In human health care, hospital-acquired infections increase morbidity, duration of hospitalization, and cost of treatment, often because the infective organisms are multidrug resistant. Although prevalence of hospital-acquired infections in privately owned veterinary practices remains largely unknown, results of a recent study indicated that 82% of veterinary teaching hospitals reported outbreaks of hospital-acquired infections during the past 5 years. Hospital-acquired infections can be caused by endogenous flora from animals with breached immunity or caused by exogenous bacteria originating from environmental sources.

**Objective**—To determine the prevalence of bacterial contamination on 4 surfaces of 4 types of standard equipment in small animal veterinary hospitals.

**Design**—Surveillance study.

**Sample**—10 small animal veterinary hospitals.

**Procedures**—Each hospital was visited 3 times at 4-month intervals; at each visit, a cage door, stethoscope, rectal thermometer, and mouth gag were swabbed. Swab samples were each plated onto media for culture of enterococci and organisms in the family Enterobacteriaceae. Enterococci were identified via a species-specific PCR assay and sodA gene sequencing; species of Enterobacteriaceae were identified with a biochemical test kit. Antimicrobial susceptibility was assessed via the disk diffusion method. Enterococci were screened for virulence traits and genotyped to assess clonality.

**Results**—Among the 10 hospitals, enterococci were isolated from cage doors in 7, from stethoscopes in 7, from thermometers in 6, and from mouth gags in 1; contamination with species of Enterobacteriaceae was rare. Enterococci were mainly represented by Enterococcus faecium (35.4%), Enterococcus faecalis (33.2%), and Enterococcus hirae (28.3%). Antimicrobial resistance was common in *E. faecium*, whereas virulence traits were present in 99% of *E. faecalis* isolates but not in *E. faecium* isolates. Clonal multidrug-resistant *E. faecium* was isolated from several surfaces at 1 hospital over multiple visits, whereas sporadic nonclonal contamination was detected in other hospitals.

**Conclusions and Clinical Relevance**—Contamination of surfaces in small animal veterinary hospitals with multidrug-resistant enterococci is a potential concern for pets and humans contacting these surfaces. Implementing precautions to minimize enterococcal contamination on these surfaces is recommended. (J Am Vet Med Assoc 2012;240:437–445)

Contaminated hospital surfaces and equipment can be sources of infection if they are not cleaned properly between patient uses. In human health-care settings, stethoscopes are known potential vectors of infection and thermometers have been implicated in multiple nosocomial outbreaks. Stethoscopes and thermometers may be fomites in veterinary medicine as well because in many privately owned hospitals, a single stethoscope and a single thermometer may be used for all patients examined in a given day. Microorganisms from the skin or feces of a patient could contaminate those device surfaces and be spread to another patient if proper disinfection is not performed between patient uses. Because enterococci and organisms in the family Enterobacteriaceae are often hospital-acquired pathogens, the recommendation is that the environmental contamination be minimized when possible.
Enterobacteriaceae are established components of the gastrointestinal flora of dogs and cats, fecal contamination of veterinary hospital surfaces could create an opportunity for nosocomial bacterial infections.8 The objectives of the study reported here were to determine the prevalence of surface contamination with enterococci and species of Enterobacteriaceae in 10 privately owned small animal veterinary hospitals, to assess antimicrobial resistance patterns of isolated enterococci and Enterobacteriaceae, to further characterize enterococcal isolates through virulence profiling and genotyping, and to use data collected by use of a questionnaire from veterinarians to compare cleaning protocols with bacterial contamination. It was hypothesized that cage doors, stethoscopes, thermometers, and mouth gags used in the participating hospitals would have bacterial contamination that could contribute to hospital-acquired infections.

