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BRIEF REPORT

Multidrug and vancomycin-resistant *Enterococcus* isolates in different productive stages of a poultry farm

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KEYWORDS
Vancomycin-resistant *Enterococcus*;
Poultry industry;
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Multidrug-resistance

Abstract The poultry industry represents an important economic sector in Argentina. In this study we recovered 26 vancomycin-resistant *Enterococcus* (VRE) isolates from different productive stages of a poultry farm located in Tandil, Argentina. Ten isolates were *Enterococcus faecium* and seven, *Enterococcus faecalis*. Total resistance to vancomycin (96.2%), erythromycin (80.8%), levofloxacin (57.7%), chloramphenicol (26.7%), penicillin (23.1%), ampicillin (7.7%) was detected and 20 isolates (76.9%) were identified as multidrug-resistant (MDR). With respect to the distribution of glycopeptide resistance genes, 57.7% of the isolates harbored the *vanC-1* gene, and 11.5%, carried the *vanC-2/C-3* gene. The *vanA* and *vanB* variants were not detected. This study provides evidence that healthy chickens from the studied region can be a reservoir of MDR–VRE. Further surveillance should be conducted to control their dissemination through the food chain.

PALABRAS CLAVE
Enterococcus resistente a vancomicina;
Industria avícola;
Genotipos *van*;
Multirresistencia

Enterococcus multirresistentes y resistentes a vancomicina en diferentes etapas productivas de una granja avícola

Resumen La industria avícola representa un sector económico importante en Argentina. En el presente estudio se obtuvieron 26 aislamientos de *Enterococcus* resistentes a vancomicina (ERV) en diferentes etapas productivas de una granja avícola ubicada en Tandil, Argentina. De los aislamientos obtenidos, 10 correspondieron a *Enterococcus faecium* y 7 a *Enterococcus faecalis*. Se detectó resistencia a vancomicina (96,2%), eritromicina (80,8%), levofloxacina (57,7%),

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cloranfenicol (26,7%), penicilina (23,1%) y ampicilina (7,7%). Veinte aislamientos (76,9%) fueron multirresistentes (MDR). En cuanto a la distribución de los genes de resistencia a glicopéptidos, el 57,7% de los aislamientos presentó el gen *vanC-1* y el 11,5% presentó *vanC-2/C-3*. No se detectaron las variantes *vanA* ni *vanB*. Este estudio aporta evidencia de que los pollos sanos de la región estudiada pueden ser reservorios de ERV MDR y destaca la necesidad de continuar con la vigilancia para controlar su diseminación a través de la cadena alimentaria.

Enterococcus, mainly *E. faecium* and *E. faecalis*, represents one of the main agents causing nosocomial infections. This genus exhibits intrinsic resistance to common antibiotics and a plastic genome which allows it to readily acquire resistance to further antibiotics, such as vancomycin, either through mutation or by horizontal transference of genetic elements[5]. Despite their low virulence, resistance to antimicrobials and adaptability have enabled enterococci to transition from intestinal commensals to major causes of healthcare-associated infections[11].

The WHO has identified the most critical antimicrobial-resistant bacteria worldwide, which must be monitored due to the urgent need for new treatments to combat them. Vancomycin-resistant *Enterococcus* (VRE) is classified as a high priority pathogen (Group 2)[14]. Vancomycin is considered a last-resort antibiotic for treating infections caused by Gram-positive bacteria. Resistance is mediated by *van* genes, with *vanA* and *vanB* located on transposons and capable of being horizontally transferred, while *vanC* and *vanD* are chromosomal and apparently not transferred in that way[7].

Enterococcus contributes to the spread of antimicrobial resistance. Antibiotic resistance genes present in mobile genetic elements of *Enterococcus* isolates of animal origin can be transmitted to *Enterococcus* isolates of human origin[10]. The poultry industry is one of the most crucial food producers globally, and Argentina is among the top 10 poultry meat producers[9]. The emergence of VRE in food production systems has been attributed not only to the hospital use of glycopeptides but also to the use of avoparcin as a growth promoter in animals[1].

