

Risk factors for vancomycin-resistant enterococci colonisation in critically ill patients

Deivid William da Fonseca Batista^{1/+}, Paulo Pinto Gontijo-Filho¹,
Natália Conceição², Adriana Gonçalves de Oliveira², Rosineide Marques Ribas¹

¹Laboratório de Microbiologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Av. Pará 1720 Bloco 4 C, 38400-902 Uberlândia, MG, Brasil ²Laboratório de Microbiologia, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brasil

Vancomycin-resistant enterococci (VRE) are important hospital pathogens and have become increasingly common in patients admitted to the intensive care unit (ICU). To determine the incidence and the risk factors associated with VRE colonisation among ICU patients, active surveillance cultures for VRE faecal carriage were carried out in patients admitted to the ICU of the University Hospital of Uberlândia, Minas Gerais, Brazil. Risk factors were assessed using a case-control study. Seventy-seven patients (23.1%) were found to be colonised with vanC VRE and only one patient (0.3%) was colonised with vanA VRE. Independent risk factors for VRE colonisation included nephropathy [odds ratio (OR) = 13.6, $p < 0.001$], prior antibiotic use (OR = 5.5, $p < 0.03$) and carbapenem use (OR = 17.3, $p < 0.001$). Our results showed a higher frequency (23.1%) of Enterococcus gallinarum and Enterococcus casseliflavus, species that are intrinsically resistant to low levels of vancomycin (vanC), without an associated infection, associated with prior antibiotic use, carbapenem use and nephropathy as comorbidity. This study is the first to demonstrate the risk factors associated with vanC VRE colonisation in ICU hospitalised patients. Although vanA and vanB enterococci are of great importance, the epidemiology of vanC VRE needs to be better understood. Even though the clinical relevance of vanC VRE is uncertain, these species are opportunistic pathogens and vanC VRE-colonised patients are a potential epidemiologic reservoir of resistance genes.

Key words: risk factors - vancomycin-resistant enterococci - vanC

Vancomycin-resistant enterococci (VRE) have emerged worldwide as important nosocomial pathogens. The prevalence and incidence of VRE colonisation vary widely among hospitals and studies have suggested that such VRE rates are higher among critically ill patients, particularly those admitted to intensive care units (ICUs), limiting the therapeutic options available (Hendrix et al. 2001, Mutnick et al. 2003, Mazuski, 2008).

Glycopeptide resistance in enterococci is associated with diverse phenotypes (Werner et al. 2008) and their resistance to several antimicrobial agents, whether intrinsic (low-level resistance to penicillin, cephalosporins and aminoglycosides) or acquired (resistance to glycopeptides and high concentrations of aminoglycosides), is of great concern (Metallidis et al. 2006).

The most infectious cases are associated with *Enterococcus faecalis* and *Enterococcus faecium*; in these species, the presence of *vanA* and *vanB* genes that encode high-level vancomycin resistance is of great concern due to their spread worldwide (Mazuski 2008).

Enterococcus gallinarum and *Enterococcus casseliflavus* are species that are intrinsically resistant to low levels of vancomycin (vanC) and have been found to colo-

nise the human intestinal tract (Toye et al. 1997, Mondino et al. 2003, Maschieto et al. 2004). Although these species have been reported to cause infections sporadically, their clinical significance remains uncertain (Mutnick et al. 2003, de Perio et al. 2006, Neves et al. 2009).

Intestinal colonisation by VRE has been associated with a variety of risk factors and asymptomatic colonisation by VRE often precedes true infection. Although many studies on the prevalence, incidence, epidemiology and risk factors of VRE have been performed in Europe and the United States, such data for Brazil are scarce (Warren et al. 2003, Furtado et al. 2005). Moreover, despite higher rates of vanC VRE colonisation, the risk factors for colonisation by enterococci of this phenotype have not been identified (Neves et al. 2009).

The purpose of this study was to determine the incidence of VRE colonisation among patients admitted to the ICU of a tertiary teaching hospital and to identify the risk factors that predispose these hospitalised patients to VRE colonisation.

PATIENTS, MATERIALS AND METHODS

Study setting and population - The University Hospital of Uberlândia, located in the state of Minas Gerais (MG), southeastern region of Brazil, is a 503-bed tertiary teaching hospital with a 15-bed medical and surgical ICU. We performed a prospective observational study to determine the incidence of VRE colonisation among patients admitted to this ICU between April 2009-January 2010.

