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ORIGINAL ARTICLE

Geographical variation in antibiotic resistance profiles of *Escherichia coli* isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida[1]

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Abstract

Aims: The aim of this study was to assess geographical variation in multiple antibiotic resistance (MAR) profiles of livestock *Escherichia coli* as well as to evaluate the ability of MAR profiles to differentiate sources of faecal pollution.
Methods and Results: More than 2000 *E. coli* isolates were collected from water retention ponds and manure of swine, poultry, beef and dairy farms in south, central and north Florida, and analysed for MAR using nine antibiotics. There were significant differences in antibiotic resistance of *E. coli* by season and livestock type for more than one antibiotic, but regional differences were significant only for ampicillin. Over the three regions, discriminant analysis using MAR profiles correctly classified 27% of swine, 49% of poultry, 56% of beef and 51% of dairy isolates.
Conclusions: Regional variations in MAR combined with moderate discrimination success suggest that MAR profiles of *E. coli* may only be marginally successful in identifying sources of faecal pollution.
Significance and Impact of the Study: This study demonstrates the existence of regional and seasonal differences in MAR profiles as well as the limited ability of MAR profiles to discriminate among livestock sources.

Introduction

Livestock, such as swine, poultry, beef and dairy cattle, are major sources of faecal pollution that can introduce human pathogens, as well as chemical pollutants, into surface and ground waters. Faecal contamination of water occurs when manure is directly deposited in streams, is transported via land runoff and/or migrates into ground water.

This pollution impairs the use of many rivers, lakes, ponds, estuaries and ground waters throughout the US (Azevedo and Stout 1974; Long and Painter 1991). Waggoner *et al.* (1995) reported that more than 100 million tonnes of dry livestock manure is produced annually in the US, translating to more than 1 billion tonnes of wet manure.

Escherichia coli, a member of the faecal coliform group, has been used as an indicator of human enteric pathogens for many years (Geldreich 1966). However, it is well established that it also inhabits the intestines of other warm-blooded animals (Leclerc *et al.* 2001). Consequently, research is needed to determine the potential characteristics of *E. coli* that can be used to identify its source from various inputs of faecal pollution. In this manner, more accurate health risks can be assessed and remediation efforts can be enhanced.

Multiple antibiotic resistance (MAR) typing, using of single or multiple concentrations of antibiotic, is a method that has been used to differentiate sources of *E. coli* and faecal streptococci by testing bacterial resistance to antibiotics commonly associated with human and animal treatment, as well as with animal feed (Cooke 1976; Kaspar *et al.* 1990; Wiggins 1996; Parveen *et al.* 1997; Parveen and Tamplin 1998; Hagedorn *et al.* 1999; Wiggins *et al.* 1999, 2003; Harwood *et al.* 2000; Kelsey *et al.* 2003). Similar to other reports (Cooke 1976; Kaspar *et al.* 1990), we previously found that *E. coli* isolated from human source were more resistant to antibiotics than nonhuman source isolates (Parveen *et al.* 1997). We also found that discriminant analysis (DA) of MAR profiles correctly classified 82% of human isolates (Parveen and Tamplin 1998). Harwood *et al.* (2000) reported that DA of antibiotic resistance patterns of *E. coli* correctly classified 54% of human, 57% of chicken, 54% of cow, 95% of dog, 73% of pig and 51% of wild *E. coli* isolates.

In Florida and many other states, livestock, especially those on commercial farms, can be significant sources of faecal pollution (Clouser *et al.* 1982; Bureau of Business and Economics Research 1990). Although much information is available on faecal pollution from dairy and beef cattle operations, there is limited information for swine and poultry (Davis *et al.* 1980; Jackson 1990). In addition, almost no information is available on the geographical variation in MAR profiles of *E. coli* isolates originating from swine, poultry, and beef and dairy cattle farm water retention ponds and manure. This study describes the geographical variation in MAR profiles of *E. coli* isolated from livestock in three geographical regions of Florida.

