Acquisition and persistence of antimicrobial-resistant bacteria isolated from dogs and cats admitted to a veterinary teaching hospital

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Objective—To assess antimicrobial resistance among bacteria isolated from dogs and cats admitted to a veterinary teaching hospital (VTH), determine the incidence of acquisition of and frequency of persistent colonization by antimicrobial-resistant organisms among these animals, and identify risk factors associated with these variables.

Design—Prospective longitudinal study.

Animals—622 dogs and 92 cats admitted to a VTH and expected to stay ≥ 48 hours.

Procedures—Samples were collected with rectal and nasal or oropharyngeal swabs at admission and discharge. Isolates of enterococci, staphylococci, and *Escherichia coli* were tested for antimicrobial resistance via microbroth dilution methods. A subset of isolates was analyzed with pulsed-field gel electrophoresis and multilocus sequence typing. Significant trends in proportions of organisms with antimicrobial resistance over the 3-year study period were assessed.

Results—The proportion of staphylococci with antimicrobial resistance increased, whereas the proportion of *E coli* with resistance decreased, over time; resistance among enterococci was more variable. For 506 dogs with paired admission and discharge samples, multidrug-resistant (MDR) *E coli* was acquired by 40 (8%) and methicillin-resistant *Staphylococcus aureus* (MRSA) was acquired by 7 (1.4%); hospitalization for > 3 days was significantly associated with both variables. Most (5/7 isolates) acquired MRSA was of sequence type (ST) 5.

Conclusions and Clinical Relevance—Extended hospitalization was associated with increased risk of acquiring MDR *E coli* or MRSA, although few animals acquired MRSA. It is unclear whether associations were confounded by illness severity or use of infection control measures. Additionally, MRSA of ST5, which has been associated with small animal medicine, was the most commonly acquired MRSA in this study. (*J Am Vet Med Assoc* 2013;243:990–1000)

Use of antimicrobials in human or animal medicine is often followed by development of antimicrobial resistance. The acquisition of infections among companion animals in a hospital or clinic setting is emerging as a public health threat[1] that not only impacts an infected animal's course of treatment and outcome but may substantially impact the health of humans (owners or veterinary staff) or other animals. Like human patients in hospital settings, animals housed in VTHs are often susceptible to infection,[2] and some bacteria typically found in dogs, such as *Staphylococcus pseudintermedius*, have the ability to carry and transfer resistance to a host of other pathogenic bacteria.[3] However, the number of approved antimicrobials for use in companion animals is more limited than those approved for use in human medicine.[4] This leaves fewer options when resistance does emerge.

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Abbreviations

CI	Confidence interval
ECC	Emergency and critical care
MDR	Multidrug-resistant
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSP	Methicillin-resistant *Staphylococcus pseudintermedius*
PFGE	Pulsed-field gel electrophoresis
ST	Sequence type
VRE	Vancomycin-resistant *Enterococcus* spp
VTH	Veterinary teaching hospital

A qualitative risk assessment of acquisition of MRSA by patients at a VTH found that contact with veterinary personnel poses the greatest risk, followed by environmental surfaces.[5] Adherence to established infection control practices can help to prevent transmission of infections in a hospital setting. However, other factors may confound this, such as the circumstances of hospital admission (eg, emergency),[6,7] duration of hospitalization,[7–9] prior antimicrobial use,[9] or combinations of these. It has been reported that for each day a dog is hospitalized, the odds of being colonized with *Escherichia coli* resistant to 1 or more antimicrobials in-

creased by a factor of 1.5, regardless of antimicrobial treatment.[9] In contrast, results of another study[6] indicated that use of fluoroquinolones was associated with increased risk of colonization with MDR *E coli* in dogs during hospitalization.

Understanding the reasons for transmission of important pathogens such as MRSA, MDR *E coli*, or even VRE could provide evidence for improved infection control methods. Our hypothesis was that patient characteristics or aspects of their stay at a VTH would affect the likelihood of acquiring bacterial infection during hospitalization. The purpose of the study reported here was to assess antimicrobial resistance profiles of bacterial isolates obtained from animals at the time of admission to a VTH; determine the incidence of acquisition and frequency of persistent colonization of MRSA, MRSP, VRE, and MDR *E coli*; and identify the epidemiological risk factors associated with acquisition and persistent colonization of these pathogenic bacteria.

Materials and Methods

Animals and study design—A longitudinal study of dogs and cats admitted to the ECC ward, soft tissue and internal medicine ward, or orthopedic ward of the Michigan State University VTH was conducted from February 5, 2007, through December 29, 2009. A sample size of 280 animals was required to achieve a power of 80% with a significant probability level of 5%. Dogs or cats admitted to 1 of the 3 described VTH locations were considered for inclusion in the study if the attending clinician anticipated a hospital stay ≥ 48 hours. Five animals that were initially admitted to other wards (dermatology, oncology, and ophthalmology) and then subsequently admitted to 1 of the 3 included locations were also included in the study. Each animal was considered for enrollment once during the study period.

After owner consent was obtained, samples were collected within 24 hours after admission and again at discharge but at least 48 hours after the admission sample. During the 3-year study period, changes to protocol only occurred in collection of feline samples. Sample collection consisted of 1 rectal swab and 2 nasal swabs (dogs only) or 1 rectal swab and 1 oropharyngeal swab (cats only). Initially, nasal samples were collected from both dogs and cats; however, an assessment of bacterial recovery during the first year of the study revealed very low recovery rates from feline nasal swabs (data not shown). Subsequently, only oropharyngeal samples were collected from cats to increase recovery rates, as supported by studies[10,11] in humans. The study was approved by the Michigan State University Institutional Animal Care and Use Committee; because it was part of a larger study that included humans, it was also approved by the Michigan State University Institutional Review Board for Research on Human Subjects.

Biological sample collection—Rectal swabs were collected with a sterile swab and tube containing a transport medium.[a] Swabs were inserted into the colon 1 to 2 cm, just beyond the rectum, and rotated until feces adhered to the swab. Nasal and oropharyngeal samples were also collected with a sterile swab and tube containing a transport medium.[a] Nasal samples from dogs (and cats in year 1) were collected with a sterile swab moistened with transport media and placed 2 to 3 mm into the nares and rotated. This process was performed in both nares with new swabs for each. Oropharyngeal samples from cats were collected with a sterile swab moistened with transport media and placed in the lateral oropharynx and rotated. Collected samples were then immediately transported (at ambient temperatures) to the Center for Comparative Epidemiology Microbial Epidemiology Laboratory at Michigan State University for processing.

