INTRODUCTION
The emergence of antimicrobial resistance is a priority for human health. In this sense, the detection of reservoirs of potentially pathogenic bacteria with transferable resistance mechanisms that may spread from the environment to man is an important challenge. In animal husbandry, antimicrobials are heavily used for prevention and treatment of bacterial diseases; but also as growth promoters in farm animals, such as poultry. Enterococci are recognized as part of the indigenous microbiota of animal and human gastrointestinal tract. In fecal samples from farm animals, such as poultry, resistance to antimicrobials used in humans (e.g. aminoglycosides, glycopeptides) has been observed.

Enterococci can acquire high-level gentamicin resistance (HLGR) determinants by horizontal gene transfer. Their expression result in the loss of synergistic bactericidal effect achieved with cell wall-active agents, posing a serious therapeutic problem for invasive infections such as enterococcal endocarditis.

Among enterococci with HLGR, \textit{E. faecalis} is the most prevalent gene, which encodes a bifunctional enzyme, AAC(6')-APH(2''). That confers resistance to aminoglycosides, except for streptomycin. Other monofunctional chromosomal [e.g. \textit{aph}(2'')] and plasmidic [e.g. \textit{aph}(2'')-\textit{Ic}] genes also encode gentamicin resistance.

Glycopeptide resistance in enterococci is mediated by van genes. Expression of vanA gene leads to inducible high-level vancomycin resistance (Minimum Inhibitory Concentration, MIC > 64 \mu g/mL) and teicoplanin (MIC > 16 \mu g/mL) resistance.

In farm animals, such as poultry, \textit{E. faecalis} are antimicrobial resistance determinants' reservoirs (HLGR, glycopeptide). In addition, there is evidence supporting the feasibility of genetic determinants transfer between poultry and human \textit{E. faecalis}. In Argentina, however, there is no available information in this regard.

The aim of this study was to investigate HLGR and vancomycin resistance determinants in fecal poultry enterococci isolated from farms located at a region in Argentina.

Material and methods
Fecal samples (N = 120) were randomly collected from two poultry farms (GPT-A; GPT-B) in Tandil district, Buenos Aires Province (Argentina), from March to September 2015. GPT-A: a 9,000 broilers/year average production. GPT-B: a 7,000 broilers/year average production.

Samples (0.5 g) were added to 5 mL of Azide-Glucose broth. Cultures at 35 \degree C for 18 h in Bile-Escolin Azide agar (BEA) were carried out. Black pigmented colonies were selected for characterization at species level. Genotypic confirmation by PCR for \textit{tuf} and \textit{ddl} genes (Table 1) was performed.

Resistance to vancomycin, teicoplanin, gentamicin and streptomycin was investigated. MIC was determined by the agar dilution method. Quality control strains: \textit{E. faecalis} ATCC 29212 and \textit{E. faecalis} ATCC 51299.

Detection of glycopeptide resistance (\textit{vanA}, \textit{vanB}) and HLGR [\textit{aac} (6')-\textit{le-aph} (2'')-\textit{Ia}, \textit{aph} (2'')-\textit{Ib}, \textit{aph} (2'')-\textit{Ic}] genes was performed by PCR (Table 1).

\begin{table} [H]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Gene} & \textbf{Primers (sequence 5' to 3')} & \textbf{Reference} \\
\hline
\textit{Tuf} & TACTGACAAACCATCCTATGGT AACCCTGCACCAACGGGAAAC & 20 \\
\textit{ddl}_{\textit{E. faecalis}} & ATCAAGTACATAGTCT AGCTTAAAGCTTACAG & 21 \\
\textit{ddl}_{\textit{E. faecalis}} & TGAAGACATIGTACATGGC TCAGAATGCTCACAATC & 20, 22 \\
\textit{vanA} & GGGAAAGCCAGATGTC GTAAGTCGCGGCGCGT & 20 \\
\textit{vanB} & ATGGGGCAAGCGATAGTC GATTTCGTCCTCGACC & 20 \\
\hline
\end{tabular}
\caption{Primers employed in PCR.}
\end{table}
RESULTS

Enterococci were isolated from 80% (961/120) of the poultry fecal samples. Phenotypic and genotypic characterization showed that E. faecalis (n = 105) was the predominant enterococcal species found (52.5%), followed by E. faecium (n = 70).

In 19% (20/105) of poultry E. faecalis (FLS-29, FLS-38, FLS-59, FLS-77, FLS-81, FLS-84, FLS-90, FLS-91, FLS-100, FLS-103, FLS-110, FLS-115, FLS-122, FLS-128, FLS-138, FLS-146, FLS-150, FLS-153, FLS-164, FLS-171), HLGR was observed (MIC\textsubscript{gentamicin} = 512-1,024 µg/mL). In high-level gentamicin resistant E. faecalis (100%), presence of aac(6\prime)-le-aph(2\prime\prime)-la gene was confirmed by PCR (Fig. 1a).

High-level streptomycin resistance (HLSR) was not detected in E. faecalis isolates (MIC\textsubscript{streptomycin} = < 2,000 µg/mL). Glycopeptide resistant E. faecalis were not recovered from any of the fecal samples analyzed in this study (MIC\textsubscript{vancomycin} = < 4 µg/mL; MIC\textsubscript{teicoplanin} = < 8 µg/mL).

