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RESEARCH ARTICLE

**Xiomara Pérez-Hernández · Sebastián Méndez-Álvarez
Teresa Delgado · Antonio Moreno
Jose Antonio Reyes-Darias · Antonio Sierra López
Jesús Villar · Agustín González
Antonio Manuel Martín Sánchez · Manuel Macía
Félix Claverie-Martín**

Low prevalence of vancomycin-resistant enterococci in clinical samples from hospitalized patients of the Canary Islands, Spain

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Abstract Over the last decade vancomycin-resistant enterococci (VRE) have emerged as nosocomial pathogens. The aim of this study was to determine the prevalence of VRE in clinical samples from hospitalized patients in the Canary Islands. From April to November 2000, 437 enterococci were isolated from patients hospitalized at the four main health care centers in those islands. Identification to the species level was performed with the GPS-TA (Vitek 1) or the Wider I system. A PCR assay was used to determine the genotype of glycopeptide resistance (*vanA*, *vanB*, *vanC1*, and *vanC2*/*C3* genes). Only three (0.7%) VRE were detected: one *vanA Enterococcus faecalis*, and two *vanC1 Enterococcus gallinarum*. To our knowledge, this is the first VRE study carried out in the Canary Islands hospitals, and the results showed a low prevalence of VRE.

Keywords *Enterococcus* · Prevalence · Glycopeptide · Antibiotic resistance

X. Pérez-Hernández · S. Méndez-Álvarez · J.A. Reyes-Darias
J. Villar · M. Macía · F. Claverie-Martín (✉)
Research Unit,
Hospital Universitario Ntra. Sra. de Candelaria,
38010 Santa Cruz de Tenerife, Spain
E-mail: fclamar@gobiernodecanarias.org
Tel.: +34-922-600675
Fax: +34-922-600562

S. Méndez-Álvarez
Department of Cellular Biology and Microbiology,
University of La Laguna,
La Laguna, Spain

M. Macía
Nephrology Service,
Hospital Universitario Ntra. Sra. de Candelaria,
La Laguna, La Laguna, Spain

T. Delgado · A. Sierra López
Microbiology Service,
Canarian University Hospital,
Santa Cruz de Tenerife, Spain

A. Moreno
Microbiology Service,
Hospital Universitario Ntra. Sra. de Candelaria,
Santa Cruz de Tenerife, Spain

A. González
Microbiology Service,
Hospital Dr. Negrín,
Las Palmas de Gran Canaria, Spain

A.M. Martín Sánchez
Microbiology Service,
Hospital Insular,
Las Palmas de Gran Canaria, Spain

Present address: X. Pérez-Hernández
Unidad de Investigación,
Hospital Dr. Negrín,
Las Palmas de Gran Canaria, Spain

Introduction

Vancomycin-resistant enterococci (VRE) have emerged as a major cause of nosocomial infections [2, 13, 14, 21]. Since their initial isolation from patients in the United Kingdom and France, VRE infections have been increasingly detected throughout the world [23]. These bacteria are often resistant to multiple antibiotics, thus limiting the number of therapeutic options available to the physician [9]. Among isolates from United States hospitals, resistance to vancomycin rose from 0.3% in 1989 to 15.4% in 1997 [5, 14]. In contrast, the prevalence of VRE in European hospitals is low, being 1.8% in Spain [3, 22].

Vancomycin is a member of a class of antibiotics referred to as glycopeptides. These antibiotics interfere with peptidoglycan biosynthesis by binding to the terminal D-alanyl-D-alanine residue of the peptidoglycan precursor [23]. Seven glycopeptide resistance genotypes have been described in enterococci [2, 12, 13]. Five of these (*vanA*, *vanB*, *vanD*, *vanE* and *vanG*) are acquired mechanisms and the other two (*vanC1* and *vanC2*/*C3*)

are intrinsic properties. The *vanA* and *vanB* resistance genotypes are the most commonly found. The *vanA* gene cluster encodes proteins that confer high-level resistance to both vancomycin and teicoplanin [1]. The expression of *vanA* genes is induced by either vancomycin or teicoplanin. The *vanB* genes confer resistance to various concentrations of vancomycin but not teicoplanin [6, 13], and are induced only by vancomycin and not by teicoplanin. The VanA phenotype is distributed worldwide, and is by far the predominant type of vancomycin resistance reported in Europe [20]. Although VanA is the predominant type, VanB strains are fairly common in the Unites States [2]. The *vanC1* and *vanC2/C3* genes are specific to the motile VRE species *Enterococcus gallinarum* and *E. casseliflavus/E. flavescens*, respectively [4]. The other genotypes, *vanD*, *vanE* and *vanG*, have been detected in only a few enterococcal strains [8, 12, 16, 19].