Materials and Methods

Study design and participants—Ten privately owned small animal veterinary hospitals in Kansas whose owners gave written informed consent to participate were included in the study. The hospital owners of 20 primarily small animal hospitals in Kansas (within 2 hours’ driving distance from the authors’ university) were initially contacted by phone by the primary investigator for recruitment. Eleven hospital owners were available to discuss possible enrollment; 10 agreed to participate, and 1 declined due to hospital construction (15 miles, 2 hours’ driving distance from the authors’ university) at the time of the study period. For the purposes of the study, the hospitals were designated as hospitals 1 through 10. Each hospital was visited for sample collection 3 times at 4-month intervals. Each visit was announced. Each visit was scheduled for early morning, prior to the start of daily appointments, and the staff was asked not to change routine cleaning protocols prior to visits. A cage door, stethoscope, thermometer, and mouth gag were chosen for investigation because those items regularly come into direct contact with patients; moreover, those items can act as fomites and be sources of bacterial contamination if their surfaces are not cleaned properly between patient uses. Surfaces selected for sample collection were unknown to veterinarians or staff at the time of the study, with cage doors that were as close as possible to the exam room or treatment room by rolling the applicator across the inside surfaces of the door bars and handle. A sample was collected from a stethoscope by rolling the applicator over the diaphragm. A sample was collected from a thermometer by rolling the applicator over its entire surface. Samples were collected from the same cage door, stethoscope, thermometer, and mouth gag at each visit in each hospital. Cotton-tipped applicators bearing samples were each stored in 2 mL of PBS solution in a tube that was placed in a cooler containing an ice pack at 4°C during transit and processed on the day of sample collection.

Isolation—One hundred microliters of each 2 mL of sample-storage PBS solution was spread on 3 plates: a TSA plate, a plate with selective media for growth of enterococci,9 and a plate with selective media for organisms in the family Enterobacteriaceae. This resulted in a 20X dilution for enumeration. Additionally, 450 µL of the sample-storage PBS solution was added to 1 tube of 5 mL of trypticase soy broth, 1 tube of 5 mL of selective enrichment broth for growth of enterococci, and 1 tube of 5 mL of selective enrichment broth for gram-negative bacteria.10 Preparations containing none selective media were incubated at 28°C for 48 hours and preparations containing selective media were incubated at 37°C for 48 hours. From each individual plate, CFUs were counted manually (JVS and KML). Bacterial growth in enrichment broth was recorded as positive or negative.

Identification—Up to 30 putative enterococcal colonies/swab were isolated on selective medium and were confirmed at the genus level by use of an esculin hydrolysis test in enterococcal selective broth (4-hour incubation period at 44°C). These isolates (up to 30/swab) were stored for antimicrobial susceptibility and virulence profiling. Enterococci were identified at the species level by use of a species-specific multiplex PCR assay for Enterococcus faecium and Enterococcus faecalis or sequencing of the sodA gene, as previously described.9,10 Gram-negative bacteria were identified by use of a biochemical identification kit.11 Colonies that grew on TSBA were not further characterized.

Antimicrobial susceptibility testing—Isolates were screened for phenotypic antimicrobial susceptibility via the disk diffusion method on Mueller-Hinton agar. For enterococcal isolates, only E. faecium, E. faecalis, Enterococcus hirae, and Enterococcus avium were screened for susceptibility because these are considered the most common species to contribute to disease in dogs and cats. Standard disk sizes, as recommended by the CLSI (formerly known as the National Committee for Clinical Laboratory Standards), were used in the study. Nine antimicrobial agents were selected for testing of enterococci, including ampicillin (10 µg), doxycycline (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (120 µg), nitrofurantoin (300 µg),
quinoypristin-dalfopristin (for testing of *E. faecium* only; 15 µg), tigecycline (15 µg), and vancomycin (30 µg). Nine antimicrobials were selected for testing of gram-negative bacteria, including amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cefotaxime (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), and trimethoprim-sulfamethoxazole (30 µg). Resistance control of antimicrobial disks was performed with control strains obtained from the ATCC (*E. faecalis* ATCC 19433 and *E. faecalis* ATCC 19434). Zone diameters and interpretive breakpoints to determine susceptible, intermediate, and resistant isolates were based on CLSI guidelines for *Enterobacteriaceae* and *Enterococcus* spp for small animals when available; otherwise, guidelines for human patient isolates were used. Because there is no validated enterococcal-specific disk diffusion breakpoint for enrofloxacin, the veterinary breakpoint established for *Staphylococcus* spp was used. The breakpoint used for tigecycline was that specified by the manufacturer on the basis of a recommendation from CLSI. Multidrug resistance was defined as resistance to 3 or more antimicrobial agents, regardless of class.

**Virulence profiling**—All enterococcal isolates were evaluated for gelatinase activity on Todd-Hewitt agar with 1.5% skim milk, and the clearance zone around colonies was evaluated. Each *Enterococcus faecium* and *E. faecalis* isolates were further assessed by use of a multiplex PCR assay and a previously published protocol for the presence of genes *gelE*, *cyIA*, *asa1* (*E. faecalis* only), and esp. Briefly, each 25-µL volume of PCR mixture consisted of 1.5 µL of bacterial suspension, 0.1 µM of each of the primers specific for *asa1* and *gelE*, 0.2 µM of each of the primers specific for *cyIA* and *esp*, and 12.5 µL of PCR master mixture. An initial denaturation step at 95°C for 4 minutes was followed by 30 cycles of denaturation (94°C for 1 minute), annealing (56°C for 1 minute), and extension (72°C for 1 minute), followed by a cycle consisting of 10 minutes at 72°C. The amplicons were separated on 1.3% agarose gel.