Isolation and identification of bacteria—Nasal or oropharyngeal swabs were streaked onto a Columbia colidixin and nalidixic acid agar plate supplemented with 5% sheep blood. Rectal swabs were streaked onto 1 MacConkey plate and 1 Columbia colidixin and nalidixic acid agar plate (1 side of the swab/plate). MacConkey plates were incubated for 18 to 24 hours at 37°C, and Columbia colidixin and nalidixic acid agar plates were incubated for 48 hours at 37°C. One to 5 colonies/plate with typical *Enterococcus* spp, *Staphylococcus* spp, and *E coli* morphology were chosen for identification.

Identification of enterococci and staphylococci were completed following previously described biochemical testing methods.[12] Identification of *E coli* was completed following methods described elsewhere.[13] After the present study was initiated, evidence was published concerning misclassification of *Staphylococcus intermedius*[14]; therefore, these isolates were identified as *S pseudintermedius*. Each positively identified isolate of *Enterococcus* spp, *Staphylococcus* spp, or *E coli* was suspended in tryptic soy broth using all available culture to create a very turbid suspension, and 0.5 mL of the suspension was added to 0.5 mL of a 65% glycerol solution; this mixture was frozen at –70°C and later regrown for DNA isolation and molecular evaluation.

Antimicrobial susceptibility testing—Of the 5 *Enterococcus* spp, *Staphylococcus* spp, or *E coli* colonies isolated/sample, 3 isolates of each species were randomly chosen for antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed with an automated microdilution system[b] on 2 commercially prepared plates (gram-positive[c] and gram-negative[d] format plates). Antimicrobials on the gram-positive testing plate were tested against enterococci and staphylococci and included ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, 2 concentrations of gentamicin, levofloxacin, linezolid, oxacillin (as a proxy for methicillin), penicillin, quinupristin-dalfopristin, rifampin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, and vancomycin. Antimicrobials on the gram-negative testing plate were tested against *E coli* and included amikacin, ampicillin, amoxicillin–clavulanic acid, ceftiofur, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline. These panels were chosen to ensure inclusion of antimicrobials used in both human and animal medicine. *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), and *E coli* (ATCC 25922) were

used for quality-control purposes.[e] Quality-control results were reviewed for each batch of tests, all of which were within acceptable limits. Inducible clindamycin resistance was not evaluated.

The minimum inhibitory concentration at which no bacterial growth occurred was determined with a fluorescence technology–based automated reading system,[f] and an antimicrobial susceptibility and resistance profile was generated. Susceptibility, intermediate resistance, and resistance to antimicrobials were determined by comparison with Clinical and Laboratory Standards Institute breakpoints.[15] Enterococcal resistance to gentamicin, ceftriaxone, clindamycin, trimethoprim-sulfamethoxazole, and oxacillin was not interpreted. The results of antimicrobial resistance testing were used to identify the 4 types of organisms that were further analyzed in this study: MRSA, MRSP, VRE, and MDR *E coli* (defined as *E coli* resistant to ≥ 5 antimicrobials).

PFGE—To determine the epidemiological relatedness between different isolates of the same species, MDR *E coli* isolated from both admission and discharge samples of animals with persistent colonization was evaluated via PFGE at the Michigan State University Diagnostic Center for Population and Animal Health. Bacterial DNA was isolated and purified[g] and then digested with the restriction enzyme XbaI. Electrophoresis of DNA preparations was performed with a PFGE unit[h] and achieved by ramping the switch times from 4 to 35 seconds. The overall run time was 20 hours. The PFGE patterns were then analyzed and compared with commercially available software.[i] The PFGE clone groupings were determined according to the standard of Tenover et al.[16] Briefly, similarity clustering analyses were performed with software[i] by use of the unweighted pair group-matching algorithm and the Dice correlation coefficient with a tolerance of 1.5%.

MLST—To identify genetic relatedness within each group of organisms, MLST was performed on persistent MDR *E coli* isolates as well as on isolates of acquired MRSA that were identified. To isolate bacterial DNA, samples from frozen stocks were subcultured onto trypticase soy agar with 5% sheep blood and grown overnight in tryptic soy broth at 37°C. The DNA was extracted with a commercial silica gel–based extraction column[j] per manufacturer's instructions. For MLST, PCR amplification of bacterial DNA, purification of PCR amplification products, and sequencing of 7 conserved housekeeping loci/species were performed at the Michigan State University Genomic Research Support Technical Facility according to previously described methods.[17,k] Briefly, internal fragments (400 to 500 base pairs) of uidA, mdh, lysP, idcA, fadD, clpX, and aspC for *E coli* and arcC, aroE, glpF, gmk, pta, tpi, and yqiL for *S aureus* were examined. The quality of the DNA sequence and base calling was validated with a commercial software program,[l] and consensus sequences were assembled and trimmed. Finally, allele and numeric ST assignments were made with the MLST database[m] and with a reference database[n] for MRSA and MDR *E coli*, respectively. Neighbor-joining trees were constructed with concatenated sequence data by use of a commercial software program.[o]

Epidemiological data collection—Data on signalment (eg, sex and age), housing at home (indoor, outdoor, or other) and status of the animal (pet or other), reason for admission, whether clinical abnormalities were detected on physical examination (yes or no), antimicrobial usage, length of hospitalization, and locations visited or consultations received during hospitalization were extracted from the medical record of each animal enrolled in the study. An animal admitted to a ward may have been temporarily housed elsewhere (eg, a patient admitted to the ECC ward may have been initially housed in the soft tissue and internal medicine ward on the basis of space availability); thus, data on both the ward admitted to and the location of initial housing was collected.

Statistical analysis—Because up to 5 typical colonies of each *Enterococcus* spp, *Staphylococcus* spp, and *E coli* were chosen from each sample, antimicrobial susceptibility patterns produced by applying the Clinical and Laboratory Standards Institute breakpoints[15] to all antimicrobials tested were compared for each group of species isolated from each animal for each sampling event. Any species with identical susceptibility patterns, sample collection dates, and animal were restricted, and 1 isolate was randomly chosen for inclusion in the analysis. Nasal and oropharyngeal samples were treated equally in the selection process. Random selection was completed within the statistical software program[p] by grouping data by isolate species, susceptibility patterns, sample collection, and animal identification number, then selecting the first observation.