In this report, we provide data on the prevalence of VRE in clinical samples from hospitalized patients in the Canary Islands. These islands, which constitute an autonomous region of Spain, are located in the Atlantic ocean off the coast of North-West Africa, and have a population of approximately 1,700,000. In our hospitals, vancomycin is frequently prescribed due to the high prevalence of methicillin-resistant *Staphylococcus aureus* [18]. To our knowledge, this is the first study on VRE carried out in the Canary islands.

Materials and methods

Patients, bacterial isolates and reference strains

The four main hospitals in the Canary Islands are 500- to 900-bed health care centers, two of which are located in the island of Tenerife (Nuestra Señora de Candelaria University Hospital and Canarian University Hospital) and the other two in the island of Gran Canaria (Dr. Negrín Hospital and Insular Hospital). These hospitals provide tertiary care for most of the population of the islands. During a 9-month period (April–November 2000), we studied 406 enterococcal clinical isolates collected from patients hospitalized at the four hospitals. A single specimen was obtained from each subject. The isolations, approximately 100 per hospital, were carried out by the Microbiology Service of each hospital. The isolates from clinical samples came from urine (173), wounds (118), blood (46), catheter tips (29), respiratory tract (13), and other clinical materials (27). A total of 31 colonizers, isolated from rectal cultures in adults, were also included in the study. The following reference strains were used: *E. faecium* BM4147 (*van*A), *E. faecalis* V583 (*van*B), *E. gallinarum* BM4174 (*van*C*1*), *E. casseliflavus* ATCC 25788 (*vanC2*), and *E. faecalis* ATCC 29212 as a glycopeptide-susceptible control. *S. aureus* ATCC 29213 was used as a negative control.

Phenotypic identification and susceptibility testing

Bacterial isolates were processed by agar bile-esculin-vancomycin (6 μg/ml) screening to detect enterococcal colonies and vancomycin resistance. Isolates were identified to the species level with the GPS-TA (Vitek 1) system (bioMérieux, Marcy l'Etoile, France) except those from the Hospital Insular (Las Palmas de Gran Canaria), which were identified with the Wider I System (Dade Microscan, West Sacramento, Calif.). The minimal inhibitory concentrations (MIC) of vancomycin and teicoplanin for the resistant isolates were determined by broth microdilution according to National Committee for Clinical Microbiology Standards guidelines [15]; both antibiotics were tested in the range 0.25–128 μg/ml. VRE were also tested with the E-test method (AB Biodisk, Solna, Sweden) according to the manufacturer's specifications. Biochemical identification of enterococci was performed according to standard laboratory criteria [7].

PCR amplification

We used a previously described PCR protocol for the simultaneous detection of the most common vancomycin-resistance genotypes (*van*A*, vanB, vanC1* and *vanC2/C3*) and identification of *Enterococcus* isolates at the genus level [17]. Strains were grown overnight at 37°C on blood sheep agar plates (bioMérieux, Marcy l'Etoile, France). Three to five colonies of each sample were scraped from the surface of the agar and resuspended in 1 ml of sterile distilled water. The cell suspension was heated to 100°C for 15 min and was then centrifuged at 15,000 *g* for 10 min. The DNA-containing supernatant was used as a template. An aliquot of 20 μl of the supernatant was added to 80 μl of a PCR mixture consisting of 1× reaction buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8], 0.2 mM of each deoxyribonucleotide triphosphate (Promega, Madison, Wis.), 2.5 mM $MgCl_2$, 50 pmol of each primer, and 1.2 U *Taq* DNA polymerase (Bioline, UK). For each sample, the PCR reaction was performed with the *van*A, *van*B, *van*C1, *van*C2/C3 and Ent pairs of primers (Roche, Mannheim, Germany) in a single tube [4, 10, 17]. A negative control without DNA template was included in the assay. DNA amplification was carried out in a GeneAmp PCR system 9700 thermocycler (PE Applied Biosystems, Calif.) with the following thermal cycling profile: an initial denaturation step at 94°C for 2 min was followed by 25 cycles of amplification (94°C for 60 s, 55°C for 60 s, and 72°C for 60 s), and an extension at 72°C for 5 min. PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. A 100-bp DNA ladder (Roche, Mannheim, Germany) was run in each gel, and the VRE genotype was determined by the size of the amplified product.