**Genotyping**—Multiple-locus variable-number tandem repeat analysis and PFGE were performed to allow clonal analysis of enterococcal isolates within and among hospitals and over multiple visits. The MLVA typing was performed on the basis of 6 VNTR loci following a protocol described previously. In all cases, initial denaturation was at 95°C for 15 minutes and a final extension step consisted of 5 minutes at 72°C. For VNTR-1, 35 cycles (each consisting of 30 seconds at 94°C, 30 seconds at 52°C, and 30 seconds at 72°C) were performed. For VNTR-2, VNTR-8, and VNTR-9, a touchdown PCR procedure was used that included 10 cycles, each consisting of 30 seconds at 94°C, 30 seconds at 70°C decreasing to 60°C, and 30 seconds at 72°C. The annealing temperature during the first cycle was 70°C and decreased 1°C at each cycle during the next 9 cycles. During the remaining 25 cycles, an annealing temperature of 60°C was used. For VNTR-7 and VNTR-10, the initial annealing temperature was 65°C and was decreased to 55°C. Reactions were performed in 25-µL volumes with master mix. The PCR fragments were separated on 1% or 2% agarose gels with a 50-bp, 100-bp, or 1-kbp ladder as a size marker. Relationships of *E. faecium* isolates were analyzed by use of a PFGE system and the restriction enzyme *Sma*I as previously described; isolates that could not be typed by use of *Sma*I were typed by use of *Apr*I. Clonality was determined to occur when isolates had >90% similarity index.

**Statistical analysis**—Descriptive statistics were used to report results of bacterial enumeration, antimicrobial susceptibility testing, virulence profiling, and cleaning protocol data obtained from surveys. Bacterial CFUs were determined from each plate. For TSBA plates, a median CFU value for each surface from 30 samples (obtained during 3 visits to 10 hospitals) was reported. For selective medium plates, CFU values were determined for each plate and results were reported as range and median of CFU per surface where appropriate. Cleaning protocol data were summarized and described on the basis of responses from a questionnaire during the first visit from each of the 10 hospitals. The MLVA profiles obtained during the study were submitted to a central website that assigns the MLVA type and compares MLVA types from enterococcal strains worldwide. Cluster analysis of PFGE profiles was performed with a commercial software program by use of the Dice correlation coefficient and the unweighted pair group mathematical average algorithm.

**Results**

**Bacterial identification and enumeration**—Among the samples collected from the 4 surfaces (cage door, stethoscope, thermometer, and mouth gag) in the 10 small animal veterinary hospitals, bacterial growth on TSBA was common. Findings on TSBA ranged from no bacterial growth to >10⁵ CFUs for all surfaces. Over the 3 visits to each hospital (30 samples/surface), the median number of CFUs for bacterial growth on TSBA for the 4 surfaces was as follows: cage door, 1.9 × 10² CFUs; stethoscope, 1.1 × 10³ CFUs; thermometer, 0.7 × 10³ CFUs; and mouth gag, 0.4 × 10⁴ CFUs. One isolate of *Hafnia alvei* was recovered from a mouth gag during the first visit (but not subsequent visits) at hospital 10; no other organism in the family *Enterobacteriaceae* was isolated from sampled surfaces at any hospital. Fifteen isolates of *Acinetobacter baumannii* were recovered from a cage door at hospital 7 during the first visit; this organism was not isolated at follow-up visits at this hospital or from other hospitals during the study.

