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Letter to the Editor

The first clonal spread of *van*A-positive *Enterococcus raffinosus* in a nursing home

Sir,

In a recent issue of this Journal, Jolivet and colleagues reported the first nosocomial outbreak of *van*A-type vancomycin-resistant *Enterococcus raffinosus* in France [1]. We would like to report a *van*A-positive *E. raffinosus* outbreak that is not only the first in Belgium but, to our knowledge, is also the first to be reported in a nursing home anywhere in the world. We also tabulate a literature search on previous *E. raffinosus* outbreaks worldwide.

Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens and the treatment option for VRE infections is limited [2]. Invasive infections caused by VRE are associated with higher mortality than those caused by vancomycin-susceptible enterococci (VSE) [2]. Outbreaks of VRE usually occur in hospital settings caused by *E. faecium* and *E. faecalis* [2]. The most frequently reported resistance genotypes responsible for acquired resistance to vancomycin are *van*A and *van*B [2]. The *van*A gene is responsible for high-level resistance to glycopeptides vancomycin and teicoplanin.

Outbreaks of VRE due to species other than *E. faecium* and *E. faecalis* are rarely reported. *E. raffinosus* is another species of *Enterococcus* that has been linked to severe infections such as endocarditis [3].

In the spring of 2015, the Belgian National Reference Center (NRC) for Enterococci received two *E. raffinosus* strains for confirmation of vancomycin resistance from two different hospitals within a distance of 30 km (18 miles). *E. raffinosus* was confirmed in the NRC using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany). These isolates were also positive for *van*A genes as detected using specific polymerase chain reaction primers with forward sequence AAAATGTGCGAAAAACCTTG, and reverse sequence AAACATATCCACACGGGCTA [4] .

The first strain originated from a screening sample from a patient in hospital 1. The VRE screening was performed in that hospital due to an outbreak of *van*A-positive *E. faecium*. The second strain originated from a patient who received haemodialysis in hospital 2; this hospital had also implemented VRE screening due to the same *van*A-positive *E. faecium* outbreak in hospital 1.

After contacting both hospitals, new information revealed that both patients lived in the same nursing home. The Agency for Care and Health was contacted and they performed VRE screening in a first circle of close contacts in the nursing home. This investigation resulted in the identification of an additional case. All three residents colonized with *van*A-positive *E. raffinosus* lived in single rooms on the same floor in the nursing home and shared nursing and paramedical staff as well as a same dining room and physiotherapy room. The characteristics of the three patients and their isolates are presented in Table I. Residents from a second circle, having less contact with these three index cases, were screened but no additional cases were identified within this circle.

Pulsed-field gel electrophoresis (PFGE) confirmed the clonal spread of a *van*A-positive *E. raffinosus* clone (Figure 1). PFGE was performed by digesting genomic DNA of E. *raffinosus* isolates with SmaI (Life Technologies, Carlsbad CA, USA), embedded in agarose 0.75% w/v plugs and separated by using Pulsed Field-Certified Agarose. The following *E. raffinosus* strains were used as control strains: reference strain LMG 12888T, clinical isolates O8L1270 and 111-005886 (own strain collection) and 8991/64, UW 11260, UW 7358, UW 10887, C-31135 [kindly provided by R. Willems (UMC-Utrecht, The Netherlands), G. Werner (Robert Koch Institute, Germany), K. Hegstad (University Hospital of North-Norway, Norway) and P. Damborg (University of Copenhagen, Denmark), respectively]. PFGE patterns were interpreted according to Tenover et al. [4].

To investigate the previous occurrence of *E. raffinosus* outbreaks, we searched Medline up to December 29th, 2016, using search terms '*Enteroccocus raffinosus*' without language restriction. After reading the abstracts of all retrieved references and obtaining the full text of possible outbreak reports, four studies on *E. raffinosus* outbreak were included (Table I) [1,5–7]. The first outbreak was reported in the USA in 1997 and the most recent one in a hospital Paris region in France in 2014 [1]. The outbreaks were mostly short-lived, lasting around two to three months. Even though the short duration of outbreaks is perhaps due to successful infection control measures, the possibility that the outbreaks were self-limiting cannot be excluded. All isolates involved in the outbreaks were resistant to vancomycin, teicoplanin and ampicillin but susceptible to linezolid. Unlike the present study, outbreaks published in other studies occurred only in hospital settings.

