FIRST ISOLATES OF *Enterococcus faecium* VANB IN ARGENTINA: TWO CASES REPORT

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ABSTRACT

The vancomycin-resistant enterococci have emerged as important nosocomial pathogens. The first *Enterococcus faecium* vancomycin resistant (VREfm) in Argentina was reported in 1997. Since then, nosocomial outbreaks of VREfm have been reported, all of them due to *E. faecium* phenotype VanA. We describe two cases *E. faecium* VanB recovered from ambulatory patients admitted in our hospital in July of 2000. They are the first VREfm VanB isolated in Argentina.

CASE 1. A 30 years old woman with a relapsed cystic node in mamma. She referred fat injection in breasts seven years before. By sonnogram, an inflammatory process was demonstrated. The mammography revealed imagines suitable with infected necrosis. Needle aspiration of the node was performed, and VREfm was isolated from the culture.

CASE 2. A 23 years old woman who suffered pain and inflammation in external genitals. Sample from the vulva was obtained by punction. From the bacteriological study, VREfm was isolated.

Both cultures were monomicrobials and agreeing with the Gram stain of the samples. The patients were not of high –risk, have not received antibiotic previously and were assisted in different services. Susceptibility test by agar dilution method (NCCLS, M7-A5) was performed. Both strains were susceptible to teicoplanin (TEI), ampicilin (AMP), gentamicin (GEN), streptomycin (STR), intermediate to chloramphenicol (CMP) and resistant to vancomycin (VAN), tetracycline (TET), erythromycin (ERY) and ciprofloxacin (CIP).

PCR was performed to confirm the *vanB* genotype. PFGE was made to the isolates in order to establish the relationship between them and the fingerprinting revealed that the strains were identical. No further VanB strains were isolated in the hospital

INTRODUCTION

Enterococci (EN) are part of the normal flora of the gastrointestinal and female tract, however, they are associated with both community and hospital acquired infections. EN have emerged as significant nosocomial pathogens because of their natural resistance to a vast array of antimicrobial drugs and their ability to develop resistance to virtually every antimicrobial agent. EN were the first clinical important Gram positive cocci to acquire vancomycin resistance.

There are six recognized phenotypes of vancomycin–resistant enterococci (VRE): VanA, VanB, VanC, VanD, VanE, and VanG. VanA and VanB phenotypes were described primarily in *Enterococcus faecalis* and *E. faecium* and account the majority of VRE isolations. Most of VRE are recovered from hospitalized patients in intensive care units, oncology, renal or surgical units. The risk of VRE infection or colonization has been associated with previous multi-antimicrobial therapy, severe underlying disease or inmmunosuppression and long term hospitalization. Because all that reasons, VRE are strongly associated to nosocomial outbreaks.

The first isolate of *E. faecium* vancomycin resistant (VREfm) in Argentina was reported in 1997. Since then, nosocomial outbreaks of VREfm have been reported, all of them due to VREfm VanA.

We describe two cases of VREfm VanB recovered from ambulatory patients admitted in our hospital in july of 2000. They are the first VREfm VanB isolated in Argentina.

CASE 1. A 30 years old woman with a relapsed cystic node in mamma was admitted to the gynecology unit. She referred fat injection in breasts seven years before. The cystic node was 4 cm wide and appeared to be an infected haematoma. The previous diagnosis was inflammatory tumor. By echography, an inflammatory process was demonstrated. The mammography revealed an image suitable with infected necrosis. Needle aspiration of the node was performed for bacteriological studies and searching of neoplasic cells. No malignant cells were found. From the culture, VREfm was isolated. Empirical treatment with gentamicin during ten days was instalated prior to the antimicrobial susceptibility test results. After the therapy, the node was removed by surgery. The patient had a successful recovery.

CASE 2. A 23 years old woman, who suffered pain and inflammation of external genitals, was admitted to the emergency unit. Sample obtained by punction of the vulva was cultured. VREfm was isolated from the bacteriological study. None previous records of the patient were found and she did not return for further treatment and control. Both cultures were monomicrobials and agreeing with the Gram stains of the samples.

METHODS

@ BIOQUIMICAL IDENTIFICATION:

The strains were identified by conventional biochemical tests and the GPI card (VITEK, Bio-Merieux).