Materials and methods

Sample sites and collection

Samples were collected from swine, poultry, dairy and beef cattle farms (one farm per type of livestock from each region) in three geographical regions of Florida [South (SF), Central (CF) and North (NF)] over a 1 year period (winter, spring and fall) (Table 1). Each farm was visited thrice and one sample was collected from each farm per visit. Swine samples were collected from retention ponds located in Grand Ridge (NF), Gainesville (CF) and Dade City (SF), and were at least 80 miles apart (maximum 230 miles). Poultry samples were collected from retention ponds located in Bushnell (NF), Dade City (CF) and Zolfo Springs (SF), and were at least 30 miles apart (maximum 110 miles). Samples from beef cattle farms were collected from composite manure pits and flush water retention ponds in Lake City (NF), Alachua (CF) and Okeechobee (SF), and were at least 50 miles apart (maximum 200 miles). Dairy samples were collected from retention ponds containing stall flush water located in Greenville (NF), Hague (CF) and Okeechobee (SF). The dairy farms were at least 100 miles apart (maximum 200 miles). To detect recent pollution, sample location was near the discharge pipe from the retention pond and sample was taken beneath the slime layer of the retention pond from the same spot each time. After collection, all samples were stored at 4°C, transported to the laboratory in refrigerated (4°C) coolers and processed within 24 h. A summary of the source of isolates sampled is shown in Table 1.

Table 1 Number and sources of *Escherichia coli* isolates

Livestock	Total # of isolates	Site (# of isolates)			Sample type
		South	Central	North	
Swine	351	163	76	112	Retention pond water
Poultry	550	144	211	195	Retention pond water
Beef	512	214	118	180	Manure and retention pond water
Dairy	595	237	147	211	Retention pond water

Isolation and identification of *E. coli*

Sample preparation and bacteriological tests of *E. coli* were performed by standardized procedures (American Public Health Association 1984,1989; Parveen *et al.* 1997). In brief, all samples were streaked onto MacConkey agar (Difco) and incubated for approximately 16–18 h at 37°C. All lactose-fermenting *E. coli*-like colonies were screened with 4-methylumbelliferyl-β-D-glucuronide (MUG) (Sigma) (Hernandez *et al.* 1993). Presumptive (MUG-positive) *E. coli* isolates were confirmed by indole, methyl red, Voges-Proskauer and citrate (IMViC) tests. About 11–121 isolates were collected per sample event.

Multiple antibiotic resistance

The MAR profiles for the *E. coli* isolates were determined as previously described (Parveen *et al.* 1997), except that a different panel of antibiotics was used: ampicillin (10 μg ml^{-1}), amoxicillin (10 μg ml^{-1}), chlortetracycline (25 μg ml^{-1}), erythromycin (15 μg ml^{-1}), oxytetracycline (25 μg ml^{-1}), penicillin G (75 U ml^{-1}), streptomycin (12·5 μg ml^{-1}), sulfathiazole (500 μg ml^{-1}) and tetracycline (25 μg ml^{-1}) (Sigma). The concentrations of antibiotics were selected based on the results of previous studies used for differentiating sources of faecal pollution (Kaspar *et al.* 1990; Parveen *et al.* 1997). In brief, aliquots of stock solutions were added to tempered (46°C) Muller–Hinton agar (Difco), mixed, poured into petri

dishes and stored at 5°C for no longer than 2 weeks. *E. coli* isolates were grown in 96-well plates containing tryptic soy broth (Difco) at 35°C for 4–6 h, replica plated onto antibiotic-containing agar plates and control plates without antibiotic and incubated at 35°C for 18 h. *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as positive and negative controls, respectively. Isolates were recorded as resistant to an antibiotic if growth was indistinguishable from that on the control plate without antibiotic.

Statistical analyses

Antibiotic resistance was coded as a binary value. Predominant resistance patterns were identified and an attempt was made to determine if there were livestock, regional and seasonal differences in MAR profiles. A generalized linear model (McCullagh and Nelder 1989) for binomial data having main effects for livestock source, region and season and including the livestock source by region interaction was fitted to the isolate antibiotic resistance patterns. *P*-values for Type III Wald *F*-tests were used to assess the significance of effects. The index of association developed by Jaccard (Ludwig and Reynolds 1988) was used to measure the degree of association in antibiotic resistance patterns between isolates. Averages of the values for isolates from each combination of livestock sources and regions were determined. Statistical discriminant analysis (DA; McLachlan 1992) was used to determine whether the MAR pattern could be used to identify livestock source. The results of the DA were summarized as the percentage of correctly classified and misclassified isolates, respectively. All computations were performed with the SAS© System for Windows version 8·02 (SAS Institute, Cary, NC, USA).