A discharge sample was not available from all patients enrolled in the study (because of early discharge [< 48-hour stay], euthanasia or other patient condition that precluded collection, or failure to coordinate sample collection at the time of discharge). Therefore, analysis of data was performed separately for animals that had an admission sample only and for those that had paired admission and discharge samples. For admission samples, all variables of interest were evaluated for the entire study population, stratified by animal species. Differences between dogs and cats were assessed via a 2-sample *t* test for continuous variables and χ^2 or Fisher exact test for categorical variables. Prevalence of antimicrobial resistance among all admission sample isolates by organism was described. Additionally, significant increasing or decreasing trends in the proportion of resistance by organism and year of the study were measured with a Cochran-Armitage test for trend.

Proportions of isolates with antimicrobial resistance were calculated, and values were summarized for those isolates obtained from admission samples only. Incidence of acquisition and persistence of bacteria were analyzed for animals that had both admission and discharge samples collected. For study purposes, acquisition of an organism was identified when a patient had an admission sample that tested negative for an organism but had a discharge sample that tested positive. Persistence was identified when admission and discharge samples from the same animal tested positive for the same organism. Incidence of acquisition and persistence of *E coli*, MDR *E coli*, *Enterococcus* spp, VRE, *Staphylococcus* spp, MRSA, and MRSP were analyzed.

Additionally, multivariable logistic regression was performed with statistical software[p] to evaluate risk factors for outcomes of acquisition and persistence for organisms of interest. For this analysis, persistent organisms in the same animal were further identified as having matching molecular profiles in both admission and discharge samples, and organisms that did not meet this criteria (as well as those identified in the discharge sample only) were classified as acquired. First, univariable (χ^2) analysis was performed for all independent variables to assess suitability of inclusion in multivariable regression (data not shown). Variables with a value of $P < 0.2$ were included in each of the full models. The final model of each multivariable analysis was achieved

Table 1—Characteristics of 714 dogs and cats evaluated during a 3-year study to assess antimicrobial resistance profiles of bacterial isolates obtained via nasal or oropharyngeal and rectal swabs at the time of admission to a VTH; determine the incidence of acquisition of and frequency of persistent colonization by MRSA, MRSP, VRE, and MDR *Escheria coli*; and evaluate epidemiological risk factors associated with acquisition of and persistent colonization by these pathogenic bacteria.

Variable	Dogs (n = 622)	Cats (n = 92)	*P* value
Weight at admission (kg)	26.0 ± 16.3	4.4 ± 1.7	< 0.001
Length of stay (d)	2.6 ± 1.6	3.2 ± 2.1	0.030
Age at admission (y)	5.7 ± 3.7	7.5 ± 5.5	0.003
Sex			—
Male	306 (49.2)	52 (56.5)	
Female	316 (50.8)	40 (43.5)	
Reason for admission			0.001
Emergency	222 (35.7)	49 (53.3)	
Elective or referral	400 (64.3)	43 (46.7)	
Contact with other animals at home	407 (65.4)	65 (70.7)	
Housing			—
Indoor only	411 (66.1)	60 (65.2)	
Other (outdoor, indoor and outdoor, or unknown)	211 (33.9)	32 (34.8)	
Animal status			—
Pet	449 (72.2)	67 (72.8)	
Other	173 (27.8)	25 (27.2)	
Abnormal findings on physical examination	513 (82.5)	75 (81.5)	—
History of prolonged antimicrobial use	237 (38.1)	33 (35.9)	—
Antimicrobial administered ≤ 10 days prior to admission	95 (15.3)	10 (10.9)	—
Receiving antimicrobial at admission	125 (20.1)	21 (22.8)	—
Given antimicrobial while hospitalized	285 (45.8)	56 (60.9)	0.007
Given antimicrobial during surgery	374 (60.1)	30 (32.6)	< 0.001
Prescribed antimicrobial at discharge	253 (40.7)	30 (32.6)	—
Ward admitted to			< 0.001
ECC	214 (34.4)	48 (52.2)	
Orthopedic	225 (36.2)	4 (4.3)	
STIM	179 (28.8)	39 (42.4)	
Other (dermatology, oncology, or ophthalmology)	4 (0.6)	1 (1.1)	
Location of initial housing			< 0.001
ECC ward	137 (22.0)	31 (33.7)	
NCU-ICU	140 (22.5)	31 (33.7)	
Orthopedic ward	217 (34.9)	4 (4.3)	
STIM ward	118 (19.0)	24 (26.1)	
Isolation ward	1 (0.2)	0 (0.0)	
Wards visited or consultations performed during stay			
Surgery	373 (60.0)	48 (52.2)	—
Radiology and ultrasound	451 (72.5)	73 (79.3)	—
NCU-ICU	218 (35.0)	45 (48.9)	0.010
STIM	228 (36.7)	58 (63.0)	< 0.001
Ophthalmology	20 (3.2)	0 (0.0)	—
ECC	215 (34.6)	48 (52.2)	0.009
Oncology	13 (2.1)	7 (7.6)	< 0.001
Orthopedics	252 (40.5)	10 (10.9)	—
Cardiology	45 (7.2)	12 (13.0)	—
Physical therapy	10 (1.6)	0 (0.0)	—
Neurology	11 (1.8)	0 (0.0)	—
Dermatology	8 (1.3)	3 (3.3)	—
Isolates in admission sample			
E coli	554 (89.1)	74 (80.4)	0.009
MDR *E coli*	60 (9.7)	7 (7.6)	—
Staphylococcus spp	231 (37.1)	10 (10.9)	< 0.001
MRSA	3 (0.5)	1 (1.1)	—
MRSP	5 (0.8)	0 (0.0)	—
Enterococcus spp	529 (85.0)	52 (56.5)	< 0.001
VRE	2 (0.3)	1 (1.1)	—

Data are shown as mean ± SD or as No. (%) of animals.

— = Nonsignificant. NCU-ICU = Nursing care unit or intensive care unit. STIM = Soft tissue and internal medicine.

Samples were collected from 2007 through 2009; each animal was considered for study inclusion only once. Prolonged antimicrobial use was defined as the patient having received > 1 course of antimicrobial treatment for the current condition. Significance was assessed with a 2-sample *t* test for continuous variables and χ^2 test for categorical variables. The Fisher exact test was used for categorical variables with cell counts < 5.

Table 2—Resistance over time against selected antimicrobials among *Enterococcus* spp and *Staphylococcus* spp isolates obtained from admission samples of the same 714 animals as in Table 1.