Rectal swabs were obtained within 48 h of ICU admission to detect VRE colonisation that might have occurred prior to entering the unit. Samples of patients who

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+ Corresponding author: deividwfb@yahoo.com.br

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remained in the ICU for more than 48 h were acquired once a week until a positive VRE culture was achieved, the patient was discharged from the ICU, or the patient died. *Enterococcus* was considered to be ICU-acquired if it was detected from a patient who had been on the unit for more than 48 h. Patients who had serious medical conditions that precluded the collection had left the ICU prior to collection, were non-consenting or who had non-consenting guardians were excluded. The study protocol was approved by the hospital's ethical committee.

Isolation, identification and susceptibility testing

The samples obtained were transported to the microbiology laboratory for selective culturing of VRE. The swabs were inoculated on BBL™ Enterococcosel™ Agar plates (BD, Becton, Dickinson and Company, France), supplemented with vancomycin (6 µg/mL) and incubated aerobically at 35°C for 48 h. All enterococci samples that were not sensitive to vancomycin were considered VRE. From each plate, some suspected enterococci colonies were initially identified by Gram staining, growth in 6.5% NaCl broth and bile esculin hydrolysis. All presumed enterococci were further identified as previously described (Teixeira et al. 2007) using the following phenotypic tests: yellow-pigment production, motility, L-pirrolidonil-β-naftilamida hydrolysis, acid formation from sorbitol and arabinose and arginine hydrolysis.

Antimicrobial susceptibility testing was performed on Mueller-Hinton Agar (BD, Becton, Dickinson and Company) according to recommendations of the Clinical and Laboratory Standard Institute (CLSI 2009). The vancomycin minimal inhibitory concentration (MIC) was evaluated by the E-test method (AB Biodisk) and isolates with a MIC ≥ 32 µg/mL were selected for testing by polymerase chain reaction (PCR). The high level resistance to aminoglycosides was carried out by disc-diffusion with gentamicin (120 µg) and streptomycin (300 µg) (Laborclin Ltda).

The isolates identified as *E. gallinarum* and *E. casseliflavus* with low-level resistance to vancomycin (MIC = 2-32 µg/mL) were considered as the vanC phenotype.

Epidemiological investigation - A case-control study was then performed. Cases were defined as patients with VRE colonisation, but without other infections, and the controls were defined as patients with neither VRE colonisation nor infection from any microorganism. The following sociodemographic, clinical and laboratory data were evaluated: age, gender, duration of hospitalisation, prior ICU hospitalisation, underlying diseases, surgery, infections and antibiotic treatment. This information was obtained by a review of medical records and computer systems databases.

Prior antibiotic use was defined as the administration of antimicrobials in the 15 days before ICU admission. Prior ICU hospitalisation was defined as an inpatient admission to the ICU for any reason in the 12 months before the current ICU admission.

The defined daily dose (DDD) of antibiotics per 1,000 patients-day was calculated as previously described (Moreira et al. 2009).

Molecular methods - To assess the presence of the *vanA* and *vanB* genes in enterococci strains with high MIC for vancomycin (≥ 32 µg/mL), the PCR was performed as previously described (Woodford et al. 1993). Briefly, genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The amplification primers for *vanA* gene were A₁ ATGGCAAGTCAGGTGAA-GATGG and A₂ TCCACCTCGCCAACAACCTAACG, and for *vanB* gene, B₁ TCTGTTTGAATTGTCTGGTAT and B₂ GACCTCGTTTAGAACGATG. The cycling conditions for the PCR were as follows: 5 min at 94°C, 30 cycles of 25 s at 94°C, 40 s at 52°C and 50 s at 72°C and a final step of 10 min at 72°C in a GeneAmp® PCR System 9700 (Applied Biosystems, USA). The PCR products were detected in 1.5% agarose gel by gel electrophoresis and ethidium bromide staining.