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Table I
Literature review on *van*A-positive *Enterococcus raffinosus* outbreak

Authors, year	Geographic location	Setting	No. of involved patients	Period of outbreak	MIC of antibiotics (mg/L)[a]	Method used in identifying *E. raffinosus*
Present study	West Flanders, Belgium	Nursing home	Three (all screening isolates)	May to August 2015	Ampicillin: 32–128 (R) Vancomycin: 256 (R) Teicoplanin: 16 (R) Linezolid: 1–2 (S)	MALDI-TOF MS
Jolivet et al., 2016 [1]	Paris region, France	Hospital; intensive care unit	Four (one clinical (probably cholecystitis), three screening)	September to October 2014	Ampicillin: 32 (R) Vancomycin: >256 (R) Teicoplanin: 32 (R) Linezolid: 2 (S)	MALDI-TOF MS
Samuel et al., 2008 [5]	Newcastle, UK	Hospital; haematology ward	17 (all screening)	August 2007 to February 2008	Ampicillin: >8 (R) Vancomycin: >32 (R) Teicoplanin: >32 (R) Linezolid: 1–2 (S)	API 20 Strep Kit
Kawalec et al., 2007 [6]	Warsaw, Poland	Hospital; haematology, surgery and nephrology ward	11 (all screening)	March 2005 to June 2006	Cannot be derived since the data were presented together with *E. faecium*	VITEK2 compact version
Wilke et al., 1997 [7]	Iowa, USA	Hospital; internal medicine and vascular surgery wards	Four (all clinical, bloodstream infection)	April 1995 to June 1996	Ampicillin: no data available Vancomycin: >16 (R) Teicoplanin: 16 (not interpreted) Linezolid: no data available	Reference biochemical method

MIC, minimum inhibitory concentration; S, susceptible; R, resistant; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
[a] Interpretation according to guidelines by the authors.

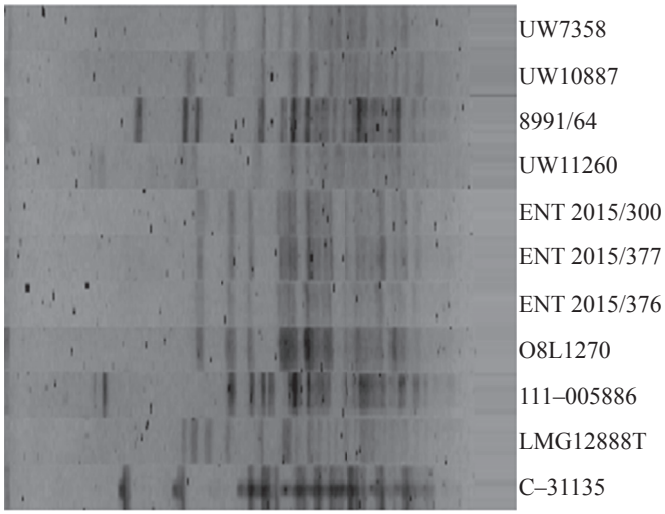

Figure 1. Pulsed-field gel electrophoresis pattern of *E. raffinosus* isolates. ENT 2015/300, ENT 2015/376, and ENT 2015/377 show a similar pattern; they are identical strains involved in this clonal outbreak.

The *van*A-positive *E. raffinosus* isolates mostly originated from screening, not from clinical samples. Considering that invasive infections due to VRE are associated with a higher mortality than those due to VSE, and because *E. raffinosus* may be an important reservoir of *van* genes and may contribute to the dissemination of vancomycin resistance, infection control measures should be taken to curb the spread of *van*A-positive *E. raffinosus* [8]. Such strains ought therefore to be submitted to specialized centres for confirmation and surveillance.

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None declared.

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