Absence of intestinal colonization by vancomycin-resistant enterococci in nonhuman primates[1]

Diego Batista Xavier[2], *Adriana Helena Rosa[2], Hilana dos Santos Sena[2],* Danillo Simonini Teixeira[3], Carlos Tomaz[3] *and* Ricardo Titze-de-Almeida[2*]

ABSTRACT.- Xavier D.B., *Rosa A.H., Sena H.S.,* Teixeira D.S., Tomaz C. *&* Titze-de-Almeida R. 2010. **Absence of intestinal colonization by vancomycin-resistant enterococci in nonhuman primates.** *Pesquisa Veterinária Brasileira 30(6):491-496.* Microbiologia Molecular e Biotecnologia, Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Cx. Postal 04508, Brasília, DF 70910-900, Brazil. E-mail: ricardo.titze@hotmail.com

The animal reservoirs of vancomycin-resistant enterococci (VRE) have important role in the epidemiology of the bacteria and resistant genes. The present work searched fecal samples taken off nonhuman primates for the presence of VRE. Resistance profiles, virulence traits, and genetic variability among enterococci isolates were also analyzed. The samples included Capuchin monkeys (*Cebus apella*, n=28*)* and Common marmoset (*Callithrix penicillata*, n=37) housed in the Primate Center of the University of Brasília, Brazil. Most individuals were captive monkeys from the Central-West and South-East regions of Brazil (n=48). We collected rectal swabs and carried out selective isolation followed by multiplex Polymerase Chain Reaction (PCR) to identify species and resistance genes. No *vanA* or *vanB*-containing enterococci were found. The carriage rates ranged from 1.5% for the VanC-type *E. casseliflavus* and *E. gallinarum* until 12.3% (n=8) for *Enterococcus faecalis*. All *E. faecalis* isolates showed susceptibility to vancomycin, teicoplanin, ampicillin, gentamicin, and streptomycin. The virulence genes *ace* and *esp* were prevalent (100.0%, 87.5%). Multilocus variable number of tandem repeats (MLVA) revealed diversity in the number of repeats among *E. faecalis* isolates and targets, which was higher for *espC*, *efa5*, and *efa6*. We identified six different MLVA genotypes that were divergent from those described in human beings. Also, they were clustered into two genogroups that showed host-specificity for the species *Cebus apella* or *Callithrix penicillata.* In conclusion, no vanA- or vanB-containing enterococci were found colonizing those primate individuals. This finding suggested that the primate individuals investigated in our study are not directly involved in the epidemiological chain of high-level vancomycin-resistant genes vanA or vanB in Brazil. Our study also showed that *E. faecalis* isolated from nonhuman primates carry virulence traits and have ability to spread their lineages among different individuals.

INDEX TERMS: *Enterococcus*, vancomycin-resistance, MLVA, monkey.

RESUMO.- [**Ausência de enterococos resistentes à vancomicina na microbiota intestinal de primatas não-humanos.**] Os reservatórios animais de Enterococos Resistentes à Vancomicina (VRE) têm um importante papel na epidemiologia destas bactérias e dos respectivos genes de resistência. O presente estudo examinou a presença de VRE em amostras fecais obtidas de primatas não-humanos. Foram analisados os perfis de resistência, as características de virulência e a variabilidade genética dos isolados. A amostragem incluiu macacos Prego (*Cebus apella*, n=28*)* e Sagüis do cerrado (*Callithrix penicillata,* n=37) alojados no Centro de Primatologia da Universidade de Brasília, Brasil. A maioria dos indivíduos amostrados foram macacos apreendidos na região Centro-Oeste e Sudeste do Brasil (n=48). Assim, foram coletados swabs retais e realizado o isolamento seletivo, seguido da Reação de Polimerização em Cadeia (PCR) multiplex para identi-

[1] Received on May 20, 2009.
Accepted for publication on April 27, 2010.