Antimicrobial class and type	*Enterococcus* spp			*Staphylococcus* spp		
	2007 (n = 414)	2008 (n = 350)	2009 (n = 347)	2007 (n = 74)	2008 (n = 129)	2009 (n = 122)
Fluoroquinolone						
Ciprofloxacin	42 (10.1)	36 (10.3)	24 (6.9)	1 (1.4)	8 (6.2)	12 (9.8)*
Gatifloxacin	24 (5.8)	12 (3.4)	9 (2.6)*	1 (1.4)	9 (7.0)	11 (9.0)*
Levofloxacin	27 (6.5)	16 (4.6)	12 (3.5)*	1 (1.4)	8 (6.2)	8 (6.6)
Glycopeptide						
Vancomycin	1 (0.2)	3 (0.9)	0 (0)	0 (0)	1 (0.8)	0 (0)
Macrolide						
Erythromycin	53 (12.8)	43 (12.3)	42 (12.1)	17 (23.0)	31 (24.0)	35 (28.7)
β-Lactam						
Ampicillin	68 (16.4)	53 (15.1)	37 (10.7)*	1 (1.4)	18 (14.0)	15 (12.3)*
Oxacillin	NI	NI	NI	1 (1.4)	17 (13.2)	11 (9.0)
Penicillin	70 (16.9)	56 (16.0)	39 (11.2)*	9 (12.2)	44 (34.1)	47 (38.5)*
Rifampin						
Rifampin	136 (32.8)	135 (38.6)	146 (42.1)*	0 (0)	2 (1.6)	1 (0.8)
Streptogramin						
Quinupristin-dalfopristin	207 (50.0)	164 (46.9)	211 (60.8)*	0 (0)	2 (1.6)	2 (1.6)
Tetracycline						
Tetracycline	165 (39.9)	143 (40.9)	125 (36.0)	22 (29.7)	32 (24.8)	35 (28.7)

Data are reported as No. (%) of isolates.
*Values were significantly ($P < 0.05$) different over time.
NI = Minimum inhibitory concentrations of these antimicrobials were not interpreted.
Antimicrobial resistance was determined on the basis of Clinical and Laboratory Standards Institute breakpoints.[15]

via backward stepwise elimination, which included assessments of interaction and confounding (assessed as a > 25% change in the value of a coefficient; thus, variables that were not significant may have been retained). For all models, the potential interaction of duration of hospitalization with all independent variables was considered but not found to be significant. Odds ratios and 95% CIs were reported; values were considered significant if the 95% CI did not cross the null of 1.0.

Results

Characteristics of patients and admission samples—Admission samples were collected from 714 animals (622 dogs and 92 cats; **Table 1**). Paired admission and discharge samples were collected from 570 (79.8%) of these animals (506 dogs and 64 cats). Cats enrolled in our study were significantly ($P = 0.003$) older (mean, 7.5 years vs 5.7 years) and were hospitalized significantly ($P = 0.030$) longer (mean, 3.2 days vs 2.6 days) than were dogs. Additionally, cats were more frequently admitted on an emergency basis, rather than being admitted for an elective procedure or being referred from another institution, compared with dogs (49/92 [53.3%] vs 222/622 [35.7%]; $P = 0.001$). Dogs were admitted in fairly equal proportions to the ECC ward, orthopedic ward, and soft tissue and internal medicine ward, whereas cats were primarily admitted to ECC ward or soft tissue and internal medicine ward ($P < 0.001$). In accordance with this, there was also a significant ($P < 0.001$) difference in areas of the VTH where cats and dogs were initially housed.

Areas visited in the VTH as well as specialty consultations obtained were recorded for enrolled animals. Significantly ($P \leq 0.01$ for all comparisons) greater proportions of cats visited the nursing care or intensive care unit, soft tissue and internal medicine ward, ECC ward, and oncology area, compared with dogs (Table 1). Additionally, a greater percentage of cats received antimicrobials during their stay (56/92 [60.9%] vs 285/622 [45.8%]; $P = 0.007$), compared with dogs. However, a greater percentage of dogs than cats received antimicrobials during surgery (374/622 [60.1%] vs 30/92 [32.6%]; $P < 0.001$), although there was no significant difference in having had a surgical procedure. *Escherichia coli*, staphylococci, and enterococci were more commonly isolated from canine samples than from feline samples ($P < 0.01$ for all comparisons).

Table 3—Resistance over time against selected antimicrobials among *E coli* isolates obtained from admission samples of the same 714 animals as in Table 1.

Antimicrobial class and type	*E coli*		
	2007 (n = 294)	2008 (n = 244)	2009 (n = 228)
Aminoglycoside			
Gentamicin	10 (3.4)	13 (5.3)	13 (5.7)
Kanamycin	25 (8.5)	14 (5.7)	10 (4.4)*
Cephalosporin			
Ceftriaxone	27 (9.2)	15 (6.2)	3 (1.3)*
Cefoxitin	46 (15.6)	28 (11.5)	16 (7.0)*
Ceftiofur	41 (13.9)	22 (9.0)	10 (4.4)*
Fluoroquinolone			
Ciprofloxacin	31 (10.5)	23 (9.4)	12 (5.3)*
Nalidixic acid	38 (12.9)	26 (10.7)	18 (7.9)*
β-Lactam			
Ampicillin	107 (36.4)	77 (31.6)	44 (19.3)*
Amoxicillin–clavulanic acid	47 (16.0)	33 (13.5)	19 (8.3)*
Sulfonamide			
Trimethoprim-sulfamethoxazole	46 (15.6)	35 (14.3)	17 (7.5)*
Sulfisoxazole	59 (20.1)	42 (17.2)	26 (11.4)*
Chloramphenicol	23 (7.8)	20 (8.2)	8 (3.5)*
Tetracycline			
Tetracycline	57 (19.4)	36 (14.8)	26 (11.4)*

See Table 2 for key.

Antimicrobial susceptibility testing of isolates obtained from all admission samples revealed that resistance

among enterococci (n = 1,111 isolates) was most commonly detected against quinupristin-dalfopristin (582 [52.4%]), tetracycline (433 [39.0%]), and rifampin (417 [37.5%]). Four of 1,111 (0.4%) isolates were VRE. Only 1 of 1,111 (0.1%) enterococci isolates were resistant to linezolid, and none were resistant to daptomycin. Resistance among staphylococci (n = 325 isolates) was most commonly detected against penicillin (100 [30.8%]), tetracycline (89 [27.4%]), and erythromycin (83 [25.5%]). Six of 325 (1.8%) *Staphylococcus* spp isolates were identified as MRSA isolates and 5 (1.5%) as MRSP. Resistance among *E coli* (n = 766 isolates) was most commonly detected against ampicillin (228 [29.8%]). Of 95 isolates of MDR *E coli*, 68 (71.6%) were resistant to ampicillin, a sulfonamide, and tetracycline, and 57 (60.0%) were also resistant to nalidixic acid.