Enterococci were isolated from at least 1 surface at all 10 hospitals during the study. The distribution of species among the 325 enterococcal isolates was as follows: *E. faecium* (115/325 [35.4%]), *E. faecalis* (108/325 [33.2%]), *E. hirae* (92/325 [28.3%]), *Enterococcus gallinarum* (8/325 [2.5%]), and *E. avium* (2/325 [0.6%]). Enterococci were recovered from 10 of 30 cage door swabs, with 7 of 10 hospitals having enterococcal cage door contamination during at least 1 of the 3 visits. Cage doors in 3 of these hospitals were contaminated at multiple visits. Of the 10 cage door swabs with enterococcal growth, 1 cage door sample yielded positive results via enrichment only, whereas enterococcal contamination detected from the remaining 9 enterococcal-
positive samples ranged from $0.2 \times 10^2$ CFUs to $2.4 \times 10^3$ CFUs. Enterococci were recovered from 12 of 30 stethoscope swabs; 7 of the 10 hospitals had enterococcal stethoscope contamination detected during at least 1 of the 3 visits. Four hospitals had enterococcal contamination of the selected stethoscope at multiple visits. Of the 12 stethoscope swabs with enterococcal contamination, 7 (originating from 5 hospitals and detected throughout the study) had enterococci detected by enrichment only. Enterococcal contamination recovered from the remaining 5 stethoscope swabs (originating from 2 hospitals and detected at multiple visits) ranged from $0.2 \times 10^2$ CFUs to $1.9 \times 10^3$ CFUs (median, $0.4 \times 10^2$ CFUs). Enterococci were recovered from 8 of 30 thermometer swabs; 6 of the 10 hospitals had enterococcal thermometer contamination detected during at least 1 of the 3 visits. The thermometer swab from hospital 6 had enterococcal contamination at all 3 visits. One thermometer swab (from 1 hospital collected at 1 visit) yielded enterococcal growth via enrichment only, whereas the remaining 7 thermometer swabs with enterococcal growth had enterococcal contamination that ranged from $0.6 \times 10^2$ CFUs to $5.3 \times 10^2$ CFUs (median, $2.7 \times 10^2$ CFUs). The mouth gag assessed at 1 of the 10 hospitals had enterococcal contamination, which was detected by use of the enrichment technique on 2 consecutive visits.

**Antimicrobial susceptibility**—Among the 115 *E. faecium* isolates, resistance to enrofloxacin (73.0%), erythromycin (53.9%), ampicillin (51.0%), and doxycycline (42.9%) was detected (Figure 1). *Enterococcus faecalis* and *E. hirae* isolates were less often resistant. Of the 2 *E. avium* isolates, both were resistant to doxycycline and 1 was resistant to enrofloxacin. No enterococcal isolates were resistant to vancomycin or tigecycline. Sixty-one (53.0%) *E. faecium* isolates were multidrug resistant; of those isolates, 37 originated from cage doors, 20 originated from stethoscopes, and 4 originating from thermometers. One *E. faecalis* isolate obtained from a sample collected from a cage door at hospital 1 was resistant to enrofloxacin, erythromycin, and nitrofurantoin. The *A. baumannii* isolates were resistant to cefotiofur (15/15 isolates) and doxycycline (10/15), had intermediate susceptibility to enrofloxacin (11/15), and were susceptible to other antimicrobial agents tested.

**Virulence**—All 115 *E. faecium* isolates were negative genotypically for tested virulence traits, but 99 (86.1%) were weakly positive phenotypically for gelatinase. Virulence genes were common in the 108 *E. faecalis* isolates: *gelE* (107 [99.1%]), *asa1* (83 [76.9%]), *esp* (60 [55.6%]), and cyLA (28 [25.9%]). All *E. faecalis* isolates were positive (78 [72.2%]) or weakly positive (30 [27.8%]) for gelatinase. *Enterococcus hirae* (79/82 [96.3%]), *E. gallinarum* (8/8), and *E. avium* (2/2) isolates were also weakly positive for gelatinase.

**Genotyping**—Hospitals 1, 3, and 5 had contamination with *E. faecium* identified at multiple visits, and those isolates were further analyzed by genotyping. The remaining hospitals had inconsistent patterns of enterococcal contamination during the study. At the first visit to hospital 1, *E. faecium* isolates were obtained from samples collected from a cage door, stethoscope, and thermometer. These 54 isolates were determined to be clonal (assigned to MLVA type 131). Pulsed-field gel electrophoresis analysis of isolates from hospital 1 over the 3 visits revealed that the *E. faecium* detected on the stethoscope, cage door, and thermometer at the first visit were clonal with *E. faecium* detected on the stethoscope and cage door at the second visit. However, these clones were different from the *E. faecium* isolated from the stethoscope during the third visit (Figure 2). At hospital 3, *E. faecium* contamination of the stethoscope was evident at the first visit (1 isolate) and third visit (11 isolates). The isolate obtained during the first visit was untypeable by use of *Smal*; thus, PFGE was performed with *Apa*I on *E. faecium* from these 2 visits. *Enterococcus faecium* in samples collected during the first and third visits to hospital 3 were not clonal (data not shown). At hospital 3, *E. faecium* was isolated from the cage door (2 isolates) at the second visit and from the thermometer (5 isolates) at the third visit; those isolates were nonclonal on the basis of results of PFGE analysis. No evidence for clonal spread of *E. faecium* among different hospitals was found.