Statistical analysis - Statistical analyses were performed using GraphPad Prism v.4 (GraphPad Software, San Diego, CA) and Bioestat 5.0 (Belém, PA, Brazil). For determining the risk factors for VRE colonisation, the control and case groups were compared using the Mann-Whitney U test for continuous data and the Fisher exact test or chi-squared test for categorical data, when appropriate. We used a Pearson's correlation test to calculate the correlation between VRE colonisation per 1,000 patients-day and DDD of antibiotics per 1,000 patients-day. Multiple logistic regression was performed for the significant variables in the univariate analysis. Statistical significance was defined as $p \leq 0.05$.

RESULTS

During the study period, 422 patients were admitted to the adult ICU at the University Hospital of Uberlândia. Of these patients, 328 stayed there for more than 48 h. Eighty-nine patients were excluded for the reasons listed above. From 333 patients included in the study, 78 (23.4%) were VRE-colonised (18.3/1,000 patients-day) and 35.9% (28/78) of these VRE-colonised patients had acquired the infection in the ICU. We observed that 64.1% (50/78) were already colonised with VRE upon ICU admission, including 46% (23/50) who were transferred from general wards and 54% (27/50) who were from outside of the hospital.

Enterococcus spp were isolated in 90 (27%) of the 333 patients included in this study and two of them (2.2%) presented with more than one species of enterococci in the faecal samples, resulting in a total of 92 samples isolated. The enterococci species isolated in our study included *E. casseliflavus* (55.4%), *E. gallinarum* (28.3%), *E. faecium* (12%) and *E. faecalis* (4.3%). We isolated only one *E. faecalis* strain, which carried the *vanA* gene.

The distribution of antimicrobial resistance among enterococcal species is presented in Table I. The results showed that only one *E. faecalis* isolated was resistant to high-level vancomycin (MIC ≥ 256 µg/mL). In general, the samples showed a multiresistance profile. A resistance to erythromycin was the most frequent form of antibiotic resistance (45.7%), followed by resistance to tetracycline (32.6%), ciprofloxacin (17.3%) and chloramphenicol (10.9%). Only two strains were peni-

cillin resistant and none were ampicillin resistant. A high-level resistance to aminoglycoside was observed in 16.3% of the strains.

From 78 VRE-colonised patients, 21 of them were included in the case group, because they did not present any infection and the vanA VRE-colonised patient was excluded of the case group because acquired other infections. From 255 VRE non-colonised patients, 143 (56.1%) had no colonisation or infection from any organism and were included in the control group. Twenty-eight VRE-colonised patients were VRE-negative at admission and became colonised during their hospital stay. None of the VRE-colonised patients developed other infections during the observation time of the study.

The results of the univariate and logistic regression analysis are shown in Tables II, III, respectively. In the univariate analyses, the cases and control groups differed significantly in the following areas of their medical histories: nephropathy ($p < 0.001$), diabetes mellitus ($p = 0.05$), prior antibiotic use ($p = 0.001$), vancomycin use ($p = 0.02$) and carbapenem use ($p < 0.001$). In the multivariate analysis, significant independent risk factors for VRE colonisation were nephropathy ($p < 0.001$), prior antibiotic use ($p = 0.03$) and carbapenem use ($p < 0.001$).

The consumption of vancomycin, carbapenems and third and fourth generation cephalosporins in the ICU represented by the DDD per 1,000 patients-day varied during the study period (Fig. 1). On average, the DDD of cephalosporins, carbapenems and vancomycin were 589.33/1,000, 243.53/1,000 and 152.78/1,000 patients-day, respectively. Despite the high density of cephalosporin use, only the use of vancomycin (Fig. 2) and carbapenems (Fig. 3) was related to the increased incidence of VRE-colonisation per 1,000 patients-day, according to the Pearson's correlation ($r = 0.61$, $p = 0.03$ and $r = 0.53$, $p = 0.05$, respectively).

DISCUSSION

This study of ICU patients found a high prevalence of VRE vanC colonisation among patients admitted to the ICU (23.1%). Prior antibiotic use, including car-

bapenem and vancomycin use in the ICU, as well as diabetes mellitus and nephropathy, were associated with VRE colonisation in ICU patients by univariate analysis. Despite the high rates of colonisation by vanC VRE enterococci among hospitalised patients, no patients infected with bacteria of these phenotypes were detected in the ICU in this study. However, it is important to report that during the study period, two strains of *E. gallinarum*-causing bacteraemia were isolated in patients admitted to other hospital units, illustrating their role as potential opportunistic pathogens (Reid et al. 2001, Dargere et al. 2002, Choi et al. 2004).