[2] Laboratório de Microbiologia Molecular e Biotecnologia, Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília (UnB), Cx Postal 04508, Brasília, DF 70910-900, Brazil. *Corresponding author: ricardo.titze@hotmail.com

[3]Centro de Primatologia e Laboratório de Neurociências e Comportamento, UnB, Brasília, DF.

ficar espécies e genes de resistência. Não foram isolados enterococos contendo os genes vanA ou vanB. A porcentagem de enterococos variou de 1,5% para *E. casseliflavus* e *E. gallinarum* VanC até 12,3% (n=8) para *Enterococcus faecalis*. A totalidade dos isolados da espécie *E. faecalis* demonstrou sensibilidade aos antimicrobianos vancomicina, teicoplanina, ampicilina, gentamicina e estreptomicina. Os genes de virulência *ace* e *esp* foram prevalentes (100%, 87.5%). A análise em multilocus de repetições em *tandem* de número variável (MLVA) revelou diversidade no número de repetições entre os isolados de *E. faecalis*, que foi mais alta para *espC*, *efa5* e *efa6*. Foram identificados seis diferentes genotipos de MVLA, divergindo daqueles já descritos em humanos. Os genotipos foram ainda agrupados em dois genogrupos, demonstrando especificidade de hospedeiro para as espécies *Cebus apella* ou *Callithrix penicillata*. Concluindo, não foram isoladas linhagens de enterococos contendo os genes *vanA* ou *vanB* colonizando as espécies de primatas analisadas. O presente estudo demonstrou que os isolados de *E. faecalis* obtidos de primatas não-humanos apresentam determinantes de virulência e possuem a habilidade de disseminar linhagens entre diferentes indivíduos.

TERMOS DE INDEXAÇÃO: *Enterococcus*, resistência à vancomicina, MLVA, macacos.

INTRODUCTION

Two decades after the first isolation reports, the epidemiology of vancomycin-resistant enterococci (VRE) remains not fully understood (Uttley et al. 1988, Cetinkaya et al. 2000, Nordmann et al. 2007). The bacteria colonize pets and food production animals and have shown remarkable ability to spread hospital-adapted lineages (Aarestrup et al. 2002, Klare et al. 2003, Willems et al. 2005, Rice 2006).

Most epidemiological studies in animals were focused on the food production chain and found relationships between the use of growth promoters and the rate of resistance development (Bager et al. 1997, Aarestrup et al. 2001). These studies also identified virulence genes in *E. faecalis* strains isolated from food (Franz et al. 2003, Moreno et al. 2006). However, resistance and virulence traits in enterococci isolated from wild animals were seldom reported (Livermore et al. 2001, Butaye et al. 2002, Poeta et al. 2005). This study was aimed to examine whether VRE colonize individuals from the species *Cebus apella* and *Callithrix penicillata* housed in a Primate Center from Brazil. We also analyzed genes for virulence and resistance, as well as the genetic variability among *Enterococcus faecalis* isolates.

MATERIALS AND METHODS

Individuals. We examined 65 fecal samples from Capuchin monkeys (*Cebus apella*, n=28*)* and Common marmoset (*Callithrix pennicilata*, n=37) housed in the Primate Center of the University of Brasília (CPUnB), Brazil, during July and August 2006. The samples included animals breed at the CPUnB (n=17) and captive monkeys from the Central-West and South-West Regions of Brazil (n=48) (Table 1). The animals were feed daily with tropical fruits (banana, orange, pineapple, mango, and others), vegetables (potato, carrot, tomato, pumpkin, cucumber, cabbage, cauliflower, spring greens, and others), and commercial cat food. Twice a week they received insect larva as a protein source. Water was provided *ad libitum*. We administrated twice a year either Ivermectin or a combination of Pyrantel, Oxantel and Praziquantel as antiparasitics for the species *C. apella* and *C. pennicillata*, respectively. Individuals eligible for this study received no antibiotic treatment within 12 months prior the sampling procedure.

