pollution from unknown origins were analyzed, 72% of the isolates from one stream and 68% of the isolates from another were classified as cattle isolates. Because the correct classification rates of these fecal streptococci are much higher than would be expected by chance alone, the use of discriminant analysis appears to hold promise as a method to determine the sources of fecal pollution in natural waters.

Differentiation of sources of fecal contamination of ground and surface waters is an important problem, especially for waters receiving mixed agricultural and human waste. In most contaminated waters, the presence of fecal indicator organisms can be demonstrated, but the nature of the source of the pollution is unknown. The contamination of natural waters with untreated fecal material may result in an increased risk of transmission of diseases to the humans who use those waters (20). Because the risk to humans is greater from human than from animal waste (21), the knowledge of the source of the pollution is an important factor in determining the degree of risk. It would thus be desirable to be able to determine the source of the fecal material, both to assess the risk to the people who are exposed to the waters and to assist in the location of the sources of pollution.

Several attempts have been made to develop methods to determine the sources of fecal pollution. Initially, the ratio of fecal coliforms to fecal streptococci was used as an indicator of the source: a high ratio (>4) was considered to indicate an animal source, while a low ratio (<0.7) suggested a human source (6). This ratio has since proven unreliable, and the method has been abandoned (1).

Bacteriophages have also been proposed as indicators of the source of fecal pollution. Furuse et al. (7) and Osawa and coworkers (18) observed that animal and human wastes contained different serotypes of RNA coliphages. However, the usefulness of this observation is limited because only a small percentage of fecal samples contained the phages (18). More recently, bacteriophages of *Bacteroides fragilis* have been suggested as an indicator of human sources because they have been found exclusively in human feces (24).

Other studies have shown that there are differences in the species composition of fecal streptococci among various types of animals. Devriese et al. (4) found different percentages of various fecal streptococci in the feces of poultry, cattle, and other animals. Similarly, Rutkowski and Sjogren (19) observed quantifiable differences in the distribution of fecal streptococci: *Streptococcus bovis* was the major constituent in cattle, and *Enterococcus avium* was the primary component in poultry. Human samples contained both of these species but at lower percentages.

Because the use of antibiotics in animals can result in the occurrence of antibiotic-resistant bacteria in those animals (8, 16), several attempts have been made to compare the patterns of antibiotic resistance in fecal coliforms with the sources of the isolates. Krumperman (15) showed that the multiple-antibiotic-resistance (MAR) index of *Escherichia coli* from wild animals was generally low, while human and poultry isolates had higher MAR indices. Similarly, Kaspar et al. (10) demonstrated that there were fewer MAR strains of *E. coli* isolated from rural than from urban sources.

Fecal streptococci have also been used to try to identify sources of pollution. Kibbey et al. (13) observed higher levels of antibiotic resistance in fecal streptococci that were isolated from sewage than in those that were found in soil or on vegetation. Knudtson and Hartman (14) measured antibiotic resistance of fecal enterococci isolated from humans, pigs, and natural waters but found only slight differences among the various sources.

Although these and other studies have measured antibiotic resistance of fecal isolates from various sources, it has been difficult to use that information to identify the sources of fecal pollution. Discriminant function analysis is a variation of multivariate analysis of variance and can be used to classify individuals into groups on the basis of the values of several classification variables (22, 23). In this paper, discriminant analysis is used to classify fecal streptococci from known human and

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TABLE 1. Characterization of strains isolated from various sources by use of Enterococcosel broth[a]

Source	% Of all isolates catalase neg and esculin pos	% Of 30 randomly selected esculin-pos isolates with growth characteristics[b]			
		45°C+, 6.5% NaCl+	45°C−, 6.5% NaCl+	45°C+, 6.5% NaCl−	45°C−, 6.5% NaCl−
Turkeys	100	93	0	7	0
Chickens	92	100	0	0	0
Dairy cattle	98	100	0	0	0
Beef cattle	99	100	0	0	0
Sewage	95	90	0	0	10
Pristine streams	56	97	3	0	0
Polluted streams	86	65	15	0	20

[a] neg, negative; pos, positive.
[b] Isolates were either positive (+) or negative (−) for growth at 45°C and/or in 6.5% NaCl.

animal sources on the basis of their patterns of antibiotic resistance and to classify unknown isolates from polluted streams on the basis of the patterns of the known isolates.