Results

A total of 2008 *E. coli* were isolated from swine, poultry, beef and dairy cattle farm water retention ponds and manure in Florida, and were analysed for MAR profiles (Table 1). Among the four livestock sources, 84% of the isolates were resistant to one or more antibiotics (Table 2). Predominant single and MAR patterns of *E. coli* isolates are shown in Table 5. Seventy-three, 107, 82 and 92 different resistance patterns were observed for isolates from swine, poultry, beef and dairy farms, respectively.

The distribution of resistance to specific antibiotics was not uniform among livestock sources (Table 2). Ampicillin resistance was the least variable across livestock sources with an average of 38·2% resistant. The most variable responses were to chlortetracycline and oxytetracycline at 48% and 41·5%, respectively, where the resistance in swine and poultry isolates (approximately 65% and 50%, respectively) was twice that of dairy and beef isolates (at about 33% and 25%, respectively). Antibiotic resistance among four livestock sources was not uniform across the three regions, with SF locations producing a higher proportion of isolates that were resistant to most of the antibiotics (Table 3). The analysis of variance associated with a multi-factor linear model used to test for statistically significant livestock, region and season effects (Table 4) showed highly significant livestock-by-region interactions for two antibiotics, one of which, amoxicillin, also has significant regional effects. All antibiotics with the exception of tetracycline displayed strong seasonal differences in resistance.

Table 2 The percentage of *Escherichia coli* isolates resistant to single antibiotics from swine, poultry, beef and dairy cattle farms

Antibiotics	Number of resistant isolates	Percentage of resistant strains				
		Swine, *n* = 351	Dairy, *n* = 595	Poultry, *n* = 550	Beef, *n* = 512	Significance*
Ampicillin	781	34	43	39	37	0·03
Amoxicillin	460	27	24	25	17	<0·01
Chlortetracycline	923	65	31	63	33	<0·01
Erythromycin	299	21	10	20	11	<0·01
Oxytetracycline	809	57	30	57	22	<0·01
Penicillin G	907	42	33	47	59	<0·01
Streptomycin	330	19	31	26	8	<0·01
Sulfathiazole	164	9	6	5	14	<0·01
Tetracycline	715	52	26	51	20	<0·01
Total (resistant to at least one antibiotic)		85	81	91	80	<0·01

*Probability that the source proportions are equal using a chi-square test for equality of proportions.

Table 3 The percentage of *Escherichia coli* isolates resistant to single antibiotics from north, central and south Florida farms

	Region				
Antibiotics	North Florida	Central Florida	South Florida	Total	Significance*
Ampicillin	27·6	33·3	39·0	781	NS
Amoxicillin	32·2	32·8	35·0	460	0·04
Chlortetracycline	33·6	30·1	35·7	923	<0·01
Erythromycin	27·1	27·4	45·5	299	<0·01
Oxytetracycline	32·8	31·9	35·3	809	<0·01
Penicillin G	17·6	37·7	44·7	907	<0·01
Streptomycin	30·3	29·7	40·0	330	0·10
Sulfathiazole	15·8	23·2	61·0	164	<0·01
Tetracycline	31·0	32·6	36·4	715	0·02

*Significance via a chi-square test for homogeneity of proportions.

We also examined the data to determine if there were combinations of MAR that could be associated with different livestock, region or season differences. The predominant MAR patterns of *E. coli* isolates (Table 5) illustrate the high variability in patterns observed. Note that very few patterns comprised more than 5% of all isolates. Most frequent MAR profiles for swine isolates were chlortetracycline–oxytetracycline–tetracycline, chlortetracycline–penicillin G–erythromycin–oxytetracycline–tetracycline and chlortetracycline. Prevalent MAR profiles for poultry isolates were penicillin G, penicillin G–erythromycin and chlortetracycline–penicillin G–oxytetracycline–tetracycline. Dominant MAR profiles for beef and dairy isolates were penicillin G, penicillin G–erythromycin, ampicillin–chlortetracycline–penicillin G, and penicillin G, ampicillin, ampicillin–amoxicillin, respectively. An overall estimate of similarity in MAR profiles among isolates is provided by the average of Jaccard association indices for all pairs of isolates within each livestock source by region (Table 6). The overall average value of 0·71 suggests that 71% of isolates were resistant to two or more antibiotics. Levels of average similarity were uniform across livestock sources and regions.