Specimen collection, isolation of enterococci and species identification. The rectal swabs (n=65) were inoculated in the selective media *BBL Enterococcosel broth* (Becton Dickinson and Co., Sparks, MD) supplemented with vancomycin 8μg/mL. After 24-48h of incubation at 35°C, the positive samples were seeded onto sheep blood agar plates. The colonies showing typical morphology and positive phenotypic testing for *Enterococci* were submitted to multiplex PCR as described earlier (Titze-de-Almeida et al. 2004a; Table 2). This PCR identifies the species *E. faecalis*, *E. faecium*, *E. gallinarum* (*vanC1*), *E. casseliflavus* (*vanC2/3*) and, in addition, the resistance genes *vanA* and *vanB* (Xavier et al. 2006).

Virulence genes detection and multilocus variable number of tandem repeat analysis - MLVA. We used a previously described MLVA scheme to analyze genetic polymorphisms in seven different repeat regions (Titze-de-Almeida et al. 2004b). The method was also used to type the isolates and to identify the virulence genes *ace* and *esp* that code for microbial surface proteins with function of cellular adhesins. To obtain the DNA, the strains were cultured at 37°C in brain heart infusion broth; 1.0 mL of this media was then centrifuged at 13.000 G for 3 min. The pellets were diluted in 100μL of sterile milli-Q water, boiled for 10 min., and centrifuged at 13.000 G for 3 min. To prepare the PCR mixture, we added 5μL of this supernatant, 25pmol of the previously described primers *aceB*, *espC*, *espA*, *efa2*, *efa3*, *efa5* and *efa6* (Titze-de-Almeida et al. 2004b; Table 2), 10 mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.25 mM of each deoxynucleotide triphospate (dATP, dCTP, dGTP, dTTP), and 1.2U of Taq DNA polymerase. We used the following PCR program: initial preheating step at 94° for 2 min; initial denaturation step at 94°C for 4min; 35 cycles of amplification (denaturation 94°C for 1 min., annealing at 55°C for 1min, extension at 72°C for 2min); and a final extension at 72°C for 5min. The PCR products were electrophoresed through a 1% agarose gel stained with ethidium bromide for 1 hour at 60V. The gel was photographed under UV light.

Antimicrobial susceptibility testing. The disk diffusion susceptibility testing was used to determine antimicrobial resistance to vancomycin, teicoplanin, and ampicillin, according to the NCCLS guidelines (NCCLS, 2002). High-level resistance to gentamicin and streptomycin (Oxoid, Basingstoke, UK) was also tested.

RESULTS

Sixty-five rectal samples from Capuchin monkeys and Common marmoset from *the* Primate Center of the University of Brasília were examined for the presence of VRE. No *vanA*- or *vanB*-containing enterococci were found in this study. The most prevalent species was *Enterococcus faecalis* (n=8, 12.3%), followed by *E. gallinarum* (n=1, 1.5%, *vanC1*), and *E. casseliflavus* (n=1, 1.5%, *vanC2/3*).

Six of the seven MLVA loci showed genetic variability (Table 3). The number of repeats differed strongly among the isolates

Table 1. Individuals distribution according to the species and geographic origin

Specie	Origin	Year										
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
Callithrix penicillata	CPUnB[a]	-	1	0	2	1	2[f]	4	-	4	2	16
	Captive (CW)[b]	-	-	3[e]	1	4	1	-	1	5	1	16
	Captive (SE)[c]	-	3[d]	-	-	-	-	-	-	2	-	5
Cebus paella	CPUnB[a]	-	-	-	-	-	-	-	-	1	-	1
	Captive – (CW)[b]	8	2	2	-	6[g]	5	-	-	2	2	27
Total		8	6	5	3	11	8	4	1	14	5	65

[a] Individuals breed at the Primate Center of the University of Brasília (CPUnB), according to the date of birth.
[b] Captive monkeys from the Central-West Brazilian Region, according to the year they were brought to CPUnB.
[c] Captive monkeys from the South-East Brazilian Region, according to the year they were brought to CPUnB.
[d] Samples from which the isolates M1 and M8 were obtained.
[e] Samples from which the isolates M2, M4, and M7 were obtained.
[f] Samples from which the isolate M3 was obtained.
[g] Samples from which the isolates M5 and M6 were obtained.

