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Absence of VanA- and VanB-Containing Enterococci in Poultry Raised on Nonintensive Production Farms in Brazil

Diego Batista Xavier, Francisco Ernesto Moreno Bernal, and Ricardo Titze-de-Almeida*

Molecular Microbiology and Biotechnology Laboratory, University of Brasília, Brasília, Brazil

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We examined cloacal samples from poultry raised on nonintensive production farms in Brazil for the presence of vancomycin-resistant enterococci. No VanA- or VanB-containing enterococci were identified in a total of 200 cloacal swabs. The most prevalent species were *Enterococcus gallinarum* (*vanC1*; 13.0%) and *E. casseliflavus* (*vanC2/3*; 5.5%).

Vancomycin-resistant enterococci (VRE) are emerging pathogens of nosocomial and community-acquired infections (4). These bacteria also colonize healthy animals that are considered reservoirs of the bacteria and of resistance genes (2). Here we present the first surveillance study aimed at examining the presence of VRE on nonintensive poultry farms in Brazil.

In our investigation, we selected poultry farms that employ nonintensive production techniques and raise animals without antibiotic growth promoters (AGP). All farms (n = 40) were in the Federal District, which is located in the central west region of Brazil. From each poultry farm, we collected five cloacal swabs during August and September 2005, totaling 200 samples. The swabs were inoculated in BBL Enterococcosel broth (Becton Dickinson and Co., Sparks, MD) supplemented with 8 μg/ml vancomycin. This medium allows the selective isolation of species showing low-level vancomycin resistance (e.g., *Enterococcus gallinarum* and *E. casseliflavus*, with the *vanC* genotype) as well as strains with high-level vancomycin resistance that harbor the *vanA* or *vanB* genotype. After 24 to 48 h of incubation at 35°C, the positive samples were plated onto sheep blood agar plates. A total of 47 strains of gram-positive cocci with negative catalase reactions were isolated. From each of these 47 plates, 10 typical colonies were subjected to multiplex PCR. We used a carefully adjusted combination of primers to identify the following species-specific and vancomycin resistance genes in the same reaction mix, as previously described (12): $ddl_{E.\ faecalis}$, $ddl_{E.\ faecium}$, *vanC1*, *vanC2/3* (18 pmol of each primer), *vanA* (3pmol of each primer) (5), and *vanB* (1.5 pmol of each primer) (15). This method allows the simultaneous identification of the species *E. faecalis*, *E. faecium*, *E. gallinarum* (*vanC1*), and *E. casseliflavus* (*vanC2/3*) and, in addition, the vancomycin resistance genes *vanA* and *vanB*.

In the present surveillance study, *vanA*- and *vanB*-containing enterococci were absent in all 200 samples studied. Thirty-seven of the 47 isolates subjected to PCR harbored VanC-type genes. Of these, the *vanC1* and *vanC2/3* genes were present in 26 (13.0%) and 11 (5.5%) isolates, respectively. Only one isolate was positive for the $ddl_{E.\ faecalis}$ gene, and it was identified as *E. faecalis*.

Although the majority of surveillance studies with selective media for VRE have isolated mainly the intrinsically vancomycin-resistant species *E. gallinarum* and *E. casseliflavus*, they have found remarkable differences in species and resistance gene prevalence. *E. gallinarum* was prevalent in chicken litter from farms in Arkansas, which agreed with our findings from fecal samples (7). VanC-type enterococci also predominated in fecal samples recovered from chickens in the United States (11). The level of carriage was similar to that in our study (11.0%), but all of the positive samples were *E. casseliflavus* (*vanC2*), which contrasts with the predominance of *E. gallinarum* (*vanC1*; 13.0%) in our study. Another discrepant result was found in Malaysia, where *E. faecalis* (*vanA*) and *E. casseliflavus* (*vanC2*) were the most prevalent resistant species in retail market poultry samples (10). Differences in species prevalence may reflect the abilities of the enterococcal lineages to colonize the microbiota of animals raised under different environmental conditions that generate distinct evolutionary pressures.

In Brazil, few studies have investigated the presence of enterococci in animals before and after the ban of avoparcin in 1998. This AGP was used in Brazil for 10 years, but only on intensive poultry farms. Leme et al. (8) found an absence of VRE in a total of 100 samples collected from one poultry farm located in the state of São Paulo that used avoparcin as an AGP (8). The isolation was carried out without selective medium for VRE, which may have affected their results. In contrast, VRE were found contaminating poultry meat products exported by Brazil to Japan (6).

A high prevalence of VRE has been previously reported for poultry farms that used avoparcin (2). Furthermore, the ban of avoparcin dramatically reduced the VRE prevalence in various countries, as occurred in Denmark between 1995 (72.7%) and 2000 (5.8%) (1). However, Norway and New Zealand found a persistence of VRE after the ban of avoparcin, which implies that the selective pressure of this antibiotic is not a prerequisite for the maintenance of VRE in poultry farms (3, 9). Indeed, animals not treated with avoparcin, such as cats and dogs, can carry VRE (13). Here we demonstrate that poultry raised without growth-promoting antibiotics in nonintensive production systems is not colonized by VRE. These contrasting results reinforce the importance of surveillance studies aimed at un-

* Corresponding author. Mailing address: Microbiologia Molecular & Biotecnología, Universidade de Brasília, Faculdade de Agronomia e Medicina Veterinária, C. Postal 04508, CEP 70910-900 Brasília-DF, Brazil. Phone: (55) 61 33073512. Fax: (55) 61 32736593. E-mail: titze@unb.br.

derstanding enterococcal epidemiology according to the country investigated.

In most European countries, avoparcin was used in poultry as well as swine production, and in particular, swine-derived VRE may have colonized nonhospitalized persons (14). The fact that, in Brazil, avoparcin was only used in poultry and not in swine production may explain why animal-derived VRE in Brazil have not disseminated to humans in the community. How epidemiological factors determined the lack of human and nonhuman community reservoirs of VRE in Brazil remains to be investigated.

The absence of *vanA*- and *vanB*-containing enterococci found in our study implies that poultry raised through nonintensive production systems are not reservoirs from which vancomycin resistance has spread in Brazil. Further investigation would elucidate whether poultry or other food animals raised on intensive production farms from Brazil are reservoirs of VRE.

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