**Data regarding cleaning protocols**—One veterinarian at each hospital completed a questionnaire at the first visit. Five of the 10 hospitals had a written standard operating procedure for hospital cleaning. A variety of disinfectants were used by hospitals in the study, including sodium hypochlorite, quaternary ammonium compounds, chlorhexidine, and isopropyl alcohol. No analysis of the association between type of disinfectant and bacterial contamination could be made because of the small number of hospitals enrolled in the study and the large variety of disinfectants used.

Cage doors were cleaned between patient uses at 7 of 10 hospitals. Two of 10 hospitals allowed a 10-minute contact time with disinfectant on cage door surfaces; in these hospitals, no enterococcal contamination of the cage doors was detected in any samples collected.

![Figure 1](https://example.com/fig1.png) —Antimicrobial resistance among isolates of *Enterococcus faecium* (n = 115; black bars), *Enterococcus faecalis* (108; white bars), and *Enterococcus hirae* (92; gray bars) obtained from swab samples collected from the surface of a cage door, stethoscope, thermometer, and mouth gag at each of 10 small animal veterinary hospitals on 3 occasions at 4-month intervals (30 samples/visit). The same items were swabbed at each visit. AM = Ampicillin; D = Doxycycline; E = Erythromycin; ENO = Enrofloxacin; GM = Gentamicin; NF = Nitrofurantoin; Q-D = Quinupristin-dalfopristin; TGC = Tigecycline; VA = Vancomycin.
At 8 of 10 hospitals, cage doors were routinely removed for more thorough cleaning at various intervals ranging from daily to quarterly.

Of the 10 surveyed veterinarians, 5 reported almost never or never cleaning his or her stethoscope, whereas 1 cleaned it between patient uses and 2 others cleaned it daily. For each of 3 veterinarians who never cleaned his or her stethoscope, resistant enterococci were isolated from the stethoscope at 1 visit (1 hospital) or 2 visits (2 hospitals); each of 2 veterinarians who reported never cleaning his or her stethoscope had no enterococcal contamination detected from the stethoscope at any of the 3 visits. At 8 of 10 hospitals, digital thermometers were used; at hospitals 7 and 8, mercury thermometers were used. At 7 hospitals, thermometers were cleaned between patient uses; this included hospital 9 where disposable covers were used and the thermometer was cleaned between patient uses. For 1 of the 10 hospitals (hospital 7), daily cleaning of thermometers was reported, and at 2 other hospitals (hospitals 5 and 10), disposable covers were placed over digital thermometers instead of disinfection. Both hospitals that used mercury thermometers stored them in alcohol as a means of disinfection between patient uses. All 6 thermometers from which collected samples yielded enterococcal growth were digital. Of the 3 thermom-

Figure 2—Dendrogram based on results of PFGE of SmaI-digested E faecium isolated from swab samples collected from the surface of a cage door, stethoscope, thermometer, and mouth gag at each of 10 small animal veterinary hospitals on 3 occasions at 4-month intervals (30 samples/surface). The same items were swabbed at each visit. One hundred fifteen E faecium isolates were detected. The scale (percentage similarity index) indicates the level of pattern similarity; lines and brackets identify relationships between isolates, and numbers indicate percentage similarity. The surface from which each E faecium–contaminated sample was obtained, isolate strain, and hospital visit (first, second, or third) at which each sample was collected are included for each PFGE lane. Hospital (designated as 1 through 10) from which each sample was collected is indicated at the far right.
eters with which disposable covers were routinely used, 1 had no enterococcal growth at any visit (thermometer not cleaned between patient uses; hospital 10), 1 had *E faecium* (5.2 × 10^3 CFUs) resistant to doxycycline at 1 of 3 visits (thermometer not cleaned between patient uses; hospital 5), and 1 had *E faecalis* (5.3 × 10^4 CFUs) resistant to doxycycline and erythromycin at 1 of 3 visits (thermometer cleaned between patient uses; hospital 9). In 4 of 10 hospitals, mouth gags were scrubbed with a brush and then soaked in chlorhexidine solution between patient uses. Of the other hospitals, mouth gags were scrubbed but not soaked (2), soaked but not scrubbed (2), or autoclaved (1); at the remaining hospital, procedures for cleaning mouth gags were not reported. At the hospital that had an enterococcal-contaminated mouth gag, mouth gags were scrubbed with disinfectant but were not soaked in a disinfectant between patient uses.