Table 2. Oligonucleotide primers and target genes for PCR reaction

Amplified gene	Sequence (5'- 3')[a]	Repeat / Ampliconsize (bp)	Reference
aceB	F: AAAATGTGGAAATGCCAACAGAAGAAAGTC R: ATTTAATTTTTGAATTGGTTCACTAAGCAG	141[b]	Nallapareddy et al., 2000
esp2/5	F: CAGATGGATCATCTGATGAAGT R: GTAACGTTACTGTTACATCTGC	252[b]	Shankar et al., 1999
esp46/47	F: TTACCAAGATGGTTCTGTAGGCAC R: CCAAGTATACTTAGCATCTTTTGG	246[b]	Shankar et al., 1999
efa2	F: TTCGCTGGTTCTTCAGG R: CGTTGAGGCAGTAGATATTTTAAC	393[b]	Titze-de Almeida et al. 2004b
efa3	F: TGTCACGCCATCTAAATTG R: CACCAGTGGGGTATGTATTAG	282[b]	Titze-de Almeida et al. 2004b
efa5	F: TTGTTTCGTACCAGTTTGATC R: GTTAAATGGCAGCTCGC	213[b]	Titze-de Almeida et al. 2004b
efa6	F: AGTACCAAGGACCGTGC R: ATTTAGATGCTCGTTTTGTAGG	270[b]	Titze-de Almeida et al. 2004b
vanA	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732[c]	Dutka-Malen et al. 1995
vanB	F: ACCTACCCTGTCTTTGTGAA R: AATGTCTGCTGGAACGATA	300[c]	Zanella et al. 2003
vanC-1	F: GGTATCAAGGAAACCTC R: CTTCCGCCATCATAGCT	822[c]	Dutka-Malen et al. 1995
vanC-2, *vanC-3*	F: CTCCTACGATTCTCTTG R: CGAGCAAGACCTTTAAG	439[c]	Dutka-Malen et al. 1995
$ddl_{E.\ faecalis}$	F: ATCAAGTACAGTTAGTCT R: ACGATTCAAAGCTAACTG	941[c]	Dutka-Malen et al. 1995
$ddl_{E.\ faecium}$	F: TAGAGACATTGAATATGCC R: TCGAATGTGCTACAATC	550[c]	Dutka-Malen et al. 1995 M

[a] F, sense primer; R, antisense primer.
[b] Size of each repeat amplified through the MLVA typing method.
[c] Size of the amplified sequence used for enterococci speciation and vancomycin resistance detection.

and targets, which varied from one repeat in *espA* until nine in *espC* (Table 4). Except for *aceB*, all loci showed any negative repeat results. The variability in the number of repeats was higher for *espC*, *efa5*, and *efa6*, in comparison with *aceB*, *espA* and *efa2*. Six different MLVA genotypes were found and they were clustered in four genogroups of isolates sharing 6-7 identical number of repeats (Table 3). Two of them composed major genogroups of isolates. The genogroup I comprised enterococci with MLVA types 1 and 2, all of them isolated from *C. penicillata* individuals. Within this genogroup, three isolates showed identical number of repeats for all the seven alleles tested (M1- M3); the remaining one (M4) differed only in *efa5* target. MLVA types 3 and 4 were clustered in the genogroup II. This genogroup contained two isolates from the species *C. apella* that differed only in *espC* repeats.

Genes coding for the virulence traits *aceB* and *espC* were identified in *E. faecalis* isolates, as part of the MLVA method. All isolates harboured *aceB* and seven of the eight isolates carried *espC*.