Over time, significant trends were seen among the proportions of antimicrobial-resistant isolates for many organisms obtained from admission samples (**Tables 2 and 3**). Notably, resistance of enterococci to the 2 β-lactam drugs tested and to 2 of 3 fluoroquinolones decreased during the study period. The percentage of *Entercococcus* spp isolates with resistance to rifampin and quinupristin-dalfopristin increased by 27% and 22%, respectively, from 2007 to 2009. Resistance trends for *Staphylococcus* spp were significant for the β-lactam drugs tested and for 2 of 3 fluoroquinolones, but the most notable trend was that of resistance against penicillin, which increased 225% during the same period. Significant trends in antimicrobial resistance were most commonly detected for *E coli*; however, all significant trends over time were decreasing for this organism.

Epidemiological relatedness of acquired MRSA— Seven isolates of acquired MRSA (all from dogs) were collected between March 2008 and March 2009. Examination of a consensus dendrogram of MLSTs for these isolates revealed that 5 belonged to the ST5 group. Three of the 5 samples were from dogs housed in the ECC ward, and 2 were from dogs housed in the nursing care or intensive care unit at the time the sample was collected. Two MRSA isolates from dogs in the ECC ward were collected within 1 week of each other, but none were collected on the same day. One isolate was of the ST72 group and was collected from a dog housed in the soft tissue and internal medicine ward. The remaining isolate generated bad sequence data for housekeeping genes and thus an ST was not assigned; the dog that

Table 4—Antimicrobial resistance phenotype, PFGE group, and ST of MDR *E coli* isolates collected at VTH admission and discharge from 15 dogs persistently colonized with the pathogen.

Dog	Admission date	Initial housing location	PFGE group		ST		Resistance phenotype	
			Admission	Discharge	Admission	Discharge	Admission	Discharge
1	3/21/2007	NCU-ICU	A	A	1047	1047	AMX-AMP-FOX-CFT-SIX	AMX-AMP-FOX-CFT-SIX
2	7/24/2007	STIM ward	M	M	83	83	AMP-KAN-SIX-TET-SMX	AMX*-AMP-KAN-SIX-TET-SMX
3	7/23/2007	NCU-ICU	E	E	288	288	AMX-AMP-FOX-CFT-CRO-CHL*-CIP-GEN*-KAN*-NAL-SIX-TET-SMX	AMX-AMP-FOX-CFT-CRO-CHL-CIP-GEN*-KAN*-NAL-SIX-TET-SMX
4	10/29/2007	STIM ward	C	C	855	855	AMX-AMP-FOX-CFT-CRO*-CHL*-CIP-NAL-TET	AMX-AMP-FOX-CFT-CRO-CIP-NAL-TET
5	1/16/2008	Orthopedic ward	B	B	722	722	AMP-CHL-SIX-TET-SMX	AMX*-AMP-CHL-SIX-TET-SMX
6	4/30/2007	STIM ward	—	—	653	171	AMX-AMP-FOX-CFT-CRO-CIP-NAL-TET	AMX-AMP-FOX-CFT-CRO-CHL-CIP-GEN-KAN-NAL-SIX-TET-SMX
7	8/8/2007	STIM ward	—	E	692	288	AMX-AMP-FOX-CFT-CRO-CHL*-CIP-KAN*-NAL-SIX-TET-SMX	AMX-AMP-FOX-CFT-CRO*-CHL*-CIP-NAL-SIX-TET-SMX
8	8/13/2007	ECC ward	—	—	NA	86	AMX*-AMP-CHL-CIP-KAN-NAL-SIX-TET-SMX	AMX-AMP-FOX-CFT-CRO-CIP-GEN-KAN-NAL-SIX-TET-SMX
9	7/12/2007	NCU-ICU	E	J	288	171	AMX-AMP-FOX-CFT-CRO-CHL-CIP-GEN-KAN-NAL-SIX-TET-SMX	AMX-AMP-FOX-CFT-CRO-CHL*-CIP-GEN*-KAN*-NAL-SIX-TET-SMX
10	8/29/2007	Orthopedic ward	—	J	171	171	AMX*-AMP-CFT-CRO-CIP-GEN-KAN*-NAL-SIX-TET-SMX	AMX*-AMP-CFT-CRO-CIP-GEN-KAN-NAL-SIX-TET-SMX
11	10/5/2007	NCU-ICU	—	E	1050	288	AMX-AMP-FOX-CFT-CRO-CIP-NAL-TET	AMX*-AMP-CHL-GEN-KAN-SIX-TET
12	10/29/2007	Orthopedic ward	E	—	288	1051	AMX*-AMP-FOX-CHL-CIP-NAL-SIX-TET-SMX	AMX*-AMP-FOX*-CHL-CIP-KAN-NAL-SIX-TET-SMX
13	1/9/2008	ECC ward	—	—	1052	160	AMP-CHL-CIP-KAN-NAL-SIX-TET-SMX	AMP-CHL-CIP-KAN-NAL-SIX-TET-SMX
14	4/30/2008	Orthopedic ward	—	—	287	1048	AMP-CHL-GEN-SIX-TET-SMX	AMP-CHL-GEN-KAN-SIX-TET-SMX
15	10/6/2008	NCU-ICU	B	J	1049	171	AMP-CHL-CIP-NAL-SIX-TET-SMX	AMP-CHL*-CIP-NAL-SIX-TET-SMX

*Intermediate resistance.
— = Not part of any identified group. AMP = Ampicillin. AMX = Amoxicillin–clavulanic acid. CRO = Ceftriaxone. CFT = Ceftiofur. CHL = Chloramphenicol. CIP = Ciprofloxacin. FOX = Cefoxitin. GEN = Gentamicin. KAN = Kanamycin. NA = Not applicable. NAL = Nalidixic acid. NCU-ICU = Nursing care unit or intensive care unit. SIX = Sulfisoxazole. SMX = Trimethoprim-sulfamethoxazole. STIM = Soft tissue and internal medicine. TET = Tetracycline.

this isolate was collected from had been housed in the ECC. Low numbers prevented analysis for associations.

