Dogs Should Be Included in Surveillance Programs for Vancomycin-Resistant Enterococci

Enterococci are commensal bacteria of the intestinal microbiota in humans and animals. Multidrug-resistant enterococci are among the most important pathogens responsible for nosocomial infections in humans (8). During the last decade vancomycin-resistant enterococci (VRE) have emerged as a worldwide health problem, i.e., as reservoirs of genes coding for antimicrobial resistance and as possessors of the ability to spread these resistance genes to other bacterial species (17). There are numerous reports on the presence of VRE in farm animals (2, 3). Some epidemiological studies suggest that animals carrying VRE in their gastrointestinal tract could be the source of VRE infections of humans (19). These VRE of animal origin can colonize humans, being able then to transfer their resistance genes to other intestinal bacteria of humans (3, 14). However, there are only a limited number of studies dealing with the occurrence of VRE in companion animals, even though direct contact with such animals is a recognized source of pathogenic bacteria for humans (15, 20).

The presence of fecal VRE was investigated in 87 dogs randomly selected between 1998 and 2003 among those being treated at the Animal Hospital of the School of Veterinary Medicine in Madrid, Spain. All dogs included in the study were living in households located in different neighborhoods of the metropolitan area of Madrid. Their feeding habits included homemade as well as commercial dry feed, and they were periodically vaccinated and treated for parasites. Isolation of VRE was carried out as described previously (7). Feces (5 g) were homogenized in 45 ml of sterile saline solution, and 5 ml of this suspension was transferred to 5 ml of Enterococcus broth (Difco, Sparks, Md.) supplemented with vancomycin to a final concentration of 8 \( \mu \)g/ml. Enterococcus-like colonies were subcultured to blood agar (bioMérieux, Marcy l’Etoile, France) and were further biochemically identified to species level by using the Rapid ID 32 Strep system (bioMérieux). Antimicrobial susceptibility was determined with the Sensititre system (Trek Diagnostic System, East Grinstead, United Kingdom) according to the standards set by the National Committee for Clinical Laboratory Standards (9). The antimicrobials and interpretative resistance breakpoints (in micrograms per milliliter) used were streptomycin (>2,000), gentamicin (>500), florfenicol (>16), quinupristin-dalfopristin (>4), amoxicillin (>16), penicillin (>16), ciprofloxacin (>4), trimethoprim (>16), vancomycin (>32), bacitracin (>128), and avilamycin (>16). The presence of the vancomycin resistance genes vanA, vanB, vanC1 and vanC2/C3 was investigated by using a multiplex PCR-restriction fragment length polymorphism assay (10). Briefly, a bacterial colony was suspended in 50 \( \mu \)l of a PCR mixture containing 10 pmol of each oligonucleotide primer, 1.25 U of Taq polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), 200 \( \mu \)M concentration of each deoxynucleoside triphosphate (Boehringer Mannheim, Indianapolis, Ind.), 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl\(_2\), and 5% glycerol. Bacteria were lysed at 95°C for 10 min, followed by 36 cycles of amplification (94°C for 1 min, 56°C for 1 min, and 74°C for 1 min). Ten units of MspI per milliliter and 5 \( \mu \)l of 10X restriction buffer (Promega Corp., Madison, Wis.) were added to each tube. The mixture was incubated at 37°C overnight, and the digested products were electrophoresed on a 3% NuSieve agarose gel. Enterococcus faecium B7641 (VanA; vancomycin MIC > 256 \( \mu \)g/ml; human clinical isolate [10]), Enterococcus faecalis ATCC 700802 (VanB; vancomycin MIC, 64 \( \mu \)g/ml), Enterococcus gallinarum GS (VanC1; vancomycin MIC, 4 \( \mu \)g/ml; human clinical isolate [10]) and Enterococcus casseliflavus ATCC 25788 (VanC2; vancomycin MIC, 4 \( \mu \)g/ml) were used as VRE controls.

A total of 15 VRE strains were obtained through the study. Eleven strains were identified as E. faecium (VREF) and four as E. gallinarum. Both species are usually isolated from feces of companion animals (5, 18). All VREF strains were highly resistant to vancomycin (MICs > 128 \( \mu \)g/ml) and harbored the vanA gene, which was determined by PCR. These results suggest that VanA-mediated glycopeptide resistance may be widespread in E. faecium from dogs. Further studies including more geographically and temporally diverse samples would be necessary to elucidate this point. All VREF strains were also resistant to tetracycline (MICs > 64 \( \mu \)g/ml), and 10 strains (91%) were resistant to erythromycin (MICs > 128 \( \mu \)g/ml) and bacitracin (MICs > 128 \( \mu \)g/ml). Similar levels of resistance to tetracycline and erythromycin have been reported previously (12). In addition, six strains were resistant to chloramphenicol and ciprofloxacin, five strains showed high-level resistance to streptomycin, two strains were resistant to penicillin, and one strain was resistant to quinupristin-dalfopristin. The higher prevalence of chloramphenicol-resistant strains observed in this study than of strains of E. faecium isolated from other animal species (1) could be related with the fact that chloramphenicol is still authorized for therapy use in companion animals in Spain or with coselection by the use of other antimicrobials. The 100% and 73% of the VREF strains were resistant to four and five antimicrobials, respectively. All the VREF isolates were susceptible to amoxicillin, avilamycin, florfenicol, gentamicin, and trimethoprim.

Most of the reports documenting the presence of VRE in companion animals refer to dogs living on farms where VRE were also prevalent among the other farm animals (3, 6), but very few of them allude to dogs living in urban areas (18). Ten of the eleven dogs harboring VREF did not have any known contact with farm animals, and none had previous clinical records of treatment with vancomycin. Therefore, the origin of these VRE could not be elucidated. Whatever their origin, the fact that 13% of the dogs studied harbored VREF in their feces and that most of them were resistant to some antimicrobials routinely used in veterinary medicine indicate that dogs living in urban areas may represent a significant reservoir of multiple-antimicrobial-resistant E. faecium, favoring the emergence of this microorganism as a nosocomial pathogen in veterinary medicine (4). Some studies indicate the direct transmission of VRE from farm animals to humans (14, 16). Therefore, it is feasible that dogs could also represent an important source of VRE strains to humans through direct contact with the owners or through their inhabitants, as well as being able to contribute to the horizontal spread of resistance genes among strains of animals and humans (11, 13). These
results suggest the importance of monitoring the presence of resistant enterococci and the circulation of their resistance determinants in the population of companion animals in general and of dogs in particular as an important element in the surveillance programs of VRE.

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