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# Antimicrobial susceptibility of *Enterococcus* strains isolated from poultry faeces

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**Abstract**

We have investigated the resistance of *Enterococcus* isolated from poultry faeces to antibiotics commonly used as therapy of enterococcal infections. Identification was made by the method of Facklam and Collins. Minimal inhibitory concentrations of penicillin, ampicillin, vancomycin and teicoplanin were determined and high level aminoglycoside resistance was investigated. Genes codifying high level aminoglycoside resistance (HLAR) were determined by PCR. Fifty five *Enterococcus* strains were isolated (63.6% *E. faecalis,* 12.7% *E. mundtii,* 9.1% *E. faecium,* 7.3% *E. casseliflavus,* 3.7% *E. durans* and 3.6% *E. hirae*). None of the strains were resistant to VAN, TEC, P or AM. HLAR was found in 34.5% of strains for SM, 27.3% for KM and 7.3% for GM. The gene for the bifunctional enzyme was found only in one strain, that showed HLAR to GM and KM. Fourteen strains harboured the gene *aph*(3′)-III, being 11 resistant to KM and STR, and three resistant to GM, KM and STR. The remaining six strains showed HLAR to STR, but were negative for the three genes tested by PCR. The gene *ant*(4′4″) was not detected in any of the strains. No unexpected vancomycin resistance was detected. The resistance rates among poultry strains were lower than those found among human strains isolated from hospital patients in recent Canary studies.


*Keywords:* *Enterococcus*; Antimicrobial resistance; Glycopeptides; Aminoglycosides; Poultry

## 1. Introduction

Poultry are increasingly being associated with carriage of multiresistant bacteria that may be responsible of infection in humans. The use of antimicrobials as growth promoters in poultry has been related with the dissemination of resistant *Enterococcus* strains (Bager et al., 1997; Descheemaeker et al., 1999; Robredo et al., 1999, 2000; Joseph et al., 2001; Van den Bogaard et al., 2002). Several studies suggest that poultry products could be a source of vancomycin resistant *Enterococcus* (VRE) in humans through the food chain (Bager et al., 1997; Wegener et al., 1997; Robredo et al., 2000). Traditionally, enterococcal infections are treated with penicillins, usually in combination with an aminoglycoside. Glycopeptides, specially vancomycin, are often used to treat enterococcal infections in patients with hypersensitivity to penicillins or in infections due to β-lactam resistant enterococci (Gold, 2001). The increasing prevalence of *Enterococcus* strains with high level penicillin and aminoglycoside resistance, together with the emergence of vancomycin resistance highly limited the therapeutical options. Prevalence of VRE in human strains was 15.4% among isolates from US in 1997 (Murray, 2000). In Spain, Cisterna et al. (1999) found a prevalence of VRE of 1.8% in a study that did not include Canary Islands. In hospitalised patients from

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Canary Islands, prevalence of VRE was found to be 0.7% (Pérez-Hernández et al., 2002).

The aim of this study was to investigate the resistance of *Enterococcus* strains isolated from poultry to the antibiotics commonly used as therapy of enterococcal infections.

## 2. Materials and methods

### 2.1. Bacterial strains

Samples of fresh poultry faeces were obtained from 100 animals from 5 different farms. Samples were processed within 2 h from collection. One fraction (0.5 g) of each sample was inoculated in KF broth (Difco, Mi., USA) with 6 mg/L of vancomycin (Dista SA, Madrid) and when the medium turned yellow, 100 μL were plated onto mEnterococcus Agar (Difco, MI, USA) with 6 mg/L of vancomycin. The rest of the sample was directly plated onto mEnterococcus Agar (bioMérieux) without antibiotic.