MATERIALS AND METHODS

Sample collection. Samples were obtained from six types of known sources: beef cattle feces, dairy cattle feces, turkey feces, chicken feces, the influent of a municipal sewage treatment plant, and pristine streamwater. For each agricultural animal source (i.e., beef, dairy, chicken, and turkey), one sample was collected from fresh feces from three different farms. For the sewage samples (i.e., human), two samples were collected from the Fisherville, Va., wastewater treatment plant. This facility processes only human waste, but the possibility of agricultural input from surface runoff cannot be excluded. Three samples were collected from two pristine streams (Briery Branch and Upper Dry River) in the George Washington National Forest, both of which receive no anthropogenic inputs (11). The pristine stream samples were assumed to contain bacteria from the feces of wild animals, and because these streams are not significantly impacted by humans, these pristine stream isolates were called wild. Additionally, samples were taken from two polluted streams (Cooks Creek and Muddy Creek) in Rockingham County, Va. These streams flow through land receiving heavy agricultural use and supporting many homes with septic systems. After collection, all samples were placed on ice and processed within 6 h.

Isolation and characterization of fecal streptococci. Various amounts of fecal samples (0.1 to 1.0 g) were suspended in 1 liter of saline buffer (consisting of 8.5 g of NaCl, 0.3 g of KH_2PO_4, and 0.6 g of Na_2HPO_4 per liter [pH 7.3]) and filtered through 0.45-μm-pore-size filters (type GN-6; Gelman Sciences). The filters were then transferred to a 50-mm petri dish containing an absorbent pad soaked with 2 ml of Enterococcosel broth (BBL). The filters were incubated for 48 h at 37°C. After incubation, 96 colonies from each sample were picked at random with sterile toothpicks, transferred to microwell plates containing 0.2 ml of Enterococcosel broth, and incubated for another 48 h at 37°C.

All isolates were screened for the production of catalase and the ability to hydrolyze esculin. To further determine the characteristics of the isolates obtained by this method, 10 isolates from each sample were randomly chosen for characterization. Each isolate was tested for the production of catalase, Gram reaction, growth at 37°C in brain heart infusion broth (BBL) containing 6.5% NaCl, and growth in brain heart infusion broth at 45°C.

Antibiotics. Five antibiotics were selected because of their widespread use in animals and humans (5, 17); chlortetracycline hydrochloride (CTC; Sigma), halofuginone hydrobromide (HAL; Hoechst-Roussel), oxytetracycline hydrochloride (OTC; Sigma), salinomycin sodium (SAL; Agri-Bio), and streptomycin sulfate (STR; Sigma). Stock solutions (10 mg/ml) of each drug were prepared in water, filter sterilized, and added to Trypticase soy agar (BBL) after autoclaving. The following concentrations were used: 20, 40, 60, and 80 μg/ml for CTC, OTC, and STR; 20, 40, 70, and 100 μg/ml for HAL; and 1, 5, 10, and 15 μg/ml for SAL. These concentration ranges were initially chosen arbitrarily and subsequently modified on the basis of the results from preliminary tests. The concentration ranges for each drug were considered appropriate for this method if isolates from some sources were resistant and isolates from other sources were sensitive at the highest concentration.

Antibiotic resistance was determined by use of a modification of the method of Kelch and Lee (12). The isolates were transferred with a 48-prong replica-plater from the Enterococcosel-containing microwells to a set of antibiotic-containing Trypticase soy agar plates. Each set contained one plate of each concentration of each antibiotic and one plate containing no antibiotic, for a total of 21 plates per set. The plates were incubated for 24 h, and the growth of each isolate on each concentration of each antibiotic was determined. An isolate was considered to be resistant to a given concentration of antibiotic if growth occurred on that plate. Esculin-negative isolates and isolates that did not grow on the control plates (containing no antibiotic) were not used in the analyses.

Discriminant analysis. Data on the ability of each of the known isolates to grow in the presence of each concentration of each antibiotic (20 variables per isolate) were analyzed with the SAS statistical program (VAX version 6.08; SAS Institute Inc.) by the procedure DISCRIM (prior probabilities, equal; covariance matrix, pooled). Several variants of discriminant analysis were performed by varying the combination of antibiotics and the level of pooling of source types.

