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Antimicrobial Susceptibilities and Phylogenetic Analyses of *Enterococcus hirae* Isolated from Broilers with Valvular Endocarditis

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SUMMARY. *Enterococcus hirae* is a zoonotic *Enterococcus* species that causes opportunistic infections in both humans and animals and can be transmitted by contact with animals or through contaminated food. The aim of this study was to investigate the importance of *E. hirae* in broilers with endocarditis, as well as the antimicrobial resistance patterns and genetic relatedness of the isolates. A total of 477 three- to five-week-old broilers were studied during five fattening periods on a farm with mortality due to endocarditis. Endocarditis was observed in 27 chickens (5.66%), and samples were taken for pathological, microbiological, and molecular studies. Lesions were mainly found in the right atrioventricular valve and corresponded with a fibrinous endocarditis. *Enterococcus hirae* was identified in all cases. Pulsed-field gel electrophoresis results showed clonality among some isolates, with one pulsotype harboring 11 isolates that were found throughout the study. Most of the isolates showed multi-drug-resistant phenotypes. These results confirm that *E. hirae* is a significant cause of endocarditis in broilers, and suggest that broilers may be important carriers of antimicrobial-resistant *E. hirae* that might enter into the food chain.

RESUMEN. Susceptibilidad antimicrobiana y análisis filogenético de *Enterococcus hirae* aislados de pollos de engorde con endocarditis valvular.

Enterococcus hirae es una especie zoonótica de enterococo que provoca infecciones oportunistas en el hombre y en los animales y que puede transmitirse mediante el contacto con animales o a través de alimentos contaminados. El objetivo de este estudio fue la investigación de la importancia de *E. hirae* en pollos de engorde con endocarditis, así como el estudio de sus patrones de resistencia antimicrobiana y la relación genética entre los aislados. Se estudiaron 477 pollos de engorde de tres a cinco semanas de edad, durante cinco periodos de engorde, en una granja con historial de muertes por endocarditis. Se detectó endocarditis en 27 pollos (5.66%) y se recolectaron muestras para estudios histopatológicos, microbiológicos y moleculares. Las lesiones se observaron principalmente en la válvula atrioventricular derecha, correspondiendo con una endocarditis fibrinosa. En todos los casos se identificó *E. hirae*. Mediante electroforesis en gel de campo con pulsaciones se detectó clonalidad en algunos aislados, con once aislados agrupados en un pulsotipo, los cuales fueron detectados a lo largo de todo el estudio. La mayoría de los aislados presentaban fenotipos multirresistentes a varios antibióticos. Estos resultados confirman que *E. hirae* es una causa importante de endocarditis en pollos de engorde y que estos pueden ser portadores importantes de cepas multirresistentes de *E. hirae*, las cuales podrían entrar en la cadena alimentaria.

Key words: *Enterococcus hirae*, broilers, endocarditis, PCR, multi-drug-resistant phenotype, pulsed-field gel electrophoresis (PFGE)

Abbreviations: A = ampicillin; EDTA = ethylenediaminetetraacetic acid; G = gentamycin; I = intermediate; PFGE = pulsed-field gel electrophoresis; PHS = pulmonary hypertension syndrome; SDS = sudden death syndrome; UPGMA = unweighted pair-group method with arithmetic mean; R = resistant; S = susceptible

Enterococci are ubiquitous microorganisms present in the gastrointestinal tract of humans and animals, and in natural environments (40). *Enterococcus* species can also cause opportunistic infections in humans and animals and currently are considered one of the most significant causes of nosocomial infections in humans and important transmitters of antimicrobial resistance genes (28). *Enterococcus faecium* and *Enterococcus faecalis* are the species most frequently detected in humans and domestic animals; however, the importance of unusual pathogenic species such as *Enterococcus avium*, *Enterococcus hirae*, *Enterococcus cecorum*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, or *Enterococcus durans* is unclear because they can be misidentified by standard identification techniques (4,8). However, the use of more sensitive techniques is allowing more descriptions of these species (2).

Enterococcus hirae is currently considered an emerging enterococcal species (40) with zoonotic potential (35), and it is increasingly associated with several diseases in humans, such as septicemia, endocarditis, and urinary tract infections (2,5,33). In domestic animals, *E. hirae* can be a commensal bacterium (24) or cause a variety of diseases (11,25,44). In poultry, it has mainly been associated with septicemia, endocarditis, encephalitis, and skeletal problems (3,40,43).

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