Results and discussion

A total of 437 enterococci isolates were obtained from clinical samples in the four main hospitals of the Canary Islands. Table 1 shows their phenotypic identification. *E. faecium* was the most abundant species (86.2%). The two *E. gallinarum* isolates had been initially identified as

Table 1. Enterococcal isolates and vancomycin resistance genotypes

Species	No. (%) of isolates	No. of vancomycin-resistant enterococci (VRE)	Resistance genotype (%)
Enterococcus faecalis	377 (86.3)	1	*vanA* (0.2)
E. faecium	52 (11.9)	0	–
E. avium	4 (0.9)	0	–
E. durans	2 (0.5)	0	–
E. gallinarum	2 (0.5)	2	*vanC1* (0.5)
Total	437 (100)	3	*van* (0.7)

E. faecium and *Enterococcus* sp. with the automated system. Further biochemical and motility tests identified them as *E. gallinarum*. Three VRE (0.7%) were detected: one *E. faecalis* (UIE-478, isolated from blood of a cancer patient who had had vancomycin treatment) and the two *E. gallinarum* (UIE-298 and UIE-470, isolated from a new-born ear infection and a post-surgery abscess, respectively). The automated system detected high-level resistant isolates (VanA phenotype), but not low-level resistant isolates (VanC phenotype). Using the agar dilution method, *E. faecalis* UIE-478 showed high-level resistance to vancomycin (MIC >128 μg/ml) and to teicoplanin (MIC >128 μg/ml), whereas *E. gallinarum* UIE-470 and *E. gallinarum* UIE-298 showed low-level vancomycin resistance (MIC $=8$ μg/ml) and teicoplanin (MIC $=1$ μg/ml) susceptibility. By the E-test method, *E. faecalis* UIE-478 had MICs of >256 μg/ml for vancomycin and 128 μg/ml for teicoplanin; *E. gallinarum* UIE-470 had MICs of 8 μg/ml for vancomycin and 0.38 μg/ml for teicoplanin; and *E. gallinarum* UIE-298 had MICs of 4 μg/ml for vancomycin and 0.25 μg/ml for teicoplanin. The VanA isolate, *E. faecalis* UIE-478, was sensitive to penicillin-G, high concentrations of gentamicin, and high levels of streptomycin, and therefore did not represent a problem during treatment.

The results of the PCR assay with the 437 enterococcal isolates were in accordance with the phenotypic characterization. Three VRE were detected (Table 1): one *vanA* isolate, *E. faecalis* UIE-478, and two *vanC1* isolates, *E. gallinarum* UIE-298 and UIE-470. The PCR results obtained with reference strains *E. faecium* BM4147 (*van*A) and *E. gallinarum* BM4174 (*vanC1*) were as expected (not shown). The rest of the clinical isolates were all negative for the *vanA*, *vanB*, *vanC1* and *vanC2*/*C3* genes (results not shown). The *vanB* gene was not detected in any of the isolates.

In conclusion, this is the first study of VRE isolated from clinical samples from hospitalized patients in the Canary Islands, and the results show a low prevalence (0.7%) of VRE (0.2% for *vanA* gene and 0.5% for *vanC1* gene). This prevalence was lower than that reported in other European countries and in the United States [2, 11, 14, 22, 23]. Results of a recent VRE study in Europe have shown that the United Kingdom has the highest prevalence (2.9%), while other European countries have prevalence rates of 1% or lower. In Spain, a multicenter study that did not include the Canary Islands showed that the rate of resistance to glycopeptides was 1.8% [3]. *E. gallinarum* and *E. casseliflavus* isolates are not always taken into account because their resistance to glycopeptides is intrinsic and their pathogenesis is very low. In our study, if we take out the two vanC1 *E. gallinarum*, the prevalence of VRE would be 0.2%, which is even lower than previously reported levels [22].

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