**Discussion**

The rare isolation of organisms within the family *Enterobacteriaceae* from the various surface samples collected from small animal veterinary hospital at 3 visits (at 4-month intervals) in the present study suggested that there was either a lack of contamination initially or, more likely, that the hospital cleaning protocols successfully targeted those bacteria. These study findings are reassuring for veterinarians because members of the *Enterobacteriaceae*, including *Escherichia coli*, *Enterobacter* sp, and *Klebsiella* sp, have been implicated in hospital-acquired infections in companion animals, Hafnia alvei, the only species of *Enterobacteriaceae* isolated in the study, are commensal gastrointestinal flora that can cause opportunistic infections in people and other animals. Although nosocomial transmission of *Hafnia* organisms is a concern in human health-care settings, to our knowledge, there are no reports of hospital-acquired *Hafnia* infections in small animal patients.

*Acinetobacter baumannii* is a gram-negative bacterium, which can be a component of normal skin or oral flora in humans and dogs; however, this organism can cause opportunistic hospital-acquired infections, including urinary tract infection, respiratory tract infection, and pyoderma in humans and companion animals. *Acinetobacter baumannii* is frequently multidrug resistant, and *Acinetobacter* isolates in the present study were resistant to cefotiofur and doxycycline and had intermediate susceptibility to enrofloxacin. *Acinetobacter* spp have unique properties that allow them to persist in hospital settings, such as the ability to survive in dry conditions on particles and dust for up to 6 days and the ability to adhere to the skin of health-care staff. Transmission of *Acinetobacter* organisms may occur from patient to patient or indirectly via contact with hospital staff. Results of the present study performed at small animal veterinary hospitals and results of similar surveillance studies performed at human hospitals have indicated that surfaces of equipment commonly found in hospitals may be contaminated with *A baumannii*, aiding in transmission of hospital-acquired infections.

In veterinary medicine, until recently, *Enterococcus* spp have been considered resident bacteria of the gastrointestinal tract that rarely cause opportunistic infections and have a low capacity for harm. However, there is new evidence indicating that enterococci possess virulence traits and multidrug resistance, which allow survival of the organisms in clean hospital environments, assist with mechanisms of disease development, and result in infections that are difficult to resolve. One important virulence trait is gelatinase, a secreted bacterial protease that contributes to biofilm formation. All *E faecalis* isolates in the present study were positive or weakly positive for gelatinase, and 99% of *E faecalis* isolates possessed the *gelE* gene. This is important because gelatinase-positive enterococci can form a biofilm on surfaces, such as cage doors, that can then reduce penetration of disinfectants and promote persistent enterococcal contamination. Similarly, enterococcal biofilms could contaminate indwelling urinary catheters, leading to increased risk of hospital-acquired urinary tract infections that are difficult to treat with antimicrobials. The enterococcal surface protein (encoded by the *esp* gene) may also be associated with biofilm formation and is involved with colonization of the urinary tract. Other genes, such as the aggregation substance protein (*asA*) and cytolysin (*cylA*) genes, also provide *E faecalis* with virulence and pathogenic capacity by increasing bacterial adherence to renal tubular cells, macrophages, and extracellular matrix proteins, thereby allowing the organisms to lyse erythrocytes, leukocytes, and other gram-positive bacteria, respectively.

In the present study, 53% of 115 *E faecium* isolates collected from hospital surfaces were multidrug resistant to antimicrobial agents commonly used in veterinary medicine, including enrofloxacin, ampicillin, and doxycycline. Selection pressure in patients receiving these antimicrobial agents may result in resistant fecal enterococci, which may subsequently contaminate the veterinary hospital environment. The results of the present study are similar to the findings of a study by Jackson et al. In that study, dogs and cats brought to veterinary clinics in the United States for various reasons (eg, grooming, boarding, and patient services including neutering or other surgery, routine physical examination, vaccination, clinicopathologic analyses, and dental examinations) and stray or resident clinic animals were screened for enterococci; of the 57 *E faecium* isolates obtained from dogs, 9 (15.8%) were resistant to ciprofloxacin and 27 (47.4%) were resistant to penicillin. It is unclear why increased fluoroquinolone resistance was detected in the present study, compared with findings of the study by Jackson et al. Although previous antimicrobial use was not disclosed in that study, it was shown that healthy dogs (and cats) can be carriers of resistant enterococcal species that may be shed and contaminate environmental surfaces.

