# First case of *vanA*-positive *Enterococcus mundtii* in human urinary tract infection in Iran

**M. Sharifi-Rad[1], S. Shadanpour[3], A. van Belkum[4], A. Soltani[5] and J. Sharifi-Rad[2]**

1) *Zabol University of Medical Sciences,* 2) *Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol,* 3) *Department of Microbiology, Faculty of Biological Science, Tehran North Branch, Islamic Azad University, Tehran, Iran,* 4) *bioMérieux SA, Unit Microbiology, R&D Microbiology, La Balme Les Grottes, France and* 5) *Department of Genetics and Biotechnology, Osmania University, Hyderabad, India*

## Abstract

We cultured enterococci from urinary tract infections in Iranian hospitals. Seven different *Enterococcus* species (E. *raffinosus, E. durans, E. hirae, E. avium, E. mundtii, E. faecium* and *E. faecalis*) were found. Seven strains were vancomycin resistant, leading to an overall vancomycin resistance rate of 3.9%. The enterococcal infection rate was high and vancomycin-resistant enterococci incidence low. We report the first *vanA*-positive *E.* mundtii urinary tract infections.



**Keywords:** Enterococcal infection, E. mundtii, urinary tract infection, *vanA*-positive, Vancomycin-resistant enterococci
**Original Submission:** 12 January 2016; **Revised Submission:** 13 February 2016; **Accepted:** 16 February 2016
**Article published online:** 24 February 2016

**Corresponding author:** J. Sharifi-Rad, Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol, Iran
**E-mail:** Javad.sharifirad@gmail.com

Enterococci are members of the gastrointestinal flora and emerged in the 1970s as leading causes of multidrug-resistant hospital acquired infections [1]. Enterococcal colonization may result in asymptomatic bacteriuria but also in overt urinary tract infection (UTI) [2]. Vancomycin-resistant enterococci (VRE), the most important class of drug-resistant enterococci, mainly belong to the species *Enterococcus faecium* and contain *vanA* and/or *vanB* genes [3]. There are four additional vancomycin resistance genes: *vanC*, *vanD*, *vanE* and *vanG* [4,5]. Detection of VRE is possible with culture or PCR [6]. We investigated UTI-related VRE and their *vanA* and *vanB* content from samples of East Iranian origin.

We obtained 177 isolates from 358 urine specimens of patients with UTI from two hospitals (Amir Al-Momenin and Imam Khomeini) in Zabol, Iran (2013–2014). Diagnostics included Gram staining, catalase production, pyrrolidonyl arylamidase detection and growth on bile-esculin/6.5% NaCl media. Vancomycin susceptibility testing included screening on brain–heart infusion (BHI) agar (Difco, USA) containing 6 μg/mL vancomycin (Sigma, Germany). Teicoplanin susceptibility testing was performed using 30 μg teicoplanin disks on Mueller-Hinton agar (Difco; Mast, UK). Minimum inhibitory concentrations (MICs) were determined by agar dilution on BHI agar [7]. The MIC breakpoint for both vancomycin and teicoplanin resistance was $\geq$32 μg/mL. Susceptibility to vancomycin (MIC $\leq$4 μg/mL) and to teicoplanin (MIC $\leq$8 μg/mL) was according to the Clinical and Laboratory Standards Institute.

DNA was extracted according to Perez-Hernandez *et al.* [8] and used for *vanA* and *vanB* PCR. All enterococci were PCR assayed for *vanA* and *vanB* genes using previously described primers and protocols [9]. Resistant *Enterococcus* spp. strains *E. faecalis* E206 (*vanA* positive) and *E. faecium* E2781 (*vanB* positive) were used as positive controls; *Enterococcus faecalis* ATCC 29212 was used as negative control.

We identified seven different species of *Enterococcus*: *E. raffinosus* 3.3% (*n* = 6), *E. durans* 2.8% (*n* = 5), *E. hirae* 3.9% (*n* = 7), *E. avium* 4.5% (*n* = 8), *E. mundtii* 10% (*n* = 18), *E.* faecium 27% (*n* = 48), and *E.* faecalis 48% (*n* = 85) (*n* = Table 1). *E. faecalis* and *E. faecium* accounted for 75% of the isolates. Out of 94 isolates from Amir Al-Momenin hospital, 4 (4.2%) were



http://dx.doi.org/10.1016/j.nmni.2016.02.005

**TABLE 1. Frequency of enterococcal species in two hospitals in Zabol, Iran**

| Hospital | No. of *Enterococcus* spp. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. raffinosus* | *E. durans* | *E. hirae* | *E. avium* | *E. mundtii* | *E. faecium* | *E. faecalis* | Total |
| Amir Al-Momenin | 6 | 4 | 1 | 6 | 12 | 26 | 46 | 94 |
| Imam Khomeini | 0 | 1 | 6 | 2 | 6 | 24 | 39 | 83 |
| Total | 6 | 5 | 7 | 8 | 18 | 48 | 85 | 177 |

resistant to vancomycin: *E. raffinosus* ($n$ = 1), *E. faecalis* ($n$ = 2) and *E. mundtii* ($n$ = 1) (see Fig. 1). Imam Khomeini Hospital showed an even lower resistance rate of 3.6% ($n$ = 3), including two *E. raffinosus* and one *E. faecium* strains. The overall vancomycin resistance rate in the two hospitals was 3.9% (Table 2).