Epidemiological relatedness of persistent MDR *E coli*—Two cats had persistent MDR *E coli* colonization; these were not evaluated further. Fifteen dogs were identified during the study that had persistent colonization with any MDR *E coli*. Results of PFGE analysis of 30 isolates obtained from these dogs identified 6 clone groups **(Table 4)**. In 12 samples from 8 dogs, isolates obtained at admission, discharge, or both did not match any identified PFGE clone group. The most prevalent clone identified was E, which was detected in 6 isolates (3 admission samples and 3 discharge samples) collected from 5 dogs. Presence of this clone was not significantly associated with any of the epidemiological factors investigated (data not shown). Notably, 3 of the 5 dogs that had PFGE clone E organisms isolated were initially housed in the nursing care or intensive care unit, and 2 of these had resistance or intermediate resistance to the same antimicrobials. Additionally, 5 dogs initially housed in the nursing care or intensive care unit (n = 2), soft tissue and internal medicine ward (2), or orthopedic ward (1) had the same PFGE clone isolated from the discharge sample as from the admission sample.

Multilocus sequence typing was also performed on the 30 MDR *E coli* isolates. In 1 dog, sequence obtained from the admission sample was poor and could not be categorized. For the remaining samples, 16 STs were identified **(Figure 1)**. Most commonly identified were ST288 and ST171 (6 and 5 isolates, respectively). All isolates of ST288 were part of PFGE clone group E; however, ST171 isolates were less consistently grouped by PFGE clone (3 of these belonged to group J, and 2 did not belong to any unidentified group). Isolates of ST171 were persistent in 1 dog and acquired in 3 others. Similarly, isolates of ST288 were persistent in 1 dog, acquired in 2 dogs, and replaced by isolates of other STs between admission and discharge in 2 others. The neighbor joining tree indicated 1 major clonal complex made up primarily of ST288; STs 83, 1051, 722, 692, 287, 1048, and 1050 clustered together with ST288 and had significant (99%) bootstrap support (indicating confidence in the phylogenetic grouping).

Among the 15 dogs with persistent colonization of any MDR *E coli* (Table 4), 5 had isolates of identical PFGE clone groups and STs with little or no change in antimicrobial susceptibility patterns between admission and discharge samples. These dogs were subsequently identified as persistently colonized with MDR *E coli* in multivariable analysis for risk factors associated with acquisition of these organisms. The remaining 10 dogs had acquired MDR *E coli* of different clone groups, STs, or both while hospitalized; thus, they were considered to have acquired (not persistent) colonization of MDR *E coli* for this multivariable analysis. Additionally, when assessing resistance phenotypes among these MDR *E coli* isolates, we did detect apparent differences in antimicrobial susceptibility patterns. Aside from differences in breakpoint categorization (eg, intermediate resistance vs resistance), PFGE clone E group isolates of ST288 had differences in resistance to aminoglycosides (kanamycin and gentamycin), cephalosporins (cefoxitin, ceftiofur, and ceftriaxone), and fluoroquinolones (nalidixic acid and ciprofloxacin). Isolates of ST171 had more variety, but differences in resistance were apparent for aminoglycosides and cephalosporins. Notably, isolates of ST171 from admission and discharge samples from 1 dog had similar antimicrobial resistance patterns but belonged to distinct PFGE clone groups (unidentified vs clone J for admission and discharge isolates, respectively).

Changes during hospitalization—Paired admission and discharge samples were available for 570 dogs and cats; no organisms of interest were isolated from 8. The highest proportion of animals had persistent *E coli* (441 [77.4%]) or *Enterococcus* spp (417 [73.2%]) colonization **(Table 5)**. Multidrug resistant

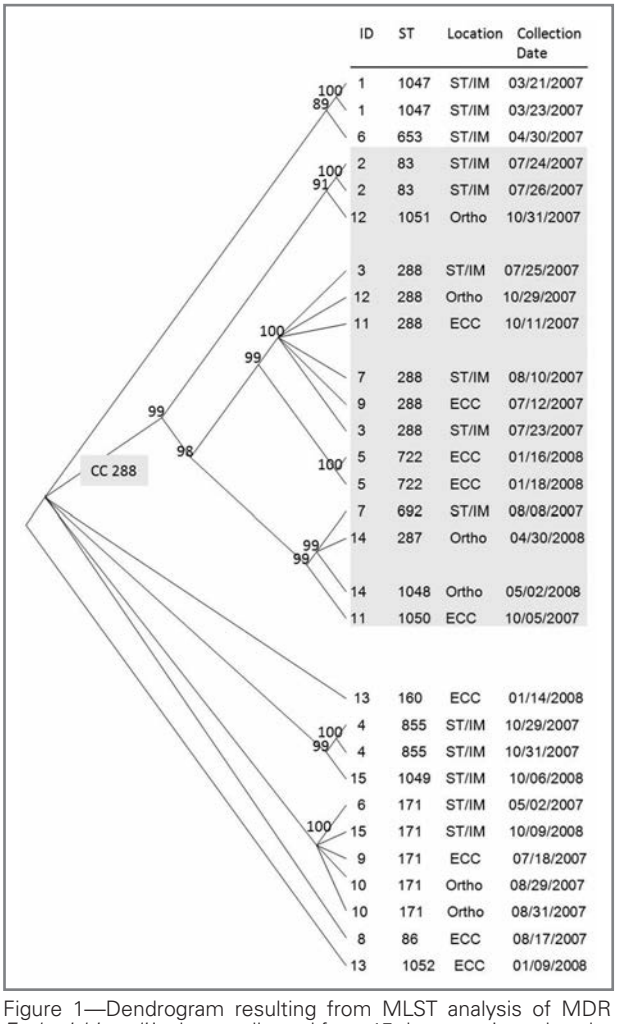

Figure 1—Dendrogram resulting from MLST analysis of MDR *Escherichia coli* isolates collected from 15 dogs persistently colonized with the pathogen. Samples were collected via nasal and rectal swabs upon admission to and discharge from a VTH on indicated dates. Dogs were admitted to 1 of 3 locations (soft tissue and internal medicine [ST/IM], orthopedic [Ortho], or ECC wards). One admission sample (ID No. 8) was excluded because of poor-quality DNA sequence. Sixteen distinct multilocus STs and 1 clonal complex (CC) are represented. Dendrogram is a consensus of 1,000 bootstrap trees generated with the neighbor-joining algorithm with use of sequence data for 7 housekeeping genes. The shaded area indicates inclusion in the CC; numbers to the left indicate percentage similarity.