The disk diffusion testing showed antimicrobial susceptibility to vancomycin, teicoplanin, ampicillin, gentamicin, and streptomycin.

DISCUSSION

The present study examined rectal samples from the nonhuman primates *Cebus apella* and *Callithrix penicillata* for the presen-

Table 3. Multilocus variable number of tandem repeat analysis polymorphism among the *Enterococcus faecalis* strains isolated from *Callithrix penicillata* and *Cebus apella*

Species	Sample code	Genogroup	MLVA type	Number of tandem repeats						
				aceB	*espC*	*espA*	*efa2*	*efa3*	*efa5*	*efa6*
C. penicillata	M1	I	1	4	9	1	0	0	7	0
	M2	I	1	4	9	1	0	0	7	0
	M3	I	1	4	9	1	0	0	7	0
	M4	I	2	4	9	1	0	0	0	0
C. apella	M5	II	3	4	9	0	0	0	5	4
	M6	II	4	4	2	0	0	0	5	4
C. penicillata	M7	III	5	3	0	0	2	0	5	5
C. penicillata	M8	IV	6	4	2	0	0	0	7	0

Table 4. Diversity in multilocus variable number of tandem repeat analysis allele scores as determined by the number of repeats in given loci

Gene	Number of tandem repeats (frequence in alleles)							
	0	1	2	3	4	5	7	9
aceB				1(12.5)	7(87.5)			
espC	1(12.5)		2(25.0)					5(62.5%)
espA	4(50.0)	4(50.0)						
efa2	7(87.5)		1(12.5)					
efa3	8(100.0)							
efa5	1(12.5)					3(37.5)	4(50.0)	
efa6	5(62.5%)				2(25.0)	1(12.5)		

ce of VRE. Antimicrobial resistance, genetic polymorphism, and presence of virulence genes were also evaluated.

Enterococcal species prevalence varied greatly according to the host species studied. Poeta et al. (2005) examined faecal samples (n=77) from various wild animals including birds of prey, owls, foxes, wild rabbits, European genets, forest wildcats, salamanders, storks, magpies, deer, vipers, otters, wolves, mouflon, badgers, partridge, hedgehog, pigeon, ferret, quails and wild boar. In that study, *Enterococcus faecalis* (52.1%) was the most prevalent species, followed by *E. faecium* (32.1%), *E. hirae* (10%), *E. casseliflavus* (2.8%), and *E. gallinarum* (1.4%). In contrast, the prevalence of *E. faecalis* was relatively low (1.5%) in *Sus scrofa* wild boars, in which *E. faecium* (50%) and *E. hirae* (40.3%) were prevalent (Poeta et al. 2007).

The method used for enterococci isolation may also affects the species prevalence results. In a previous study using selective media with vancomycin, *E. gallinarum* (VanC1, 13.0%) and *E. casseliflavus* (VanC2/3, 5.5%) were the most prevalent species isolated from poultry raised on nonintensive production farms in Brazil (Xavier et al. 2006). The Van-C enterococci were also prevalent in samples from wild animals (deer, duck, goose, llama, and squirrel) from USA in a surveillance study using selective media for VRE (Rice et al. 2003). Despite using the selective isolation in our study, the prevalence rate for *E. faecalis* (12.3%) was higher than those observed for *E. gallinarum* and *E. casseliflavus* (1.5% each one).

VRE have been identified from food production animals and pets (Aarestrup et al. 2002) but few studies reported their isolation from wild animals. Mallon et al. (2002) isolated lineages of *E. faecium* with vanA genotype from woodmice (*Apodemus sylvaticus*, n=6, 4.6%) and badgers (*Meles meles*, n=2, 1.2%) in the northwest England. However, the bacteria were absent in the bank voles (*Clethrionomys glareolus)* that live in the same region. High-level vancomycin resistant enterococci were also absent from 77 wild animal samples (mammals, birds, and other) from Portugal natural parks (Poeta et al. 2005). Rice et al. (2003) also failed to find VRE in a surveillance study on 14 different species of domestic and wild animals, including cow, deer, duck, goat, horse, llama, pig, rabbit, sheep, squirrel, dog, turkey, and goose. In a previous study, we also found absence of VRE in cloacal samples from poultry raised on nonintensive production farms in Brazil (Xavier et al. 2006). In the present study, no vanA- or vanB-containing enterococci were found in Capuchin monkeys and Common marmoset housed in the Primate Center of the University of Brasília, Brazil.