### 2.2. Biochemical identification

Catalase test and Gram stain were made to presumptive *Enterococcus* growing on plates with or without antibiotic. Gram positive catalase negative cocci were identified by the method of Facklam and Collins (1989), with slight modifications (Facklam et al., 1999). Briefly, the following tests were made: tolerance to bile-esculin, growth in 6.5% NaCl, tolerance to 0.04% telurite, deamination of arginine, motility, pigmentation, use of pyruvate, acid formation in methyl α-D-glucopyranoside and several carbohydrate fermentation tests (1% mannitol, sorbitol, sorbose, arabinose, sucrose, raffinose and lactose). The following strains from Colección Española de Cultivos Tipo (Spanish Type Culture Collection, CECT) were used as controls: *E. avium* CECT 968, *E. durans* CECT 411, *E. hirae* CECT 279, *E. gallinarum* CECT 970, *E. casseliflavus* CECT 969, *E. faecium* CECT 410, *E. malodoratus* CECT 971.

### 2.3. Antibiotic susceptibility tests

Minimal inhibitory concentrations (MICs) were carried out on Mueller–Hinton agar (Difco, MI, USA) containing double dilutions of the antibiotics (NCCLS, 1999). Concentrations from 0.06 to 256 mg/L were used for penicillin (P, Laboratorios Normon, Madrid), ampicillin (AM, Laboratorios Normon), vancomycin (VAN, Dista SA) and teicoplanin (TEC, Marion Merrell Down SA). Concentrations used for aminoglycosides ranged from 0.06 to 32768 mg/L. High level resistance against streptomycin (SM, Laboratorios Normon), gentamicin (GM, Laboratorios Normon) and kanamycin (KM, Sigma) was defined as a MIC of $>2000$, $\geqslant 500$ and $>2000$ mg/L, respectively, following NCCLS guidelines (M31-A). Only one bacterial colony per plate was tested.

*E. faecalis* ATCC 51299 (HLRG) and ATCC 29212 (susceptible to GM) were used as control strains.

### 2.4. PCR amplification

We used a previous described PCR protocol for the simultaneous detection of the genes *ant(4′4″)*, *aph(3′)*-III and *aac(6′)/aph(2″)* (Van de Klundert and Vliegenthart, 1993). Strains were grown overnight in 2 ml of BHI broth. The pellet from 1.5 mL of the overnight culture was resuspended in 500 μL of distilled water. The cell suspension was heated for 10 min at 94 °C (heating block) and it was then centrifugated for 5 min at 16,000*g*. One μL of the DNA-containing supernatant was used as template in a final volume of 25 μL of PCR mixture [10 mM Tris–HCl (pH 9.6), 50 mM NaCl, 2.5 mM $MgCl_2$, 0.02% (wt/vol) bovine serum albumin, 300 μM each deoxyribonucleoside triphosphate, 100 ng of each primer, 0.75 U of *Taq* polymerase (Bioline, UK)]. A negative control without DNA templated was included in the assay. Clinical strains harbouring the genes cited above were used as positive controls. DNA amplification was carried out in a TECNE thermocycler with the following thermal cycling profile: an initial denaturation step at 94 °C for 3 min was followed by 32 cycles of 30 s at 94 °C, 45 s at 60 °C and 2 min at 72 °C. The mixture was cooled and stored at 4 °C. PCR products were resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide.

## 3. Results

From 100 faecal samples, 55 *Enterococcus* strains were isolated, being 63.6% *E. faecalis,* 12.7% *E. mundtii,* 9.1% *E. faecium,* 7.3% *E. casseliflavus,* 3.7% *E. durans* and 3.6% *E. hirae* .

None of the strains were resistant to VAN, TEC, P or AM. MICs ranges, $MIC_{50}$ and $MIC_{90}$ values are summarized in Table 1. Teicoplanin showed greater intrinsic activity than vancomycin (Fig. 1), but penicillin and amipicillin had similar intrinsic activity (Fig. 2).

High level aminoglycoside resistance (HLAR) was found in 34.5% of strains for SM, 27.3% for KM and 7.3% for GM. Four different antibiotic susceptibility patterns were found for HLAR strains: $SM^R$, $SM^R$ $KM^R$, $SM^R$ $KM^R$ $GM^R$and $GM^R$ $KM^R$, with percentages of 10.9%, 20.0%, 5.5% and 1.8%, respectively. None of the *E. mundtii*, *E. hirae* or *E. durans* strains showed HLAR. Among *E. faecalis* strains, 40.0% showed HLAR, being 11.4% $SM^R$, 20.0% $SM^R$ $KM^R$,