Table 5—Comparison of bacteria isolated from samples collected from dogs and cats at VTH admission and discharge.

Organism	Sample		
	Admission only	Admission and discharge	Discharge only
E coli	68 (11.9)	441 (77.4)	27 (4.7)
MDR *E coli*	50 (8.8)	17 (3.0)	39 (6.8)
Staphylococcus spp	73 (12.8)	117 (20.5)	51 (8.9)
MRSA	4 (0.7)	0	7 (1.2)
MRSP	5 (0.9)	0	9 (1.6)
Enterococcus spp	50 (8.8)	417 (73.2)	67 (11.8)
VRE	2 (0.4)	1 (0.2)	3 (0.5)

Data are reported as No. (%) of animals.
Samples from 570 animals that had both admission and discharge samples collected were included in the analysis; 3 of 64 cats and 5 of 506 dogs had none of the organisms of interest isolated from either sample.

E coli of any type was acquired in 39 (6.8%) animals (35 dogs and 4 cats) and persistent in 17 (3.0%). Additionally, 7 (1.2%) animals acquired MRSA and 9 (1.6%) acquired MRSP while housed at the VTH. Detection of persistent and acquired MDR *E coli*, MRSA, MRSP, and VRE was too infrequent among samples from cats to warrant further analysis and thus multivariable analyses were performed with data from dogs only (n = 506).

Results of multivariable analysis of the incidence of acquired MDR *E coli* (ie, MDR *E coli* detected for the first time in the discharge sample on the basis of specific molecular typing; n = 45) and MRSA (7) among dogs indicated that 1 risk factor was common for both organisms: duration of stay at the VTH (**Table 6**). Dogs that were hospitalized for ≥ 3 days were 2.51 (95% CI,

Table 6—Results of multivariable logistic regression analysis for risk factors associated with recovery of MDR *E coli* or MRSA from dogs that acquired either organism during their stay at the VTH.

Variable	*P* value	OR	95% CI
Acquired MDR *E coli* between admission and discharge (n = 45)*			
Length of stay at VTH			
≥ 3 d	0.006	2.51	1.24–5.08
< 3 d	Referent	—	—
Location of initial housing			
ECC ward	0.247	0.53	0.12–2.26
Orthopedic ward	0.336	1.48	0.44–5.04
NCU-ICU	0.327	2.53	0.93–6.87
Other	Referent	—	—
Nature of admission			
Emergency	0.107	2.42	0.90–6.48
Elective	Referent	—	—
Received antimicrobials during stay			
Yes	0.173	1.45	0.70–2.99
No	Referent	—	—
Visited the oncology ward			
Yes	0.348	3.10	0.65–14.68
No	Referent	—	—
Had a cardiology consult			
Yes	0.105	2.07	0.77–5.56
No	Referent	—	—
Acquired MRSA between admission and discharge (n = 7)			
Length of stay at VTH			
≥ 3 d	0.041	15.13	1.12–205.10
< 3 d	Referent	—	—
Nature of admission			
Emergency	0.053	12.74	0.97–166.77
Elective	Referent	—	—
Animal housed solely indoors at home			
Yes	0.041	0.11	0.01–0.91
No	Referent	—	—
History of prolonged antimicrobial use†			
Yes	0.797	1.40	0.11–18.63
No	Referent	—	—
Was taking antimicrobials at admission			
Yes	0.251	3.61	0.40–32.14
No	Referent	—	—
Had taken antimicrobials in the past 10 days			
Yes	0.682	1.53	0.20–11.71
No	Referent	—	—
Visited the ECC ward			
Yes	0.072	0.44	0.07–2.75
No	Referent	—	—
Received antimicrobials during surgery			
Yes	0.378	0.14	0.02–1.19
No	Referent	—	—
Received antimicrobials during stay			
Yes	0.631	0.60	0.08–4.81
No	Referent	—	—

Data were obtained for 506 dogs that had samples collected at both admission and discharge from the VTH.
*For this analysis, colonization with MDR *E coli* was categorized as persistent if isolates of the same PFGE group and ST were present in admission and discharge samples and as acquired if the organisms were found only in the discharge sample (35) or if the PFGE group or ST differed between samples (10). †Prolonged antimicrobial use was defined as the patient having had > 1 course of antimicrobial treatment for the current condition.
— = Not applicable. NCU-ICU = Nursing care unit or intensive care unit.

1.24 to 5.08) times as likely to acquire MDR *E coli* and 15.13 (95% CI, 1.12 to 205.10) times as likely to acquire MRSA as were those hospitalized for < 3 days. Having been housed solely indoors at home was associated with a reduced risk (OR, 0.11; 95% CI, 0.01 to 0.91) of acquiring MRSA. Low incidence among dogs prevented regression analysis of VRE or persistent MDR *E coli* colonization; additionally, multivariable analysis of acquired MRSP was not possible because no independent variables remained after univariable analysis (all had *P* values > 0.2).

Discussion

Major objectives of the present study included evaluation of the antimicrobial resistance profiles of bacterial isolates obtained from dogs and cats at the time of admission to a VTH, which may be reflective of bacteria typically present in these companion animal species, and determining the incidence of and assessing risk factors for acquisition and persistent colonization of MRSA and MDR *E coli* at a VTH. We also identified common clonal groups and STs of acquired or persistent MDR *E coli* isolates and common STs of acquired MRSA isolates.

Interesting differences were detected between cats and dogs in our study. Cats were more likely to be admitted on an emergency basis and more commonly visited multiple specialty areas or had specialty consultations, compared with dogs. In the absence of a measurement of illness severity upon admission, these findings could be interpreted as indicating that cats in this study had more severe illnesses than dogs, which appeared to have been largely admitted through scheduled appointments. In another study,[6] investigators found that severity of illness was associated with colonization of MDR *E coli*. This appears to contradict our result that cats, which we inferred to have had more severe illnesses than dogs, less commonly had MDR *E coli* in admission samples; however, these differences were nonsignificant in the present study.