Few surveillance studies in wild animals have tested antimicrobial resistance to the clinically important antibiotics teicoplanin, ampicillin, gentamicin, and streptomycin. A previous study that analyzed 140 enterococci isolated from wild animals in Portugal revealed susceptibility to teicoplanin and ampicillin; resistance to gentamicin and, streptomycin occurred in only two isolates from an owl (Poeta et al. 2005). Low resistance rates to kanamycin, streptomycin, and ampicillin (9.0%, 6.7%, 3.7%), and susceptibility to gentamycin was found in samples from *Sus scrofa* (Poeta et al. 2007). In our study, *E. faecalis* strains isolated from the nonhuman primates *Cebus apella* and *Callithrix penicillata* were all susceptible to teicoplanin, ampicillin, gentamicin, and streptomycin. The low prevalence of antimicrobial resistance in enterococci isolated from wild animal species contrasts with those data concerned to food animals. This fact corroborates the selective pressure theory, in which the volume of antibiotic use would affect the rate of resistance development (Aarestrup et al. 2001).

In our study, we also examined whether enterococci colonizing wild monkeys carry the virulence genes *ace* (adhesin of collagen from *E. faecalis*) and *esp* (enterococcal surface

protein) (Shankar et al. 1999, Nallapareddy et al. 2000, Mundy et al. 2000). Both factors are involved in microbial adhesion to host tissue; Ace binds extracellular matrix proteins and Esp is involved in microbial infection and biofilm formation, which exact role remains controversial (Shankar et al. 1999, Nallapareddy et al. 2000, Willems & Bonten 2007). In a previous study from a broad range of wild animals, the prevalence of *ace* and *esp* was relatively low (9.6%, 4.1%) compared to values found in our study (100% and 87%, respectively). The prevalence of *esp* found in our study was also higher than those observed in *E. faecalis* isolates recovered from human (44-46%) and food (33%) samples (Eaton & Gasson 2001, Titze-de-Almeida et al. 2004b).

The MLVA revealed different levels of genetic variability among the loci studied, which was previously described in medical isolates (Titze-de-Almeida et al. 2004b). *espC*, *efa5*, and *efa6* showed three different alleles, contrasting with *aceB*, *espA*, and *efa2* that presented only two different alleles (Table 4). The six MLVA types found in nonhuman primates (Table 3) differed from those previously described in human beings (Titze-de-Almeida et al. 2004b). These genotypes were classified in major clusters named genogroups I (n=4) and II (n=2) and others composed by single isolates, genogroups III and IV. This clustering characteristic was also observed in previous work from human beings, where isolates formed major genogroups (MLVA types 9 and 37; n=13 and n=14, respectively) and minor genogroups composed by unique isolates, MLVA types 1, 2, 4-8 (Titze-de-Almeida et al. 2004b). The enterococci have shown a remarkable ability to spread epidemic clones throughout the word, as observed for the hospital-adapted *E. faecium* clonal complex-17 (Titze-de-Almeida et al. 2004a, Willems et al. 2005, Titze-de-Almeida et al. 2006, Willems & Bonten 2007). In our study, the genetically related isolates M1-M4 from the genogroup I have colonized monkeys originated from geographically distant regions which includes the South-east (M1), Central-West (M2, M4), and CPUnB (M3) (Table 1 and 3). We should remark that these primates live in distinct cages in CPUnB. However, the cage structure allowed physical contact between neighbors. In addition, the animals were treated by the same technicians, which could contribute for the lineages spreading.