The large number of observed MAR profiles and the low representation of any one profile across multiple livestock sources and regional groups made identifying discriminating profiles difficult. For example, it was observed that penicillin G was the most frequent antibiotic resistance pattern for beef and dairy isolates (Table 3). But SF beef and CF dairy isolates had very low penicillin G resistance and SF swine and NF poultry isolates had moderate resistance levels (not shown). Similar patterns were observed for single and combination of antibiotics. There were differences in percentage resistance among livestock sources and regions for a number of

Table 4 Proportion of isolates resistant to antibiotics by livestock and region with *P*-values from the analysis of effects in the generalized linear model

Livestock	Region	AMO*	AMP	CHT	ERY	OXY	PEN	STR	SUL	TET	Isolates
Beef	CF†	0·093	0·20	0·40	0·05	0·38	0·31	0·06	0·05	0·31	118
	NF	0·23	0·37	0·27	0·02	0·17	0·64	0·04	0·07	0·14	180
	SF	0·17	0·46	0·33	0·23	0·18	0·69	0·13	0·24	0·18	214
Dairy	CF	0·35	0·41	0·37	0·12	0·13	0·18	0·10	0·02	0·12	147
	NF	0·20	0·46	0·19	0·10	0·34	0·24	0·17	0·02	0·34	211
	SF	0·21	0·42	0·38	0·09	0·38	0·51	0·11	0·12	0·27	237
Poultry	CF	0·27	0·50	0·75	0·26	0·74	0·28	0·26	0·04	0·60	211
	NF	0·24	0·38	0·55	0·11	0·44	0·67	0·18	0·05	0·38	195
	SF	0·22	0·25	0·54	0·22	0·49	0·48	0·36	0·06	0·55	144
Swine	CF	0·38	0·34	0·66	0·03	0·59	0·47	0·32	0·12	0·57	76
	NF	0·18	0·21	0·77	0·32	0·63	0·41	0·16	0·11	0·54	112
	SF	0·27	0·44	0·56	0·21	0·52	0·41	0·16	0·07	0·49	163
ANOVA *P*-values											
Livestock		<0·01	0·02	<0·01	0·23	<0·01	0·51	<0·01	0·20	<0·01	
Region		0·08	<0·01	0·16	0·42	0·35	0·11	0·72	0·17	0·81	
Livestock region IA‡		<0·01	<0·01	0·23	0·43	0·06	0·56	0·91	0·23	0·05	
Season		<0·01	<0·01	<0·01	<0·01	0·03	<0·01	0·05	<0·01	0·11	
R-square		0·24	0·65	0·16	0·25	0·21	0·44	0·07	0·24	0·13	

*AMO, amoxicillin; AMP, ampicillin; CHT, chlortetracycline; ERY, Erythromycin; OXY, oxytetracycline; PEN, penicillin G; STR, streptomycin; SUL, sulfathiazole; TET, tetracycline.

†CF, central Florida, NF, north Florida, SF, south Florida.

‡Livestock by region interactions.

P-values related to type III Wald statistics determined for the generalized linear model fit via maximum likelihood.

Table 5 Predominant antibiotic resistance profiles of *Escherichia coli* isolated from swine, poultry, beef and dairy cattle farms in Florida

	Percentage with each resistant patterns			
Antibiotics*	Swine n = 351	Poultry n = 550	Beef n = 512	Dairy n = 595
PEN†	4·3	4·0	16·0	11
PEN–ERY	3·5	5·0	7·0	0·5‡
AMP–CHT–PEN	0·03‡	0·5‡	6·0	0·7‡
CHT–OXY–TET	8·0	3·0	5·0	2·4
AMP–PEN	ND	0·7‡	4·0	ND
AMP–CHT–PEN–AMX	0·11‡	1·3‡	4·0	1·0‡
AMP–PEN–AMX	0·11‡	4·0	4·0	4·0
OXY–TET	1·4‡	0·5‡	3·0	ND
AMP–CHT–PEN–SUL	ND	ND	2·0‡	ND
AMP	1·4‡	ND	0·4‡	9·0
AMP–AMX	0·9‡	2·0‡	0·2‡	6·0
ERY	1·4‡	2·2	0·9‡	4·0
CHT	5·0	2·0‡	1·2‡	3·2
CHT–ERY	0·3‡	ND	ND	3·0
CHT–PEN–OXY–TET	4·0	6·0	0·2‡	0·7‡
CHT–OXY	4·3	4·0	0·4‡	ND
AMP–CHT–PEN–AMX –OXY–STR–TET	ND	4·0	ND	ND
CHT–PEN–ERY–OXY–TET	5·0	3·0	ND	0·5‡
AMP–CHT–OXY–TET	4·0	3·0	ND	ND
CHT–OXY–STR–TET	2·0‡	2·2	ND	1·5‡
AMP–CHT–PEN–AMX–OXY–TET	3·0	2·2	ND	ND
CHT–PEN–AMX–OXY–STR–TET	4·3	0·2‡	ND	0·2‡
Other resistant patterns§	40	47	30	40
Sensitive to all antibiotics	15·4	10	20	19

