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FIRST REPORT OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECALIS* IN UBERABA, MINAS GERAIS STATE

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ABSTRACT

In this study we report the first isolation of VanA-type vancomycin-resistant *Enterococcus faecalis* strains from two different patients hospitalized in the same intensive care unit at the hospital of Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brazil.

Key words: vancomycin resistance; *Enterococcus faecalis*; *van*A genotyping

Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens worldwide in the last two decades, particularly in USA (3). In Brazil, the frequency of VRE isolation in hospitals has also increasing significantly, since the first report in 1998 (6).

Glycopeptide resistance in enterococci is associated with a variety of phenotypes but VanA-type VRE is the most prevalent in Brazil (9). VRE strains with this phenotype express high-level resistance to vancomycin and teicoplanin and harbored a mobile genetic element Tn*1546* that carries *vanA* gene cluster responsible by glycopeptide resistance (5).

In this study we report the first isolation of VanA-type VRE strains from two different patients hospitalized in the ICU at the hospital of the Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brazil.

The first VRE strain was isolated on March 11th, 2006, from an abdominal aorta catheter of a patient who underwent abdominal aortic aneurysmectomy. This patient, a 74-year-old male, was admitted to the hospital on March 5th, 2006, for the surgery. Postoperatively, he stayed in ICU for three days and was treated prophylactically with cephalothin. One day after left the ICU, he died by broncoaspiration. The second VRE strain was isolated on April 3rd, 2006, from a tracheal secretion of a 60-year-old male who was hospitalized since January, 2006, with pneumonia. In February, as the patient developed urinary tract infection and septic shock, he was transferred to ICU. He remained at ICU for fifty-nine days. This patient recovered and left the hospital at the end of April. Prior to isolation of VRE, the patient had been treated with ciprofloxacin, ampicillin/sulbactam, cefepim, imipenem/cilastin and vancomycin. These two patients were hospitalized in the same room in the ICU-adult for a period of three days in common.

The isolates were identified to species level by conventional biochemical tests (8). Minimum inhibitory concentration (MIC) for vancomycin was determined by broth

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dilution test and susceptibility to ampicillin, penicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamycin, streptomycin, tetracycline and teicoplanin was performed by disk diffusion method (4). *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used as controls.

Genomic DNA of the microorganisms was extracted by mechanical disruption of cells (1). Polymerase chain reaction (PCR) was performed in order to confirm phenotypic species level identification (7) and multiplex PCR to determine the *van* genotype (13). Control strains were *Enterococcus casseliflavus* NCTC 12361, *E. faecium* NCTC 7171, *E. faecalis* NCTC 775, *E. gallinarum* NCTC 12359, and *E. faecium* BM 4147 *vanA* genotype.

Tn*1546*-like elements and *vanRSHAX* regions were amplified by Long-PCR (L-PCR) according to Palepou *et al.* (10) using the Long PCR Enzyme Mix (Fermentas, Glen Burnie, MD, USA). First, a single primer (P1) complementary to the inverted repeats regions flanking the transposon was used for amplified Tn*1546*-like elements. Subsequently, the amplified Tn*1546*-like element was the template for L-PCR of *vanRSHAX* genes using primers P2 and P3. The L-PCR products were analysed on 1% agarose gel.

Isolates were typed by pulsed-field gel electrophoresis (PFGE) after *Sma*I-digestion of DNA (2) in a Gene Navigator apparatus (Amersham, Uppsala, Sweden) at 180V for 25h, at 7ºC with pulses of 20s for 10h, 8s for 10h and 3s for 5h. DNA banding patterns were analysed using previously described criteria (12).

Both VRE isolates were identified as *Enterococcus faecalis* and expressed high-level resistance to vancomycin (MIC $\geq$ 256 µg/ml). They showed the same antimicrobial susceptibility profile, characterized by resistance to ciprofloxacin, chloramphenicol, erythromycin, tetracycline and teicoplanin. The isolates showed susceptibility to ampicillin, penicillin, gentamicin and streptomycin.

According to multiplex PCR assay both VRE isolates harbored the *vanA* gene in the intact Tn*1546*-like element. The *vanRSHAX* genes were also detected by Long-PCR.

The molecular typing has disclosed the same PFGE pattern for both isolates (Fig. 1), suggesting clonal dissemination of this strain in ICU. On the other hand, we founded seven different bands comparing the PFGE profile of these VRE strains, isolated in 2006, with the clone predominant spread in some states of Brazil (strains isolated from 1998 to 2000) (9). As the criteria proposed by Tenover *et al*. (12) for analyzing the relationship among strains refer to those obtained for a short period of time, the difference in PFGE profile could not signify that they are unrelated.

In Minas Gerais state, the prevalence of VRE seems to be lower than that observed in other states, such as São Paulo and Rio Grande do Sul, since there is only one other report describing the isolation of a VRE strain in the Uberlândia city in 2003 (11).

In conclusion, our data showed that the VRE was transferred horizontally between patients in the same ICU room emphasizing that is very important to monitor their occurrence in hospital environment and to take measures to prevent the dissemination of nosocomial VRE among several patients.

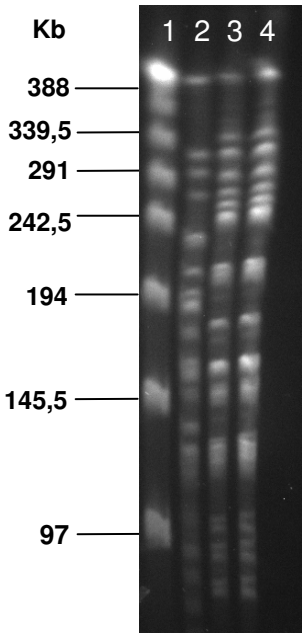

Figure 1. PFGE patterns after SmaI digestion of total DNA extracted from the VRE strains. Lanes 1: Lambda ladder PFG marker (New England Biolabs); 2: Clone of vancomycin-resistant *E. faecalis* predominant in Brazil; 3-4: Clinical isolates of vancomycin-resistant *E. faecalis* from Uberaba, Minas Gerais.

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