The classification table produced by the DISCRIM procedure was used to calculate the percentages of misclassified isolates and determine the average rate of correct classification (ARCC). The table is a source-by-source matrix in which the numbers and percentages of correctly classified isolates are found on the diagonal. The ARCC for a given combination of antibiotics was computed by averaging the percentages along the diagonal. The percentage of misclassified isolates for a given source (false negatives) was determined by adding the percentages of misclassified isolates in the appropriate row of the table (excluding the value in the diagonal). The percentage of isolates from other sources that were misclassified as a given source (false positives) was determined by taking the average of the percentages in the appropriate column (excluding the value in the diagonal).

RESULTS

Characterization of isolates. Fecal samples generally had higher percentages of esculin-positive, catalase-negative bacteria than both types of streams (Table 1). Thirty isolates were randomly selected from each type of source for further characterization. All tested isolates were gram-positive cocci, and the vast majority of them could grow at 45°C and in the presence of 6.5% NaCl (Table 1). Most of these organisms can be classified as fecal streptococci (1, 2).

Resistance patterns of fecal streptococci from various sources. From the 17 samples, a total of 1,435 isolates were obtained, and their patterns of antibiotic resistance were determined and analyzed. Chicken and turkey isolates were generally the most resistant to the five antibiotics (Table 2). Virtually all of these poultry isolates were resistant to all tested levels of CTC, HAL, and OTC, and the vast majority were resistant to all concentrations of STR. Wild isolates tended to be the least resistant, although there were moderately high percentages of these isolates that were resistant to the highest level of HAL, SAL, and STR. Human isolates were very sensitive to high levels of CTC, OTC, and SAL.

Discriminant analysis using separate versus pooled sources. When the six sources were analyzed by discriminant analysis based on resistances to the five drugs (CTC, HAL, OTC, STR, and SAL), the ARCC was 72% (Table 3). Human isolates were particularly well classified (92%), with a false-negative rate of 8% and a false-positive rate of only 2%. Chicken and turkey isolates were correctly classified much more poorly, but 43% of turkey isolates were misclassified as chicken isolates.

When chicken and turkey isolates were pooled as poultry isolates and dairy and beef isolates were pooled as cattle isolates, the ARCC improved to 82% (Table 4). Human isolates were still well classified, and classification of poultry and cattle sources improved (to 89 and 79%, respectively). Because of the

TABLE 2. Patterns of antibiotic resistance of fecal streptococci isolated from various sources

Drug and concn (μg/ml)	% Of resistant isolates from each source					
	Beef (n = 285)[a]	Chicken (n = 265)	Dairy (n = 283)	Human (n = 181)	Turkey (n = 288)	Wild (n = 134)
Chlortetracycline						
20	96	98	59	75	100	84
40	75	97	55	20	100	34
60	60	95	28	18	100	31
80	59	94	22	8	99	18
Halofuginone						
20	100	97	98	98	100	98
40	100	95	95	63	100	90
70	100	95	95	57	100	89
100	99	95	94	56	100	88
Oxytetracycline						
20	95	98	61	58	100	84
40	68	98	36	18	100	22
60	56	96	20	14	100	19
80	47	94	18	7	100	11
Salinomycin						
1	100	100	100	100	99	100
5	92	90	93	0	45	97
10	46	72	26	0	33	86
15	37	54	24	0	28	66
Streptomycin						
20	100	100	100	99	100	100
40	59	95	54	44	95	93
60	31	86	21	29	94	69
80	5	82	7	25	92	58

[a] n, total number of isolates from this source.

higher ARCCs of the pooled isolates, these sources were pooled for the rest of the analyses.

Combinations of drugs. Some combinations of drugs resulted in better discrimination between sources than others. Generally, the more drugs used in an analysis, the higher the ARCC. Single drugs resulted in generally poorer classification of isolates than combinations of drugs. For example, when resistance to OTC was used as the only variable, the ARCC for pooled sources was 54% (Table 5). Although poultry sources were well classified (97%), the average rate of false positives (other sources that were misclassified as poultry) was high, at 17%. The ARCCs for pooled sources when the other drugs were analyzed singly were as follows: CTC, 52%; HAL, 35%; SAL, 61%; and STR, 48%.

