Clonality and virulence of isolates of VRE in stem cell transplanted patients

Ana Paula Marchi[1,2], Inneke Marie van der Heidjen[1], Elisa. T. Mendes[1,2], André Doi[,2], Camila Fonseca Rizek[1], Anna Sara S. Levin[1,2], Silvia Figueiredo Costa[1,2].

(1)Department of Infectious Diseases - Laboratory of Medical Investigation (LIM54 - Bacteriology)

(2) Microbiology Laboratory of Hospital das Clinicas/Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP)

Introduction and Objective

Vancomycin-resistant enterococci (VRE) are an important agent of colonization and infection in hematology patients with high mortality, especially those undergoing Hematopoietic Stem Cell Transplantation (HSCT). The role of VRE virulence factors and clonality on the prognosis of hematologic patients is scarce and controversy. The aim of this study was to characterize VRE isolates phenotypically and molecularly, and investigate associations between the virulence factors and colonization/infection and mortality in hematologic and f HSCT patients within 6 years.

Methods

Hospital Setting: Bone Marrow Unit of Hospital das Clínicas of university of São Paulo, Brazil.

Clinical and demographic data of 64 hematologic patients colonized e or infected by VRE and 72 clinical isolates from 2005 to 2011 were collected and analyzed using EpiInfo CDC6.0 PCR was performed for resistance genes *van*A, *van*B and multiplex PCR for virulence genes (*esp*, *asa*1, *gel*E, *cyl*A and *hyl*). Minimum inhibitory concentration (MIC) was performed by microdilution for vancomycin, teicoplanin, gentamicin (HLAR), streptomycin (HLAR) and linezolid according with CLSI. Clonality of VRE isolates was characterized by Pulsed Field Gel Electrophoresis (PFGE). The genetic sequence was compared with the database available on the Internet (BLAST-http://www.ncbi.nlm.nhi.gov/blast/).

Results

The data of 64 patients enrolled in this study are show in table 1 and the characteristics of 72 clinical isolates in table 2. All isolates were positive for *van*A gene (figure 2). All isolates were resistant to vancomycin and the other MIC results are show in table 4. There is a predominant clone named clone A, present in 23 (47%) of infection isolates and none of colonization isolates. The mortality of patients with this predominant profile was 65%. One isolate were resistant to linezolid, besides that, this sample doesn't belong to the predominant clone and only has the *esp* virulence gene. The virulence PCR results are show in table 3 and the figure 1.

Table 1: Characteristics of 64 hematological patiens infected/colonized by VRE

Characteristics	Patients (n=64)
Males	35 (55%)
Average hospitalization (days)	39 (4-116 days)
Average colonization to infection (days)	13 (1-38 days)
Bone marrow transplant	34 (53%)
Allogeneic transplant	31 (74%)
Average age (years)	44 (1-70 years)

Table 2: Characteristics of 72 clinical VRE isolates

Characteristics	Isolates (n=72)
E. Faecium	70 (97%)
E. Faecalis	2 (3%)
Blood	50 (69%)
Urine	1 (1%)
Feces, sputum	22 (29%)

Figure 1. Virulence genes of VRE isolates detected by PCR multiplex

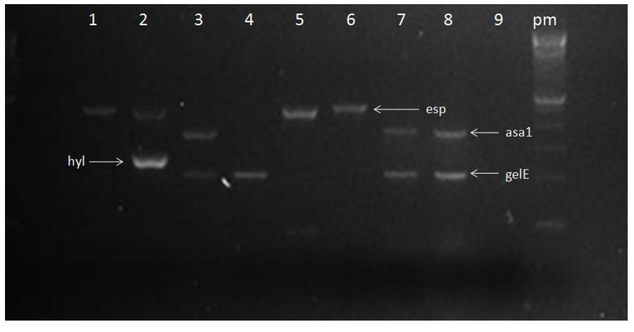

Figure 2. *van*A gene of VRE detected by PCR

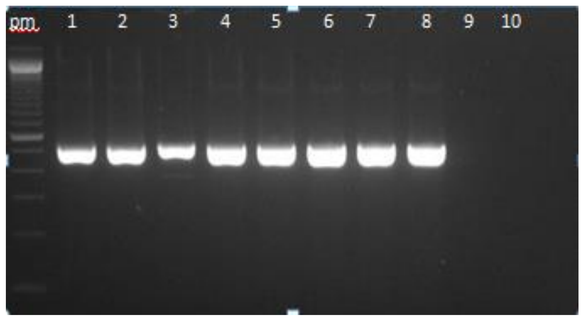

Table 3. VRE Virulence genes detected by PCR

Virulence gene	Presence (%) n=72 isolates
esp	94
gelE	69
asa1	72
cylA	0
hyl	1

Table 4. Antibiotics MIC against VRE isolates

Antibiotic	Resistance (%) n=72 isolates
Vancomycin	100
Teicoplanin	86
Linezolid	1
Gentamycin	11
Streptomycin	81

Table 5: Distribution of PFGE profiles and virulence genes of 55 VRE isolates of Bone Marrow Transplant Unit-HC-FMUSP 2006-2011.

Clonality	PCR for virulence genes					
	Infection % (n=49)			Colonization % (n=6)		
	esp	***asa*1**	***gel*E**	***esp***	***asa*1**	***gel*E**
Clone A	47	39	41	0	0	0
Closely related N= 24	43	35	37	50	0	0
Possibly related N= 3	6	2	0	0	0	0
Different N= 5	4	4	4	50	33	17

Conclusions

In our hospital, *E. faecium* was the most frequently specie identified. There was a predominant clone that harbored the virulence genes *esp*, *asa*1 and *gel*E, associated with high mortality rate in infected patients. Colonized isolates were less virulent than the infection ones. Linezolid resistance was detected in only one isolated that belonged to a different clone and harbored only *esp*.

References

PETRICH, A. et al. Direct detection of vanA and vanB genes in clinical specimens for rapid identification of vancomycin resistant enterococci (VRE) using multiplex PCR. Molecular and Cellular Probes; 13:275-281, 1999. RICE, L. B.; et al.; A Potencial Virulence Gene hylEfm, Predominates in Enterococcus faecium of Clinical Origin. The Jounal of Infectious Diseases; 187:508-512, 2003, PALMET, K.L.; et al. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. Current Opinion in Microbiology, Science Direct; 13:632-639; 2010.