Indeed, host-specificity recently described in enterococci suggests the microbial lineages evolved in parallel with the host evolution (Willems et al. 2000). In our study, we also found host-specificity among nonhuman primate enterococci isolates; the MLVA genogroups I and II shared no isolates from different primate species.

In conclusion, we found absence of VRE colonizing individuals from the species *C. apella* and *C. penicillata* housed in CPUnB. *E. faecalis* isolated from these primates showed host-specificity, ability to harbour virulence genes, and the spreading behavior already reported in other animal species.

REFERENCES

Aarestrup F.M., Seyfarth A.M., Emborg H.D., Pedersen K., Hendriksen R.S. & Bager F. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob. Agents Chemother. 45:2054-2059.

Aarestrup F.M., Butaye P. & Wolgang W. 2002. Nonhuman Reservoirs of *Enterococci,* p.55-99. In: Gilmore M.S. (Ed.), The *Enterococci:* Pathogenesis, molecular biology, and antibiotic resistance. ASM Press, Washington, DC.

Bager F., Madsen M., Christensen J. & Aarestrup F.M. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev. Vet. Med. 31:95-112.

Butaye P., Baele M., Devriese L.A. & Haesebrouck F. 2002. Comparison of susceptibility to antimicrobials of the enterococcal species isolated from pigeons (*Columba livia*). Microb. Drug Resist. 8:215-218.

Cetinkaya Y., Falk P. & Mayhall C.G. 2000. Vancomycin-resistant *Enterococci*. Clin. Microbiol. Rev. 13:686-707.

Dutka-Malen S., Evers S. & Courvalin P. 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR. J. Clin. Microbiol. 33:24-27.

Eaton T.J. & Gasson M.J. 2001. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. 67:1628-1635.

Franz C.M., Stiles M.E., Schleifer K.H. & Holzapfel W.H. 2003. *Enterococci* in foods: A conundrum for food safety. Int. J. Food Microbiol. 88:105-122

Klare I., Konstabel C., Badstübner D., Werner G. & Witte W. 2003. Occurrence and spread of antibiotic resistances in *Enterococcus faecium*. Int. J. Food Microbiol. 88:269-290.

Livermore D.M., Warner M., Hall L.M.C., Enne V.I., Projan S.J., Dunman P.M., Wooster S.L. & Harrison G. 2001. Antibiotic resistance in bacteria from magpies (*Pica pica*) and rabbits (*Oryctolagus cuniculus*) from west Wales. Environ. Microbiol. 3:658-661.

Mallon D.J.P., Corkill J.E., Hazel S.M., Wilson J.S., French N.P, Bennett M. & Hart C.A. 2002. Excretion of vancomycin-resistant enterococci by wild mammals. Emerg. Infect. Dis. 8:636-638.

Moreno M.R.F., Sarantinopoulos P., Tsakalidou E. & De-Vuyst L. 2006. The role and application of enterococci in food and health. Int. J. Food Microbiol. 106:1-24.

Mundy L.M., Sahm D.F. & Gilmore M. 2000. Relationships between enterococcal virulence and antimicrobial resistance. Clin. Microb. Rev. 13:513-522.

Nallapareddy S.R., Singh K.V., Duh R., Weinstock G.M. & Murray B.E. 2000. Diversity of *ace*, a gene encoding a microbial surface component recognizing adhesive matrix molecules, from different strains of *Enterococcus faecalis* and evidence for production of Ace during human infections. Infect. Immun. 68:5210-5217.

NCCLS. National Committee for Clinical Laboratory Standards (NCCLS). 2002. Performance Standards for Antimicrobial Susceptibility Testing. Twelfth Informational Supplement, M100-S12, 22:1. National Committee for Clinical Laboratory Standards, Villanova, PA (January).