Other studies[9,18] have estimated antimicrobial resistance in bacteria carried by the general dog population by examination of samples obtained at the time of admission to VTHs. Although it involves the use of a convenience sample, this method allows investigators to take advantage of a good opportunity to evaluate such variables. Although < 1% of admission samples in the present study tested positive for VRE, it is notable that among enterococci, the percentage of isolates resistant to quinupristin-dalfopristin was apparently greater than that for any other antimicrobial tested, and this value increased significantly over time. Although not optimal, quinupristin-dalfopristin has been used to treat VRE infections of the bloodstream in humans.[19] Daptomycin and linezolid have been shown to be effective treatments against VRE,[19] and < 1% of all enterococci isolates in our study were resistant to these agents. We isolated MRSA in very low frequency (4/714 [0.6%]) from admission samples, which is similar to findings reported in the companion animal community.[18]

During the course of the present study, the period prevalence of staphylococcal resistance to penicillin increased significantly by 225%. Although not unexpected, this finding is noteworthy because the greatest prevalence of staphylococcal resistance to penicillin (47/122 [38.5%]) was reported in the final year of our study. A study[20] of staphylococcal isolates from humans revealed that in 2011, < 5% remained susceptible to penicillin, in stark contrast to our findings. However, in a study[21] on mastitis, other investigators found increased staphylococcal resistance to penicillin in isolates from humans, compared with isolates from cattle, suggesting possible host species–related differences.

Results of a previous study[9] of antimicrobial resistance among *E coli* isolated from rectal swab samples collected from dogs upon admission to a VTH indicated high prevalence of resistance to ampicillin (32/155 [20.6%] isolates) and amoxicillin–clavulanic acid (24/155 [15.5%] isolates). We also found that the largest proportion of *E coli* isolates with antimicrobial resistance were resistant to ampicillin. However, the proportion of *E coli* isolates resistant to cephalosporins, fluoroquinolones, β-lactams, sulfonamides, chloramphenicol, and tetracycline (as well as 1/2 aminoglycosides tested) had significant downward trends during our study period. These opposing trends are interesting findings that could have been driven by a number of factors, including the types of antimicrobials used or disinfection procedures used within the VTH during the course of our study; however, the study design did not include collection of these types of data.

Whether in a human or animal hospital, the longer a patient is hospitalized, the more opportunities exist for acquisition of a health-care–associated infection.[7–9] Likewise, in our study, staying in the VTH > 3 days was associated with increased odds of acquired MDR *E coli* or MRSA in dogs. The odds for acquisition of MRSA were lower for dogs housed solely indoors at home than for those that were not; however, the results for MRSA-associated factors must be interpreted with caution, considering that the low numbers of animals with acquired MRSA resulted in extremely wide CIs.

We expected antimicrobial administration to be associated with acquisition or persistence of antimicrobial-resistant organisms, but this was not observed. Studies at animal and human hospitals have had conflicting results in this area.[9,22] One group reported that administration and type of antimicrobials administered during a dog's stay at a VTH were not significantly associated with acquisition of *E coli*.[9] In contrast, another group found that antimicrobial treatment of hospitalized humans increased the risk of acquisition of antimicrobial-resistant, gram-negative bacteria by 4.6 to 9.9 times.[7,22] Our study did not assess duration of previous antimicrobial treatment or types of those antimicrobials; these data were collected binomially (treated vs not treated). This omission may have diluted the effect of history of prolonged antimicrobial use and should be investigated further.

Although published data show that antimicrobial usage has a role in the ability for MDR *E coli* to persist, we were unable to detect any significant associations between this variable and persistence of MDR *E coli*. One study[23] found that MDR *E coli* do not compete well with normal flora in dogs in the absence of selection

pressure caused by use of antimicrobials, but once given the opportunity to thrive (via treatment with antimicrobials), MDR *E coli* persist, despite discontinuation of treatment and the return of normal flora. The manner of our data collection may have affected our ability to detect such an effect.

We did identify a predominant clone among the 15 dogs that had persistent MDR *E coli* colonization. Although most (3/5) dogs from which this clone (PFGE clone E) was isolated had it present at admission, 2 dogs acquired PFGE clone E following admission to the VTH. It is possible that clone E represents a common strain type in circulation among the general population of companion animals and that acquisition may have been the result of transmission by another patient staying at the VTH. One study[24] demonstrated an apparently greater diversity in PFGE clones of *E coli* isolated from animals and the environment of a VTH, compared with results of the present study. The authors suggested that the lack of a predominant clone was attributable to the effects of circulating genetic elements conferring resistance, rather than specific bacteria.[24] Although our study identified PFGE clone E as predominant, despite our efforts, the time gaps among admission dates of the dogs from which clone E was isolated prevented identification of potential transmission events.

Not surprisingly, the most commonly acquired MRSA ST found in the present study has been studied previously.[25,26] Isolation of MRSA of ST5 from veterinary personnel who worked with small animals was reported, and it was suggested that this isolate was distinct from isolates obtained from large animal health-care providers.[26] Additionally, results of 1 study[25] revealed that hospital-acquired MRSA ST5 represented companion animal isolates of MRSA, whereas ST8 represented equine MRSA isolates. Further study within our VTH is warranted to determine whether MRSA of ST5 is prevalent among our health-care providers.

Multidrug resistant *E coli* isolates of ST288 and ST171 in our study had differing antimicrobial resistance phenotypes. The most common differences were found in resistance to aminoglycosides, cephalosporins, and fluoroquinolones. Resistance genes are commonly shared among *E coli* by transfer of genetic elements.[27] This may be the best explanation for differences observed in isolates from PFGE clone group E (all of which were of ST288) because plasmids or transposons would not be detected by use of the PFGE or MLST techniques. However, the antimicrobial resistance patterns observed among isolates from ST171 are more difficult to characterize. More specific molecular testing would be required to discern the true differences in resistance among these isolates.

Infection control practices can help to prevent bacterial transmission, but other factors, unique to each hospitalized patient, may confound this. Although we confirmed some previously reported associations with the acquisition of health-care–associated infections, such as duration of hospital stay, the low numbers of qualifying samples in the present study likely prevented detection of other significant associations. The results of epidemiological and molecular data analysis reported in this study provide insight about the occurrences of health-care–associated infections in dogs and cats in a VTH. Although the identified associations do not necessarily imply causality, they may provide a provisional template for additional studies.

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d. CMV1AGNF, Trek Diagnostic Systems Inc, Cleveland, Ohio.
e. American Type Culture Center (ATCC), Manassas, Va.
f. AutoReader, Trek Diagnostic Systems Inc, Cleveland, Ohio.
g. Standard Operating Procedures, No. SPECIAL.001.02. Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, Mich.
h. CHEF-DR III, Bio-Rad Laboratories, Hercules, Calif.
i. BioNumerics software, version 3.5, Applied Maths, Saint-Matins-Latem, Belgium.
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