Antimicrobial-resistant *E faecium* and *E faecalis* have been implicated in hospital-acquired surgical site and urinary tract infections in veterinary medicine, and contaminated hospital surfaces may provide a source or reservoir for these infections. Enterococci are also pathogens of humans, and there is concern for potential zoonotic spread of resistant enterococci from the feces of healthy companion animals to humans. Evidently, contaminated veterinary hospital surfaces are an alter-
native source of exposure for veterinary staff to resistant enterococci. Although these are theoretical routes of transmission, the clinical importance of the enterococcal contamination detected in the present study is not known. No veterinary patient or staff member was reported ill because of hospital-acquired enterococcal infection during the study period. However, the same genotype of ampicillin-resistant *E. faecium*, identified as MLVA type 131, that was detected at hospital 1 has been isolated from human patients in The Netherlands and has been shown to belong to clonal complex-17.13,18,38 Clonal complex-17 is comprised of *E. faecium* isolates from human hospital outbreaks and clinical patients and has become a concern in human health care because of its characteristic resistance to ampicillin and fluoroquinolones.15 Further research is warranted to investigate whether a link exists between level of contamination and number of hospital-acquired infections attributable to enterococci in veterinary hospitals.

In the present study, isolate genotyping revealed that 1 hospital had repeated contamination with a moderate amount of clonal multidrug-resistant *E. faecium*, that 2 other hospitals had few nonclonal *E. faecium* isolated at multiple visits, and that the remaining hospitals had sporadic enterococcal contamination. These results suggested there may be a nidus or reservoir of an *E. faecium* clone within the hospital that had repeated contamination (hospital 1), such as a resident pet within that hospital. An alternative explanation could be a decrease in patient numbers or diversity at that hospital near the time of sample collections, perhaps allowing the 1 prevailing clone to be less diluted than those at other hospitals with a higher caseload and greater species diversity among patients. However, it was more common for hospitals to have transient contamination with various enterococcal strains, suggestive of multiple sources, such as animals treated as outpatients. Because no single *E. faecium* clone was isolated from multiple hospitals, there was no evidence for clonal spread of *E. faecium* among small animal veterinary hospitals in the area in which the present study was performed.

Although the reported cleaning protocols differed, the practices at all veterinary hospitals in the present study appeared to be highly effective in eliminating contamination with species of *Enterobacteriaceae* but were less effective against enterococci. Enterococci are hardy bacteria that can survive for 7 days on environmental surfaces, such as countertops, in healthcare settings.39 Given that enterococci were identified from the cage doors in 7 of the 10 study hospitals, increased emphasis on thorough cleaning of cage doors between patient uses is recommended for veterinarians and staff at small animal veterinary hospitals. It should be remembered that most disinfectants are deactivated by organic material, such as feces, which should be removed prior to disinfection, and that the instructions for use of most disinfectants indicate that the surface to be cleaned should remain wet with disinfectant for 10 minutes to be effective against all bacteria and viruses listed on the label. For maximum efficacy, diluted solutions of disinfectant should be prepared daily or as directed by the manufacturer. With regard to enterococcal contamination, further research is needed to determine the contact times for various disinfectants that would be optimal for elimination of the organisms, to ascertain whether cage doors require a specific type of disinfectant for effective decontamination, and to assess whether there is a benefit to removal of cage doors for more thorough cleaning.

Stethoscopes were contaminated with enterococci in 7 of 10 hospitals in the present study, and half of the 10 surveyed veterinarians reported never or almost never cleaning their stethoscopes. This finding is similar to results of a survey of emergency department personnel in a human hospital in which 13 of 43 (30%) of clinicians reported never having cleaned their stethoscope, and 45% of all responding personnel reported cleaning their stethoscope annually or never.3 Stethoscopes are considered a vector for nosocomial infections in human health-care settings, and they may act as fomites in veterinary hospitals as well.3,32 In addition to enterococcal contamination, stethoscopes in veterinary hospitals may be contaminated with *Staphylococcus* spp, as found in a recent study performed in a small animal veterinary hospital; of 100 stethoscopes examined, *Staphylococcus aureus* was detected on 7 (7%). *Staphylococcus pseudintermedius* was detected on 16 (16%), methicillin-resistant *S. aureus* was detected on 2 (2%), and methicillin-resistant *S. pseudintermedius* was detected on 5 (5%). Although it is unknown whether these bacteria originate from the veterinarians or the patients, stethoscope contamination with resistant enterococci and *Staphylococcus* spp creates a potential risk of nosocomial infections for patients as well as infection for veterinary staff using those stethoscopes. Rubbing cotton gauze soaked with propyl alcohol–based disinfectant on a stethoscope membrane for 10 seconds has been identified as the most effective method for cleaning stethoscopes, providing reduction of bacterial colonies by 99%.3