This study aimed to isolate VRE from a poultry farm located in Tandil, Pampean region, Argentina, to determine which isolates were *E. faecium*/*E. faecalis* and to investigate the antimicrobial susceptibility profiles and the glycopeptide resistance genotypes. Thirty samples obtained in 2022 in a previous study[8] from a conventional farm raising broiler chickens, with a slaughter room, were selected. Originally, the samples were grown in LB medium and stored at −80 °C with the addition of glycerol. Samples from cloacal swabs of 40-day-old and 30-day-old broiler chickens, barn beds, poultry drinker surfaces, balanced feed, carcasses and slaughter room surfaces were incubated in glucose azide broth with shaking (120 rpm) at 37 °C for 18 h. An aliquot of bacterial cultures was inoculated into bile esculin azide (BEA) agar (Britania, Argentina) supplemented with vancomycin (6 μg/ml) at 37 °C for 48 h. Presumptive colonies that grew on the selective medium were confirmed as *Enterococcus* spp. by esculin hydrolysis and catalase tests, and Gram stain. Identification of *E. faecium* and *E. faecalis* and distribution of glycopeptide resistance genes were performed by PCR, according to Dutka-Malen et al.[4], with modifications. Additionally, biochemical tests, such as PYR (L-pyrrolidonyl arylamidase) test, arabinose, mannitol, and sorbitol fermentation, were performed on isolates in which the species could not be defined by PCR. The isolates were tested for susceptibility to six antibiotics representing five antimicrobial classes, using the disk diffusion method, according to the CLSI[2] guidelines (Supplementary Table 1). The antibiotics were ampicillin (AMP, 10 μg), penicillin (PEN, 10 U), vancomycin (VAN, 30 μg), erythromycin (ERY, 15 μg), chloramphenicol (CHL, 30 μg), and levofloxacin (LVX, 5 μg). All samples analyzed showed growth on BEA agar supplemented with vancomycin and 30 isolates suspected of exhibiting antimicrobial resistance (AMR) to vancomycin were obtained.

Twenty-six isolates were confirmed as *Enterococcus* spp. (Catalase-negative/Gram-positive diplococci that hydrolyzed esculin), of which 10 were *E. faecium*, 7 *E. faecalis*, 7 *E. avium*, and 2 *E. durans*. The largest number of isolates and the greatest specific diversity (*E. avium*, *E. durans*, *E. faecalis*, *E. faecium*) were found in the slaughter room (Table 1). Total resistance to VAN (96.2%), ERY (80.8%), LVX (57.7%), CHL (26.9%), PEN (23.1%), AMP (7.7%) was detected among the 26 *Enterococcus* isolates (Table 2). Taking into account resistant and intermediate isolates, 15 AMR profiles were observed. VAN−ERY−LVX was the most predominant profile (19.2%). Twenty isolates (76.9%) were multidrug-resistant (MDR), showing resistance to three or more antimicrobial classes. One *E. faecium* isolate obtained from a slaughter room utensil (#12) was resistant to all tested antimicrobial agents (Table 1). With regard to the distribution of glycopeptide resistance genes, 57.7% of the isolates analyzed harbored the *vanC-1* gene, and 11.5%, carried the *vanC-2/C-3* (Table 1). *VanA* and *vanB* variants were not detected. Isolates from henhouse 1 showed the greatest diversity of *van* genotypes (Table 3). It can be hypothesized that the same strain may be distributed at different stages of production in some cases, for example, *E. faecalis* isolates #15 and #20 from henhouses 2 and 1, respectively (AMR profile: AMN-S/PEN-S/VAN-R/ERY-R/CHL-S/LVX-S, without *van* genes). However, this could only be confirmed after apply-

Table 1 Origin and antimicrobial susceptibility of vancomycin-resistant *Enterococcus* (VRE) isolates obtained from a poultry farm in Argentina.

Isolate	Identification	Source	Production phase	Antimicrobial susceptibility profile					*van* genotype
				AMN	PEN	VAN	ERY	CHL	LVX	
1	*E. faecalis*	Equipment	Henhouse 2	S	S	R	R	S	I	*vanC-1*
2	*E. faecalis*	Table		S	S	R	R	S	S	*vanC-1*
3	*E. faecalis*	Equipment		S	S	R	I	S	S	*vanC-1*
4	*E. faecium*	Utensil		S	S	R	S	S	S	*vanC-1*
5	*E. faecium*	Carcasses	Slaughter room	S	S	R	S	S	S	*vanC-1*
6	*E. avium*	Carcasses		S	S	R	R	S	I	*vanC-1*
7	*E. avium*	Utensil		S	S	R	R	S	I	*vanC-1*
8	*E. avium*	Carcasses		S	S	R	S	S	I	–
9	*E. faecium*	Carcasses		S	S	R	R	S	I	*vanC-1*
10	*E. durans*	Carcasses		R	R	I	R	S	R	–
11	*E. durans*	Carcasses		S	S	R	S	S	R	–
12	*E. faecium*	Utensil		R	R	R	R	R	R	*vanC-1*
13	*E. faecium*	40-Day-old broiler chicken		S	S	R	R	S	R	*vanC-1*
14	*E. faecium*	40-Day-old broiler chicken		S	R	R	R	S	R	*vanC-1*
15	*E. faecalis*	40-Day-old broiler chicken		S	S	R	R	S	R	–
16	*E. faecalis*	40-Day-old broiler chicken		S	S	R	R	I	R	–
17	*E. faecalis*	Barn bed		S	S	R	R	R	R	*vanC-2/C-3*
18	*E. faecium*	Balanced feed		S	S	R	R	R	R	–
19	*E. avium*	30-Day-old broiler chicken	Henhouse 1	S	S	R	R	R	R	–
20	*E. faecalis*	30-Day-old broiler chicken		S	S	R	R	S	R	–
21	*E. avium*	30-Day-old broiler chicken		S	S	R	R	S	R	–
22	*E. faecium*	30-Day-old broiler chicken		S	R	R	R	R	R	*vanC-1*
23	*E. faecium*	30-Day-old broiler chicken		S	S	R	R	S	R	*vanC-1*, *vanC-2/C-3*
24	*E. faecium*	Poultry drinker surface		S	R	R	R	R	R	*vanC-1*
25	*E. avium*	Balanced feed		S	R	R	R	R	S	*vanC-2/C-3*
26	*E. avium*	Barn bed		S	S	R	R	I	S	*vanC-1*