Patients colonised by vanC VRE are a potential epidemiologic reservoir of resistance genes. In Brazil, the first case of colonisation by *E. gallinarum* carrying the *vanA* gene was detected in 2002 in a hospital located in the state of Rio Grande do Sul (Camargo et al. 2004) and the first case of a clinical infection was detected in 2002 in the state of Rio de Janeiro (Merquior et al. 2008). During routine colonisation surveillance in the same hospital in RJ, seven *vanA*-carrying *E. gallinarum* isolates were detected (Neves et al. 2009).

Therapy with various antimicrobial agents may contribute to increasing colonisation by vanC VRE and others multiresistant microorganisms (Neves et al. 2009). Several studies have concluded that the consumption of antimicrobial agents is a strong indicator for multiresistant microorganism acquisition (Harthug et al. 2002). In our casuistic study, the use of antibiotics was present in approximately 70% of the 333 patients (223 patients) included in this study; most of these were broad spectrum-cephalosporins (50.4%), carbapenems (34.2%) and vancomycin (26.1%). This overuse of antibiotics, expressed as the DDD per 1,000 patients-day, reflects the high frequency of patients with infection in the ICU, although the majority of them were receiving empirical therapy with two drugs. Organisms isolated from patients in ICUs are more likely to be resistant to antibiotics than those isolated from general ward patients or outpatients (Archibald et al. 1997) because there is high antimicrobial selection pressure in these units (Vincent 2003).

TABLE I
Species distribution of enterococci species according to resistance

Species (number tested)	Antimicrobial n (%)									
	RIF	CLO	VAN	ERY	PEN	CIP	TET	AMP	HLGR	HLSR
<i>Enterococcus faecium</i> (4)	1 (25)	0 (0)	0 (0)	2 (50)	0 (0)	2 (50)	2 (50)	0 (0)	0 (0)	1 (25)
<i>Enterococcus faecalis</i> (11)	0 (0)	3 (27.3)	1 (9.1)	9 (81.8)	2 (18.2)	6 (54.5)	5 (45.4)	0 (0)	2 (18.2)	1 (9.1)
<i>Enterococcus gallinarum</i> (26)	0 (0)	1 (3.8)	0 (0)	6 (23)	0 (0)	3 (11.5)	8 (30.8)	0 (0)	0 (0)	0 (0)
<i>Enterococcus casseliflavus</i> (51)	1 (2)	6 (11.8)	0 (0)	25 (49)	0 (0)	5 (9.8)	15 (29.4)	0 (0)	1 (2)	10 (19.6)
Total (92)	2 (2.2)	10 (10.9)	1 (1.1)	42 (45.7)	2 (2.2)	16 (17.3)	30 (32.6)	0 (0)	3 (3.3)	12 (13)

AMP: ampicillin; CIP: ciprofloxacin; CLO: chloramphenicol; ERY: erythromycin; HLGR: high level gentamicin resistance; HLSR: high level streptomycin resistance; PEN: penicillin; RIF: rifampin; TET: tetracycline; VAN: vancomycin.

TABLE II
Risk factors for vancomycin-resistant *Enterococcus* spp in hospitalized patients

Risk factors	Frequency (%) or mean $\pm$ SD		Univariate analysis
	Cases (n = 21)	Controls (n = 143)	p (OR; 95% CI)
Age (years)	52.9 ($\pm$ 17.6)	50.5 ($\pm$ 18.9)	0.54
Gender			
Female	38.1	41.3	0.78 (0.87; 0.34-2.24)
Male	61.9	58.7	0.78 (1.14; 0.44-2.93)
Duration of hospitalization (days)	7.4 ($\pm$ 4.9)	5.8 ($\pm$ 3.9)	0.18
Prior ICU hospitalization	71.4	59.4	0.29 (1.70; 0.62-4.66)
Surgery	52.4	53.1	0.94 (0.97; 0.39-2.43)
Underlying diseases			
Cancer	14.3	9.8	0.46 (1.54; 0.40-5.87)
Diabetes mellitus	28.6	12.6	0.05 (2.74; 1.01-8.08)
Cardiac disease	42.9	50.3	0.52 (0.74; 0.29-1.86)
Autoimmune disease	4.8	2.8	0.50 (1.74; 0.18-16.34)
Nephropathy	23.8	4.2	< 0.001 (7.13; 1.95-26.06)
Antibiotics treatment			
Prior antibiotic use	23.8	4.9	0.001 (6.38; 1.81-22.48)
Vancomycin	19	4.2	0.02 (5.37; 1.38-20.98)
Third or fourth generation cephalosporin	38.1	27.3	0.30 (1.64; 0.63-4.26)
Fluoroquinolones	9.5	5.6	0.62 (1.77; 0.35-8.99)
Carbapenems	28.6	2.8	< 0.001 (13.90; 3.52-54.87)
Overall mortality	14.3	9	0.44 (1.63; 0.43-6.29)