The combination of drugs that resulted in the highest ARCC was CTC, OTC, SAL, and STR (ARCC, 84%) (Table 6). False negatives for human isolates were 7%, and false positives averaged just 3%. Analysis with this combination of four drugs is slightly better than the analysis using all five drugs. Because of its highest ARCC, the drug combination of CTC, OTC, STR, and SAL was used for the remaining analyses.

Pooling of all animal sources. If it is not necessary to know what type of animal caused the pollution, only that it is not human, all of the poultry, cattle, and wild isolates can be pooled (and renamed animal) and compared with the human isolates. When this discriminant analysis was performed, the ARCC increased to 95% (Table 7).

Discriminant analysis with prior knowledge of sources. The classification rate can be improved further if one or more of the possible sources can be excluded a priori. For example, in an area where there are no cattle or poultry sources, just human and wild isolates can be compared. When discriminant analysis of these two sources was performed, the ARCC was 98% (Table 8). Similarly, if the only possible sources are human and poultry, just these isolates can be analyzed (ARCC, 96%). Human and cattle isolates can be readily distinguished

TABLE 3. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from various sources[a]

Source (no. of isolates)	No. (%) of isolates classified as:					
	Beef	Chicken	Dairy	Human	Turkey	Wild
Beef (285)	199 (70)	8 (3)	47 (16)	9 (3)	8 (3)	14 (5)
Chicken (264)	39 (15)	194 (73)	4 (1)	3 (1)	18 (7)	6 (2)
Dairy (283)	51 (18)	11 (4)	192 (68)	12 (4)	2 (1)	15 (5)
Human (181)	1 (1)	1 (1)	1 (1)	166 (92)	11 (6)	1 (1)
Turkey (288)	7 (2)	124 (43)	0 (0)	0 (0)	157 (55)	0 (0)
Wild (134)	17 (12)	4 (3)	12 (9)	4 (3)	1 (1)	96 (72)
Cooks Creek (105)	62 (59)	1 (1)	19 (18)	11 (11)	0 (0)	12 (11)
Muddy Creek (88)	60 (68)	7 (8)	5 (6)	1 (1)	2 (2)	13 (15)

[a] The ARCC for this analysis (using CTC, HAL, OTC, SAL, and STR) was 72%.

TABLE 4. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from cattle, humans, poultry, and wild animals[a]

Source (no. of isolates)	No. (%) of isolates classified as:			
	Cattle	Human	Poultry	Wild
Cattle (568)	450 (79)	23 (4)	30 (5)	65 (12)
Human (181)	4 (2)	162 (90)	11 (6)	4 (2)
Poultry (552)	49 (9)	4 (1)	490 (89)	9 (1)
Wild (134)	20 (15)	12 (9)	6 (4)	96 (72)
Cooks Creek (105)	70 (67)	11 (10)	2 (2)	22 (21)
Muddy Creek (88)	63 (72)	1 (1)	8 (9)	16 (18)

[a] Beef and dairy samples were pooled as cattle. Chicken and turkey samples were pooled as poultry. The ARCC for this analysis (using CTC, HAL, OTC, SAL, and STR) was 82%.

TABLE 6. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from cattle, humans, poultry, and wild animals[a]

Source (no. of isolates)	No. (%) of isolates classified as:			
	Cattle	Human	Poultry	Wild
Cattle (568)	447 (79)	33 (6)	24 (4)	64 (11)
Human (181)	1 (1)	169 (93)	10 (5)	1 (1)
Poultry (552)	47 (8)	14 (3)	483 (88)	8 (1)
Wild (134)	25 (19)	5 (3)	4 (3)	100 (75)
Cooks Creek (105)	76 (72)	4 (4)	2 (2)	23 (22)
Muddy Creek (88)	60 (68)	4 (5)	8 (9)	16 (18)

[a] Beef and dairy samples were pooled as cattle. Chicken and turkey samples were pooled as poultry. The ARCC for this analysis (using CTC, OTC, SAL, and STR) was 84%.

(ARCC, 96%), as can cattle and poultry isolates (ARCC, 92%). The high classification rates achieved when only two sources are compared strongly support the working hypothesis that different types of animals will harbor fecal bacteria with differing patterns of antibiotic resistance.