ND, none detected.

*AMP, ampicillin; CHT, chlortetracycline; PEN, penicillin G; SUL, sulfathiazole; AMX, amoxicillin; ERY, erythromycin; OXY, oxytetracycline; STR, streptomycin; TET, tetracycline.

†Prevalent, most frequent and dominant MAR profiles are italicized.

‡Also mentioned in other resistant patterns.

§Each of the other 62 patterns for swine, 94 patterns for poultry, 73 patterns for beef, and 83 patterns for dairy isolates contained a low percentage of isolates (<3·0% for swine and beef; <2·2% for poultry and dairy) and are not shown.

Table 6 Average Jaccard index measures of association in MAR profiles among isolates in livestock by region

	Region		
Source	North	Central	South
Beef	0·79	0·75	0·74
Dairy	0·73	0·79	0·71
Poultry	0·63	0·57	0·74
Swine	0·69	0·71	0·63

combinations of antibiotics, but statistical comparisons were unreliable. In general, the average percentage of isolates represented in any one group was small, typically less than 10% and more often less than 5% (not shown).

A preliminary test of the equity of the livestock group specific between antibiotics covariance matrices was statistically significant ($\chi^2 = 1298$, $P < 0{\cdot}01$) requiring that a quadratic Fisher discrimination model be used. Using the MAR profile as predictor, the fitted discriminator correctly classified 27% of swine, 49% of poultry, 56% of beef and 51% of dairy isolates (Table 7). Swine isolates were particularly misclassified, with 33% and 21% of isolates being incorrectly classified as poultry and dairy isolates, respectively. Roughly the same fraction of beef isolates was incorrectly classified as dairy isolates, as were dairy isolates classified as beef. Examination of the results also indicated that there was very little difference between beef and dairy isolates in MAR profiles. When beef and dairy isolates were combined and recoded as 'cattle', 73% of the cattle isolates (Table 7) were correctly classified. However, correct classification rates for swine and poultry isolates remained similar (not shown). Attempts to reduce the number of antibiotics in the discrimination model to

Table 7 Discriminant analysis of MAR profiles of *Escherichia coli* isolated from swine, poultry, beef and dairy farms in Florida

	Percentage of isolates classified as			
Source (# of isolates)	Swine	Poultry	Beef	Dairy
Swine (351)	27	33	19	21
Poultry (550)	15	49	19	17
Beef (512)	7	9	56	28
Dairy (595)	8	17	24	51
Cattle* (1107)	13	14		73

*Beef and dairy isolates were pooled as cattle.

fewer than nine resulted in significant loss in discriminatory power. The most heavily weighted antibiotics in the discriminating functions were chlortetracycline, penicillin G, ampicillin and sulfathiazole, although using only these four antibiotics in a discrimination analysis increased the misclassification rate from 54% to 60%. The average rate of correct classification for discrimination using all antibiotics was different across regions with 52% for SF, 67% for CF and 60% for NF (not shown). Similarly, when classification was performed by season, only 28% of winter, 50% of spring and 46% of fall isolates were correctly classified (not shown).