CI: confidence interval; ICU: intensive care unit; OR: odds ratio; SD: standard deviation.

TABLE III
Logistic regression of risk factors for vancomycin-resistant *Enterococcus* spp in hospitalized patients

Risk factors	p	OR (95% CI)
Underlying disease		
Diabetes mellitus	0.79	1.22 (0.26-5.82)
Nephropathy	< 0.001	13.65 (3.24-57.55)
Antibiotics		
Prior antibiotic use	0.03	5.52 (1.08-28.05)
Vancomycin	0.54	1.70 (0.30-9.67)
Carbapenems	< 0.001	17.29 (3.42-87.56)

CI: confidence interval; OR: odds ratio.

The consumption of third and fourth-generation cephalosporins, vancomycin and carbapenems in our ICU is much higher than that in many other countries (Moreira et al. 2009). The use of antibiotics, particularly vancomycin, has been identified as a risk factor for VRE colonisation or infection in some, but not all studies (Fridkin et al. 2001, Furtado et al. 2005, 2006, Sakka et al. 2008). Accordingly, the positive correlation in our study between the incidence of VRE and the consumption of vancomycin and carbapenems in the ICU suggest that the

prevalence of antibiotic use is an important risk factor, particularly for the emergence and spread of VRE.

It has been reported that vancomycin use maintains the intestinal environment in a state that is favourable for VRE growth and promotes the possibility of an individual VRE carrier becoming a transmitter, additionally increasing the risk of a non-carrier becoming colonised (Furtado et al. 2006). Treatment with vancomycin was more common in VRE-colonised patients in the present study, but this association was not statistically significant in multivariate models. Unlike vancomycin use, carbapenem use in the ICU was an independent risk factor for VRE colonisation. Recent studies suggested that some carbapenems are excreted in high concentrations in the bile, resulting in the inhibition of anaerobes and overgrowth of enterococci (Stiefel et al. 2007).

Apart from the use of antimicrobial agents and inpatient care in particular units, other risk factors for VRE colonisation and infection that have been identified in other studies with samples of vanA VRE include the following: length of hospital stay, solid organ transplants, chronic dialysis, admission to the hospital at least once during the 12 months prior to ICU admission and hospitalisation for over three days prior to ICU admission (Ostrowsky et al. 1999, Warren et al. 2003, Furtado et al. 2005). We found that nephropathy was a risk factor for VRE colonisation. Patients with nephropathy are at

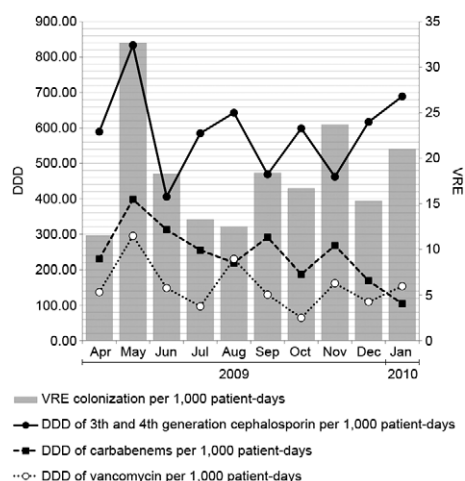


Fig. 1: relationship between the vancomycin-resistant enterococci (VRE) colonization per 1,000 patient-days (gray columns) and the density of third and fourth generation cephalosporins (filled circles), carbapenems (filled squares) and vancomycin (empty circles) use expressed as defined daily dose (DDD) per 1,000 patient-days in adult intensive care units from April 2009-January 2010.

an increased risk for acquiring VRE for several reasons, such as extensive contact with the hospitals, presence of multiple comorbid conditions, need for haemodialysis, proximity to other VRE-colonised patients, or even infected patients receiving prolonged administration of antibiotics, including vancomycin (Humphreys et al. 2004, Assadian et al. 2007).