Analysis of unknown isolates from polluted stream water. Samples were taken from two polluted streams in the Shenandoah Valley of Virginia. A total of 193 isolates were collected, and their patterns of antibiotic resistance were measured. When these isolates were classified by discriminant analysis (and the known isolates were used as reference), 72% of the isolates from Cooks Creek and 68% of the isolates from Muddy Creek were classified as cattle isolates (Table 6). When the isolates were grouped as either human or animal, 96% of the isolates in Cooks Creek and 95% of the isolates from Muddy Creek were classified as animal (Table 7). These results suggest that these streams were polluted by animal fecal material, which most likely came from cattle. On the basis of the results of the analysis using all six sources, it is likely that the majority of isolates in both streams came from beef cattle (Table 3).

DISCUSSION

These data strongly suggest that discriminant analysis can be used to differentiate among isolates from several sources of fecal pollution. Although some of the isolates were classified incorrectly, there are many more isolates that were correctly classified than would occur if the classification was random. If the isolates had been randomly classified, the ARCC would have been 17% with six possible sources and 25% with four sources. The isolates from these samples were correctly classified into one of four possible sources at an average rate of 84%. This analysis, therefore, shows correct classification rates well above those expected by chance.

The discriminant analysis method of source determination differs from previous attempts to use antibiotic resistance to identify the source of fecal pollution (the MAR index) (10, 15) in two important ways. First, the discriminant analysis method uses four concentrations for each antibiotic, while the MAR index method uses only one concentration. As shown in this study, the percentages of resistance to various concentrations of a given drug differ from source to source, and these differences can provide information that is not used in the MAR method. Second, the MAR index measures the resistances of a single isolate, and there is no established procedure for relating the MAR indices of many isolates. Criteria such as "if the MAR index is greater than x, then it is in this group" can be of some use in distinguishing between two types of sources (10) but are harder to use with multiple sources (15). The advantage of the discriminant analysis method is that it generates a classification rule based on all the isolates, and that rule is then used to actually classify each individual isolate into one of many possible sources.

When the isolates are classified into one of six possible sources, the highest percentage of correctly classified isolates is found for human sources (92%). If a human source is suspected, the high classification rate and low false-positive rate make the six-source analysis desirable. Furthermore, if a particular situation requires a differentiation between chicken and turkey sources of pollution, there is reasonable differentiation between them. However, the high percentage of chicken isolates that were misclassified as turkey isolates indicates that the pooling of the sources into a poultry category would help the

TABLE 5. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from cattle, humans, poultry, and wild animals[a]

Source (no. of isolates)	No. (%) of isolates classified as:			
	Cattle	Human	Poultry	Wild
Cattle (568)	78 (14)	126 (22)	184 (32)	180 (32)
Human (181)	7 (4)	76 (42)	12 (7)	86 (47)
Poultry (552)	5 (1)	4 (1)	538 (97)	6 (1)
Wild (134)	4 (3)	22 (17)	15 (11)	93 (69)
Cooks Creek (105)	44 (42)	0 (0)	28 (27)	33 (31)
Muddy Creek (88)	7 (8)	3 (3)	60 (68)	18 (21)

[a] Beef and dairy samples were pooled as cattle. Chicken and turkey samples were pooled as poultry. The ARCC for this analysis (using OTC only) was 54%.

TABLE 7. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from animals and humans[a]

Source (no. of isolates)	No. (%) of isolates classified as:	
	Animal	Human
Animal (1,254)	1,184 (94)	70 (6)
Human (181)	9 (5)	172 (95)
Cooks Creek (105)	101 (96)	4 (4)
Muddy Creek (88)	84 (95)	4 (5)

[a] Beef, chicken, dairy, turkey, and wild animal samples were pooled as animals. The ARCC for this analysis (using CTC, OTC, SAL, and STR) was 95%.