Nordmann P., Naas T., Fortineau N. & Poirel L. 2007. Superbugs in the coming new decade; multidrug resistance and prospects for treatment of *Staphylococcus aureus*, *Enterococcus* spp. and *Pseudomonas aeruginosa* in 2010. Curr. Opinion Microbiol. 10:436-440

Poeta P., Costa D., Sáez Y., Klibi N., Ruiz-Larrea F., Rodrigues J. & Torres C. 2005. Characterization of antibiotic resistance genes and virulence factors in faecal enterococci of wild animals in Portugal. J. Vet. Med. B. 52:396-402.

Poeta P., Costa D., Igrejas G., Rodrigues J. & Torres C. 2007. Phenotypic and genotypic characterization of antimicrobial resitance in faecal *Enterococci* from wild boars (*Sus scrofa*). Vet. Microbiol. 15:368-374.

Rice E.W., Boczek L.A., Johnson C.H. & Messer J.W. 2003. Detection of intrinsic vancomycin resistant *Enterococci* in animal and human feces. Diagn. Microbiol. Infect. Dis. 46:155-158.

Rice L.B. 2006. Antimicrobial resistance in gram-positive bacteria. Am. J. Infect. Control 34:S11-S19.

Shankar V., Baghdayan A.S., Huycke M.M., Lindahl G. & Gilmore M.S. 1999. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. Infect. Immun. 67:193-200.

Titze-de-Almeida R., Rollo-Filho M., Nogueira C.A., Rodrigues I.P., Eudes-Filho J., Nascimento R.S., Ferreira II R.F., Moraes L.M., Boelens H., Van Belkum A. & Felipe M.S.S. 2004a. Molecular epidemiology and antimicrobial susceptibility of *Enterococci* recovered from Brazilian intensive care units. Braz. J. Infect. Dis. 8:197-205.

Titze-de-Almeida R., Willems R.J.L., Top J., Rodrigues I.P., Fonseca II R.F., Boelens H., Brandileone M.C., Zanella R.C., Felipe M.S.S. & Van Belkum A. 2004b. Multilocus variable number of tandem repeats polymorphism among Brazilian *Enterococcus faecalis* strains. J. Clin. Microbiol. 42:4879-4881.

Titze-de-Almeida R., Van Belkum A., Felipe M.S.S., Zanella R.C., Top J. & Willems R.J.L. 2006. Multilocus sequence typing of hospital associated *Enterococcus faecium* from Brazil reveals their unique evolutionary history. Microb. Drug Res. 12:121-125.

Uttley A.H.C., Collins C.H., Naidoo J. & George R.C. 1988. Vancomycin-resistant *Enterococci*. Lancet 1:57-58.

Willems R.J., Top J., van Den Braak N., van Belkum A., Endtz H., Mevius D., Stobberingh E., Van Den Bogaard A. & Embden J.D. 2000. Host specificity of vancomycin-resistant *Enterococcus faecium*. J. Infect. Dis. 182:816-823.

Willems R.J., Top J., van Santen M., Robinson D.A., Coque T.M., Baquero F., Grundmann H. & Bonten M.J. 2005. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. Emerg. Infect. Dis. 11:821-828.

Willems R.J.L. & Bonten M.J. 2007. Glycopeptide-resistant *Enterococci*: deciphering virulence, resistance and epidemicity. Curr. Opin. Infect. Dis. 20:384-390.

Xavier D.B., Bernal F.E.M. & Titze-de-Almeida R. 2006. Absence of VanA- and VanB-Containing *Enterococci* in Poultry Raised on Nonintensive Production Farms in Brazil. Appl. Environ. Microbiol. 72:3072-3073.

Zanella R.C., Brandileone M.C., Bokermann S., Almeida S.C., Valdetaro F., Vitório F., Moreira M.F., Villins M., Salomão R. & Pignatari A. C. 2003. Phenotypic and genotypic characterization of VanA *Enterococcus* isolated during the first nosocomial outbreak in Brazil. Microb. Drug Res. 9:283-291.