The limited extent of the present study makes interpretation of thermometer contamination difficult because both mercury and digital thermometers were used, the latter with or without disposable covers. In human hospitals, contaminated rectal thermometers have been identified as a source of nosocomial infections, especially infections with *Enterobacter cloacae*.5,7 In an in vitro experiment as part of an epidemiological outbreak investigation, it was determined that after hurried disinfection with 80% ethyl alcohol, 1 in 10 thermometers experimentally infected with *Enterobacter cloacae* remained contaminated, and rushed use of alcoholic hand rub as a means of disinfecting thermometers led to 40% failure, likely due to inadequate contact time.6 To our knowledge, no similar studies have been performed to evaluate disinfection success rates for enterococci. Although extending alcohol contact time and encouraging hand hygiene were effective in 1 outbreak among hospitalized human neonates,8 a similar outbreak in a separate hospital continued after thermometers were exposed to prolonged exposure to ethyl alcohol and resolved when the use of disposable thermometer covers was introduced.7 These findings have influenced staff in most human health-care settings to use disposable thermometer covers as a protective barrier, and this preventative measure may be useful in
In veterinary hospitals, mouth gags are used to keep a patient’s mouth open during dental procedures; thus, they have the potential to become contaminated with bacteria from the oral cavity. The design of most mouth gags (typically, wire wrapped around a central bar that acts as a spring) makes thorough cleaning tedious. The results of the present study indicated that veterinarians are successful in properly cleaning mouth gags because very little bacterial contamination was detected by use of any of the 3 growth media. Therefore, scrubbing mouth gags with a brush prior to soaking them in disinfectant is recommended, and autoclaving mouth gags does not appear necessary.

The present study was limited by a small sample size of enrolled veterinary hospitals, which prevented statistical evaluation of antimicrobial susceptibility findings, bacterial virulence data, and questionnaire results. By scheduling visits, some hospitals may have altered their cleaning protocols prior to our arrival, especially at the second and third visits when staff knew which surfaces were to be swabbed. Lack of veterinary patient-validated enterococcal-specific disk diffusion breakpoints for all antimicrobial agents was also a limitation. Use of *Staphylococcus* zone diameter breakpoints for enterococcal species in this study was a recognized limitation because those breakpoints are not available from CLSI for enterococcal species. Human-validated breakpoints for ciprofloxacin are identical for *Enterococcus* spp in other animals as well; however, validation is required. Minimum inhibition concentration determination would also have added further clinical applicability to the susceptibility results. A final limitation was that the study focused only on species of *Enterobacteriaceae* and enterococci and that isolates from TSBA plates were not further characterized.

In the present study, although contamination with gram-negative bacteria was found to be rare, antimicrobial-resistant enterococci were isolated from common surfaces at privately owned small animal veterinary hospitals. The clinical importance of this contamination and risk of nosocomial infection with or zoonotic spread of these enterococci is not known. Preventative measures such as review and update of cleaning protocols may help minimize enterococcal contamination of these and other veterinary hospital surfaces.

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Use of contrast echocardiography for quantitative and qualitative evaluation of myocardial perfusion and pulmonary transit time in healthy dogs

Serena Crosara et al

Objective—To evaluate reproducibility of ejection fraction (EF), myocardial perfusion (MP), and pulmonary transit time (PTT) measured in a group of dogs by use of contrast echocardiography and to examine safety of this method by evaluating cardiac troponin I concentrations.

Animals—6 healthy dogs.

Procedures—2 bolus injections and a constant rate infusion of contrast agent were administered IV. Echocardiographic EF was determined by use of the area-length method and was calculated with intraobserver and interobserver variability was calculated.

Results—EF was easier to determine with the ultrasonographic contrast agent. For the first and second bolus, mean ± SD PTT was 1.8 ± 0.2 seconds and 2.1 ± 0.3 seconds and normalized PTT was 3.4 ± 0.3 seconds and 3.5 ± 0.3 seconds, respectively. A coefficient of variation <15% was obtained for global MP but not for the regional MPs. No differences were detected between precontrast and postcontrast cardiac troponin I concentrations.