TABLE 8. Discriminant analysis of antibiotic resistance profiles of fecal streptococci isolated from various sources[a]

Comparison and source (no. of isolates)	No. (%) of isolates classified as:			
	Human	Wild	Poultry	Cattle
Human vs. wild				
Human (181)	180 (99)	1 (1)		
Wild (134)	5 (4)	129 (96)		
Human vs. poultry				
Human (181)	171 (94)		10 (6)	
Poultry (552)	18 (3)		534 (97)	
Human vs. cattle				
Human (181)	179 (99)			2 (1)
Cattle (568)	41 (7)			527 (93)
Cattle vs. poultry				
Cattle (568)			37 (6)	531 (94)
Poultry (552)			498 (90)	54 (10)

[a] Beef and dairy cattle samples were pooled as cattle; chicken and turkey samples were pooled as poultry. The ARCCs for these analyses (using CTC, OTC, SAL, and STR) were 98% for human vs. wild, 96% for human vs. poultry, 96% for human vs. cattle, and 92% for cattle vs. poultry.

classification. There were consistently more correctly classified isolates (higher ARCCs) when discriminant analysis was performed with the four pooled sources than there were when all six sources were used, most likely because there are fewer possible categories. If differentiating between types of poultry or cattle sources is not required, it seems best to use the analysis that works best for all of the possible isolates, that is, the combination with the highest ARCC.

If some sources are unlikely to be present in a sample, they can be excluded from the analysis. High ARCCs (>90%) were obtained for all of the analyses when just two sources were possible. Of particular note is the separation between human and wild isolates (98%) and between cattle and poultry isolates (92%). In recreational waters that are not impacted by agriculture, it would be useful to be able to distinguish between human and wild sources of fecal pollution. There are also many streams that run through mixed-agricultural areas where it would be desirable to identify which type of animal was causing the pollution.

An indication of the power of the discriminant analysis is that the use of just one drug can sometimes result in the correct identification of a majority of the isolates from all sources as well as very high percentages of isolates from a single source. Although the use of fewer drugs is an important methodological consideration, analyses with only one drug often resulted in many misclassifications. It is important to note that for the OTC analysis, even though almost all of the poultry isolates were correctly classified, other sources were classified very poorly. This emphasizes the importance of using the ARCC as the measure of classification instead of the success rate of an individual source. Because the ARCC reflects both the false negatives from that source and the false positives from other sources, it is a better measure of the ability of a given analysis to classify the isolates.

It is interesting to note that increasing the number of drugs used in the analysis does not necessarily produce a better classification. The ARCC for all five drugs (82%) was actually lower than it was when HAL was removed (84%). Generally, the more drugs used, the higher the ARCC, but in this case, the use of HAL actually reduced the overall power of the discriminant analysis.

Once good separation of known sources is achieved, then water samples containing pollution from unknown sources can be analyzed the same way. Discriminant analysis can assign each unknown isolate to one of the known sources according to the baseline data and the unknown organism's resistance pattern. This analysis thus has the potential to determine multiple origins of isolates in a mixed sample. When streams running through agricultural land in Rockingham County were tested, a majority of the isolates from each stream were classified as coming from cattle (beef cattle, most likely, as indicated by the six-source analysis). This seems reasonable considering that the streams do pass through cattle farmland.

A successful method for source identification will facilitate the tracking of nonpoint sources and will aid in the evaluation of risk to the public health. Although the method described here will not recover all species of fecal streptococci (3, 9, 17), the method can work as long as the set of strains that are obtained can be reproducibly isolated. This method does provide a consistent and reliable pool of fecal streptococci for antibiotic resistance testing, and these isolates can be classified by discriminant analysis.

This method requires several days to obtain results. Unfortunately, because growth is required for isolation, purification, and susceptibility testing, it seems unlikely that the testing time can be reduced appreciably. This time period may be prohibitive for some water quality testing because public health officials often need to make rapid decisions on the closure of recreational waters when contamination is suspected. While the method may prove to be of limited use in these situations, it will more likely be useful in waters receiving chronic fecal pollution, where the need for quick results is not as great.

The results presented here represent the antibiotic resistance patterns for a relatively small number of samples. The patterns of antibiotic resistance may be different in other geographical areas or may vary over time, especially as a result of increases in antibiotic resistance. If geographical or temporal variation does occur, more samples will be needed to determine the extent of that variability. Nevertheless, on the basis of these data, discriminant analysis provides a strong method for classifying and identifying fecal streptococci and thus will serve to help identify the sources of nonpoint-source fecal pollution in natural waters.

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