Discussion

The MAR profiles of swine, poultry, beef and dairy cattle *E. coli* isolates showed high variability, which may be related to the widespread use of antibiotics in livestock farms. The level of antibiotic resistance observed in this study (84%) is similar to a previous report for human and non-human isolates (82%) (Parveen *et al.* 1997). Our initial hypothesis was that different antibiotic use across livestock operations should result in differences in antibiotic resistance patterns in *E. coli*. This differential resistance could then be exploited to link specific MAR pattern with specific livestock operation and eventually provide a means of identifying sources of pollution. There was significant variation in MAR profiles among *E. coli* isolated from the different livestock operations. There were regional and seasonal variations in MAR profiles, which reduced the ability to discriminate livestock source. The source of this regional and seasonal variability in MAR profiles within similar type livestock operations was not determined. Even beef and dairy operations, which demonstrated the greatest similarity in MAR profiles, had quite significant regional and seasonal differences. The percentage of resistant isolates probably will be different if NCCLS breakpoint antibiotic concentrations are used.

The most common antibiotics used in feed and for treating disease on beef and dairy cattle farms are penicillin G, erythromycin, tetracycline, oxytetracycline and sulfathiazole (Church and Kellems 2002; Huber and Bevill 1982; ww.ahi.org, http://www.ucsusa.org, http://www.fda.gov/cvm/greenbook/greenbook/html and K. Braun, University of Florida, Gainesville, FL, USA). In addition, penicillin G, ampicillin, oxytetracycline, chlortetracycline and tetracycline are commonly used in swine and poultry operations (Huber and Bevill 1982; Church and Kellems 2002; http://www.ahi.org, http://www.ucsusa.org, http://www.fda.gov/cvm/greenbook/greenbook/html, and Dr C.F. Shipley, University of Illinois, Springfield, IL, USA). Even though similar antibiotics are used in feed and for treatment of swine and poultry diseases (Huber and Bevill 1982; Church and Kellems; http://www.ahi.org, http://www.ucsusa.org, http://www.fda.gov/cvm/greenbook/greenbook/html), MAR profiles of swine isolates were no more similar to those of poultry isolates than they were to other livestock isolate profiles.

The correct classification rates from the discriminant analysis for the *E. coli* isolates in this study were similar to those reported by Harwood *et al.* (2000). They found that 57% of chicken and 54% of cow isolates were correctly classified (Harwood *et al.* 2000), compared to the 49% and 56% of this study. The similarity may be because of the fact that samples were collected from similar geographical regions. In the present study, only 27% of the swine isolates were correctly classified, whereas in a previous report (Harwood *et al.* 2000), 73% of the swine isolates were correctly identified. The reason for this difference is not known. When beef and dairy isolates were combined as cattle isolates, the correct classification rate was higher (73%) for cattle isolates, a level of discrimination that could be useful in identifying cattle source *E. coli* from that of other livestock. Using only a subset of the available antibiotics in the analysis resulted in lower correct classification rates, which is similar to results obtained by Harwood *et al.* (2000). However, in other studies, higher correct classification rates were obtained using a subset of antibiotics (Wiggins 1996; Hagedorn *et al.* 1999).

In many instances, water quality managers are primarily interested in discriminating between animal and human contamination, and secondarily interested in determining the specific source(s) of animal contamination in a watershed. Bacterial source tracking methods, such as MAR and ribotyping, are relatively novel; however, none of the available studies have demonstrated a clear utility of using these methods to discriminate potential sources. This study demonstrated the existence of regional and seasonal patterns in MAR as well as regional and seasonal differences in the ability of MAR profiles to discriminate among livestock sources. The moderate correct classification rates and regional and seasonal differences should cause regulatory agencies to consider MAR

profiles of *E. coli* as only marginally useful in their decision processes.

This study was limited in geography and seasonal coverage; hence, caution is urged in extrapolating its results to broader populations. Only a single farm was sampled in each region of Florida for each livestock operation type, with the same region being sample once for three seasons in 1 year. In addition, multiple samples were not collected from the same retention ponds per visit to understand the inter-sample variability. The protocol was designed to provide an accurate representation of the types of MAR at each site, but farm-to-farm variability in MAR within a livestock operation type was confounded with regional differences. Although major differences in MAR among geographically close farms were not expected, no data were collected to test this assumption. This is an obvious direction for future research.

Finally, we suspect that an important determinant of the prevalence of antibiotic resistance in a population is the selective pressure of antibiotic treatment on the commensal microflora of livestock (Witte 1997). If this is true discrimination, models may have to be periodically updated with new sampling data if they are to remain accurate in predicting source. The poor performance of MAR in discriminating among livestock sources suggests that other characteristics of isolates or use of multiple methods, may offer more productive avenues for future research.

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