@ SUSCEPTIBILITY TESTS:

Disk diffusion, agar dilution, epsilometer test (E- Test, AB Biodisk, Solna, Sweden) and the GPS-102 card (VITEK, Bio-Merieux) were performed. Minimal inhibitory concentrations (MICs) were determinated by agar dilution to vancomycin (VAN), teicoplanin (TEI), ampicilin (AMP), gentamicin (GEN), streptomycin (STR), chloramphenicol (CMP), tetracyclin (TET), eritromycin (ERY) and ciprofloxacin (CIP). NCCLS guidelines were followed for medium and bacterial inoculum preparations as well as for the results interpretation. To confirm the vancomycin- resistance, an in-house prepared agar screen was inoculated (brain heart infusion agar supplemented with 6 ug/ml vancomycin). The plates were incubated for 24 hours. The reference strains *Enterococcus faecalis* ATCC 29212 and 51299 were used as control.

@ GENOTYPE ANALYSIS:

PCR: the presence of *vanB* gene was confirmed by PCR, using specific primers in standard conditions. *E. faecium* TX2405 was used as positive control.

PFGE: to establish the relationship between the strains, enterococcal genomic DNA was prepared and digested with *Sma* I (GIBCO BRL), as previously described (De [illegible] 1989 [illegible] Drug, Kyst. 5:113-129). DNA fragments were separated in 0.8% agarose using a [illegible]

Susceptibility Tests

Genotype Analysis

RESULTS

- Disk diffusion: both strains showed 13 mm zone diameter to vancomycin and 20 mm to teicoplanin and were resistant to high-level gentamicin and streptomycin.
- Automated VITEK system, E-Test and agar-screen results demonstrated vancomycin resistance.
- MICs: both isolates were resistant to VAN, TET, ERY, and CIP, intermediate to CMP and susceptible to TEI, AMP and GEN. The strains should be considered susceptible to high level STR according to NCCLS break points, nevertheless , by disk diffusion screenig test for STR (300ug), were resistant (Table 1)

	VAN	TEI	AMP	GEN	STR	CMP	TET	ERY	CIP	PCR	PFGE
CASE 1 (2619)	32 (R)	0.12 (S)	8 (S)	8 (S)	1024 (S)	16 (I)	64 (R)	≥1024 (R)	4 (R)	*van* B (+)	A
CASE 2 (2620)	32 (R)	0.25 (S)	8 (S)	8 (S)	1024 (S)	16 (I)	64 (R)	≥1024 (R)	4 (R)	*van* B (+)	A

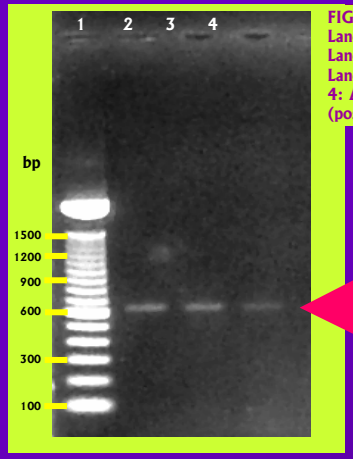

FIGURE 1. PCR to *vanB* gene.
Lane 1: ladder 100 bp;
Lane 2: *E. faecium* 2119
Lane 3: *E. faecium* 2120 Lane 4: *E. faecium* TX2405 (positive control).

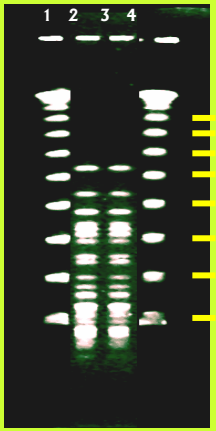

FIGURE 2. PFGE-*Sma*I
Lane 1 and 4: PFGE I ladder
Lane 2: *E. faecium* 2119 Lane 3: *E. faecium* 2120

- The *vanB* genotype was confirmed to both strains by PCR (Figure 1).
- The fingerprinting of the strains, digested with *Sma* I, revealed that they were identical (Figure 2)

CONCLUDING REMARKS

Since VRE were first reported, their importance as nosocomial pathogens have continued to increase worldwide. In the United States, VRE colonization or infection is related mainly to hospitalized high-risk patients or persons in contact with hospital environment. European studies provide evidence that VRE (commonly *E. faecium*) are wide spread in the community and farm animals, where avoparcin is used as feed supplement. It seems to be possible that colonized persons could carry the VRE from the community to the hospitals.

In Argentina, the cases reported prior these corresponded to the high-risk profile patient.

In the cases here described, VREfm were recovered from ambulatory patients, that were not of high- risk, have not received antibiotic previously and were assisted in different services.

None relationship between them could be found. Nevertheless, as the PFGE pattern of the strains were identical, the nosocomial source of the VREfm can not be discard. However, no further VREfm VanB were isolated in the hospital. The cases reported here, as well as the colonization among non hospitalized persons, suggest that the efforts to prevent the spread of VREfm, should be not only focused in hospitalized high-risk patients but also in the community.