Vancomycin and teicoplanin resistant E. faecium (7.1%, 5/70) were found (Table 2). In all cases, VanA phenotype was observed (MIC\textsubscript{vancomycin} = 64-1,024 µg/mL; MIC\textsubscript{teicoplanin} = 64-512 µg/mL). Molecular analysis confirmed vanA genotype in all glycopeptide resistant E. faecium (Fig. 1b). Glycopeptide resistant E. faecium (FCM-70) showed HLGR (20%) and HLSR (40%; FCM-08, FCM-62). In all HLGR E. faecium isolates, aac(6\prime)-le-aph(2\prime\prime)-la gene was detected.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Genotype</th>
<th>MIC\textsubscript{vancomycin}</th>
<th>MIC\textsubscript{teicoplanin}</th>
<th>HLGR genotypes</th>
<th>MIC\textsubscript{vancomycin}</th>
<th>MIC\textsubscript{teicoplanin}</th>
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<tr>
<td>FCM-70</td>
<td>vanA</td>
<td>64</td>
<td>64</td>
<td>aac(6\prime)-le-aph(2\prime\prime)-la</td>
<td>2,048</td>
<td>256</td>
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<td>FCM-93</td>
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<td>FCM-97</td>
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<td>512</td>
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<td>256</td>
<td>2,048</td>
</tr>
</tbody>
</table>

Table 2. Glycopeptide and high-level aminoglycoside resistance in poultry E. faecium.


DISCUSSION

Over the last years, E. faecalis showed a dualistic behavior. Biotechnological and immunomodulatory properties have been proven in several strains. However, nowadays, is considered as one of the most prevalent health-care associated infections pathogens.

In our investigation, E. faecalis was the most frequent isolated enterococci. In previous reports, it was the most (46.1%) or the least (11.5%) prevalent recovered species from poultry.

In this study, high-level gentamicin resistant E. faecalis (19%) were recovered and aac(6\prime)-le-aph(2\prime\prime)-la gene was detected. A low prevalence of enterococci harboring aac(6\prime)-le-aph(2\prime\prime)-la gene (2.7%) was observed for Australian poultry. Likewise, aac(6\prime)-le-aph(2\prime\prime)-la gene was detected as the only HLGR determinant in E. faecalis isolated from poultry in Korea (10.9%). In all cases, high-level gentamicin resistant enterococci showed co-expression of HLSR. Interestingly, the most frequent HLGR gene detected in human enterococci is aac(6\prime)-le-aph(2\prime\prime)-la.

Recently, our group reported the presence of HLGR in E. faecalis from food of animal origin, as well as in vivo horizontal transfer of this resistance between food and human enterococci. Therefore, horizontal transfer of resistance determinants between enterococci from different origin could not be ruled out as source of antimicrobial resistance.

In Argentina, in human enterococci, antimicrobial resistance has been studied. E. faecalis has been reported as the most prevalent enterococcal species expressing HLGR in isolates recovered from hospitalized patients.

Vancomycin-resistant E. faecalis were not recovered during this investigation. In 2007, vancomycin resistance was found in 20% of poultry enterococci from Slovakia, mainly in E. faecalis. Chan et al. also found aac(6\prime)-le-aph(2\prime\prime)-la and vanA genes in E. faecalis (4%) recovered from Korean poultry. In Europe, previous studies have shown the relevance of these genetic determinants of HLGR and glycopeptide resistance in human enterococci.

Nowadays, E. faecium is also considered another enterococcal species with clinical relevance. In addition, previous reports also found that E. faecium was the second commonest enterococcal isolates.

HLGR was observed in vancomycin-resistant E. faecium (20%). In poultry enterococcal isolates from China, aac(6\prime)-aph(2\prime\prime) gene was detected in E. faecium although HLGR was not confirmed. Furthermore, in Australian range-meat chickens and indoor-meat chickens, it was reported the recovery of gentamicin-resistant E. faecium.

Along this investigation, glycopeptide-resistant E. faecium were recovered (7.1%). VanA phenotype is encoded by gene vanA, typically carried in plasmid-harborred transposons. The most frequent glycopeptide resistance genotype in clinical enterococci is vanA. Nowadays, there is an increasing concern about vanA plasmid-mediated transfer to methicillin-resistant Staphylococcus aureus. In Chinese poultry, lower vancomycin resistance prevalence (1.3%) was detected for E. faecium, found in poultry drinking water. Recently, in Argentina, on the contrary, VanA E. faecium have been recovered from food of animal origin.

In a local nation-wide survey about resistance in clinical enterococci most of the E. faecium strains carried vanA gene (98%), expressing HLGR (77.2%) and high-level streptomycin resistance (95.8%) as well.

In countries like the United States and in the European Union the use of antimicrobials in husbandry is documented. Aminoglycosides (gentamicin) are employed in poultry.
However, glycopeptides, such as vancomycin, were banned due to its linkage with spread of vancomycin-resistant enterococci. After the ban it was observed a significant decrease of glycopeptide resistance in Dutch poultry isolates. However, in Argentina, the lack of consistent information about antimicrobial use in poultry husbandry does not allow to rule out this practice as a source of glycopeptide or aminoglycoside resistance in poultry enterococci. It is important to note that in the two studied farms, antimicrobials were frequently administered.

This study reports the presence of horizontally transferable antimicrobial resistance determinants (HGLR, vanA) in E. faecalis and E. faecium recovered from poultry in the Province of Buenos Aires, Argentina. The existence of these enterococcal reservoirs in farm animals such as poultry should be taken into account as a potential source of antimicrobial resistance genes, strengthening their potential spread to humans through the food chain.

REFERENCES