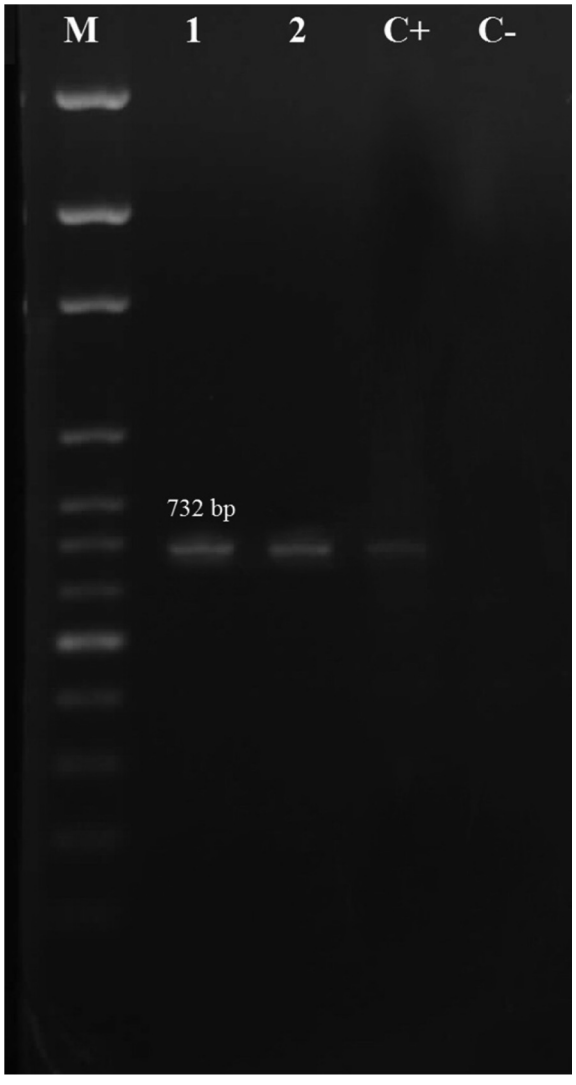


**FIG. 1.** Patterns of agarose gel electrophoresis showing PCR amplification products for vancomycin resistant enterococci (VRE) lanes 1: *E. faecalis*; lane 2: *E. mundtii*. Lane M, DNA 100 basepair molecular size marker; C+: positive control; C−: negative control.

**TABLE 2. Distribution and VRE species diversity in two hospitals in Zabol, Iran**

| Hospital | No. of isolates | VRE species, $n$ (%) | No. of *Enterococcus* spp. | | | |
|---|---|---|---|---|---|---|
| | | | *E. raffinosus* | *E. faecium* | *E. faecalis* | *E. mundtii* |
| Amir Al-Momenin | 94 | 4 (4.2) | 1 | 0 | 2 | 1 |
| Imam Khomeini | 83 | 3 (3.6) | 2 | 1 | 0 | 0 |
| Total | 177 | 7 (3.9) | 3 | 1 | 2 | 1 |

VRE, vancomycin-resistant enterococci.

All strains resistant to vancomycin showed an MIC of >512 μg/mL, while MICs obtained for teicoplanin resistant strains varied. *E. faecium* showed the highest teicoplanin MIC (98 μg/mL). *E. faecalis* (69 μg/mL), *E. mundtii* (60 μg/mL) and *E. raffinosus* (18 μg/mL) showed lower MICs.

Hospital-based VRE endemicity differs between countries, within a country and between different cities or hospitals. Here we show that the enterococcal UTI rate in two Iranian medical centers was relatively high. The VRE incidence, however, was not excessive. We show further evidence for the existence of infectious *vanA*-positive *E. mundtii* in humans beyond the single case previously reported from India [10].

## Conflict of Interest

None declared.

## References

[1] Van Tyne D, Gilmore MS. Friend turned foe: evolution of enterococcal virulence and antibiotic resistance. Annu Rev Microbiol 2014;68:337–56.
[2] Tristan OD, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist 2015;8:217.
[3] Fram D, Castrucci FM, Taminato M, Godoy-Martinez P, Freitas MCS, Belasco A, et al. Cross-transmission of vancomycin-resistant *Enterococcus* in patients undergoing dialysis and kidney transplant. Braz J Med Biol Res 2010;43:115–9.
[4] Naas T, Fortineau N, Snanoudj R, Spicq C, Durrbach A, Nordmann P. First nosocomial outbreak of vancomycin-resistant *Enterococcus*

*faecium* expressing a *vanD*-like phenotype associated with a *vanA* genotype. J Clin Microbiol 2005;43:3642–9.

[5] Zirakzadeh A, Patel R. Vancomycin-resistant enterococci: colonization, infection, detection, and treatment. Mayo Clin Proc 2006;81:529–36.

[6] Seo JY, Kim PW, Lee JH, Song JH, Peck KR, Chung DR, et al. Evaluation of PCR-based screening for vancomycin-resistant enterococci compared with a chromogenic agar–based culture method. J Med Microbiol 2011;60:945–9.

[7] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document M100–S20. Wayne, PA: CLSI; 2010.

[8] Perez-Hernandez X, Mendez-Alvarez S, Claverie-Martin F. A PCR assay for rapid detection of vancomycin-resistant enterococci. Diagn Microbiol Infect Dis 2002;42:273–7.

[9] Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995;33:24–7.

[10] Praharaj I, Sujatha S, Parija SC. Phenotypic and genotypic characterization of vancomycin resistant *Enterococcus* isolates from clinical specimens. Indian J Med Res 2013;138:549–56.