The presence of VRE in infections is more common in ICUs in North America and in parts of South America than in European ones (Guzman-Blanco et al. 2000, Mutnick et al. 2003). VRE isolation has been reported in some Brazilian hospitals, mainly in the state of São Paulo (Cereda et al. 1997); in our hospital, VRE was described for the first time in 2003 in a post-surgical infection caused by an *E. faecalis* of vanA phenotype and was the first case to be described in MG (Ribas et al. 2007). However, high level aminoglycoside resistance has not been uncommon and frequencies of 18.5% in SP (Maschieto et al. 2004), 22% in Brasília (Titze-de-Almeida et al. 2004) and 40.5% in RJ (Mondino et al. 2003) have been observed. In the present study in Uberlândia, 16.3% of the isolates cultured from colonised individuals showed high levels of aminoglycoside resistance. An increase in the frequency of enterococci with high level aminoglycoside resistance has recently been observed due to spread of plasmids and transposons that encode enzymes that inactivate aminoglycosides (Sood et al. 2008, Protonotariou et al. 2010).

Several potential limitations of the present study should be mentioned. First, we had a relatively small number of patients in the case group, thus reducing the statistical power and the ability to study subsets of patients. Another limitation of this study was that information on the management of the patient's condition and severity of the underlying illness (e.g., the APACHE II

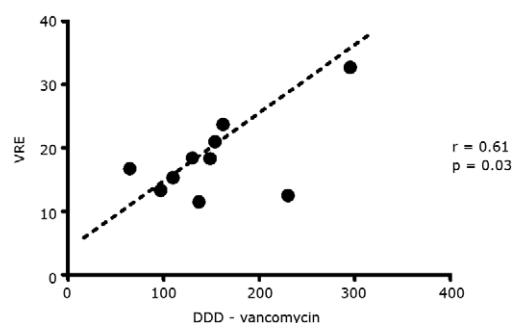


Fig. 2: Pearson's correlation coefficient between vancomycin-resistant enterococci (VRE) colonization per 1,000 patients-day and defined daily doses (DDD) of vancomycin per 1,000 patients-day.

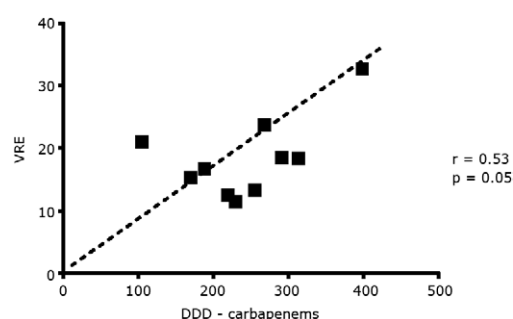


Fig. 3: Pearson's correlation coefficient between vancomycin-resistant enterococci (VRE) colonization per 1,000 patients-day and defined daily doses (DDD) of carbapenems per 1,000 patients-day.

score) was unavailable and the analysis of comorbid conditions was limited by the lack of data on the patient's underlying disease status.

In conclusion, our results showed a higher frequency of colonisation by VRE vanC, without an associated infection, associated with several risk factors, such as prior antibiotic use, carbapenem use and nephropathy as comorbidity. This study documents the emerging trend that the rates of VRE colonisation and administration of antibiotics (high DDD of antimicrobial) are very common and it may provide an accurate view of the problem in the ICUs of Brazilian university hospitals.

Despite several potential limitations, this study is the first to demonstrate the risk factors associated with vanC VRE colonisation in ICU hospitalised patients. Although vanA and vanB enterococci have the greatest clinical importance, vanC VRE epidemiology should be better understood. Despite its indefinite clinical relevance, these species are opportunistic pathogens and vanC VRE-colonised patients are a potential epidemiologic reservoir of resistance genes.

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