R, resistant; I, intermediate; S, susceptible by the disk diffusion test: AMN (ampicillin), PEN (penicillin), VAN (vancomycin), ERY (erythromycin), CHL (chloramphenicol), LVX (levofloxacin).

Table 2 Antibiotic susceptibility percentages of vancomycin-resistant *Enterococcus* (VRE) isolates obtained from a poultry farm in Argentina.

Antibiotic	Susceptible n (%)	Intermediate	Resistant
Ampicillin	24 (92.3)	–	2 (7.7)
Penicillin	20 (76.9)	–	6 (23.1)
Vancomycin	–	1 (3.8)	25 (96.2)
Erythromycin	4 (15.4)	1 (3.8)	21 (80.8)
Chloramphenicol	17 (65.4)	2 (7.7)	7 (26.9)
Levofloxacin	6 (23.1)	5 (19.2)	15 (57.7)

Table 3 Distribution of *van* genotypes in vancomycin-resistant *Enterococcus* (VRE) isolates obtained from a poultry farm in Argentina according to the production phase.

	n (%)
Henhouse 1 isolates	
vanC-1	3 (11.5)
vanC-2/C-3	1 (3.8)
vanC-1, vanC-2/C-3	1 (3.8)
Without detected *van* genes	3 (11.5)
Henhouse 2 isolates	
vanC-1	2 (7.7)
vanC-2/C-3	1 (3.8)
Without detected *van* genes	3 (11.5)
Slaughter room isolates	
vanC-1	9 (34.6)
Without detected *van* genes	3 (11.5)

ing some subtyping method. Although future studies that include the identification of sequence types (ST) will be necessary to determine the lineages and study the epidemiology, the information provided here on antimicrobial resistance profiles and *van* genotypes show genetic diversity among VRE strains circulating in the analyzed farm.

In Argentina, Delpech et al.[3] isolated *E. faecalis* and *E. faecium* with high levels of AMR to glycopeptides from chicken feces. In the present study, most of the isolates showed phenotypic total resistance to vancomycin and were MDR–ERV. Moreover, it was determined that of the isolates analyzed, 57.7% carried the gene *vanC-1* and 11.5%, *vanC-2/C-3*. These genes, unlike *vanA* and *vanB*, would be associated with low levels of resistance to vancomycin[6]. In China, Yu et al.[15] found *E. faecalis* in poultry production, but did not detect *vanA* or *vanB* genes. In contrast, Osman et al.[12] found vancomycin resistance genes (*vanA*, *vanB*, *vanC*) in *Enterococcus* obtained from chickens in Egypt. Regarding local epidemiology, the *vanA* gene was detected in *E. faecium* strains previously obtained from Argentinian chickens[3] and, vancomycin-resistant *E. faecium* strains harboring either *vanA* or *vanB* were recovered from patients with invasive infections (2010–2014) at the Public Hospital of Tandil[13].

Although conclusions should be interpreted with caution due to the limited number of samples studied, our results suggest that healthy chickens from the studied region may serve as a reservoir of MDR–VRE and this information is a call for further surveillance in the poultry sector. MDR *Enterococcus* represents a therapeutic threat to the community, and controlling its spread from poultry to humans is crucial[10]. The presence of a *van* gene pool in chickens, as observed in this study, and the colonization of humans by glycopeptide-resistant strains highlight the need to control this reservoir so that these glycopeptide-resistant strains that colonize the chicken intestine are not selected and spread through the poultry production chain into the community. The emergence of resistance is a global concern for human and animal health. For this reason, coordinated action between both sectors is essential to preserve the therapeutic efficacy of treatments used for human infections in the future.

CRediT authorship contribution statement

Juliana González: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. *Constanza Pifano García*: Validation, Investigation, Formal analysis. *Juliana Cantón*: Investigation, Writing – review & editing. *María José Izaguirre*: Investigation, Writing – review & editing. María Soledad Ríos: Investigation, Writing – review & editing. *Andrea Mariel Sanso*: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.ram.2025.10.001.

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