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A preliminary molecular epidemiologic study using analysis of variable number of tandem repeats of *Acinetobacter baumannii* OXA-23 producing strains isolated from hospitals in Rio de Janeiro State, Brazil

Estudo epidemiológico molecular preliminar usando a análise de número variável de repetições em tandem (VNTR) de cepas de *Acinetobacter baumannii* produtoras de OXA-23 isoladas em hospitais no Rio de Janeiro, Brasil

Estudio epidemiológico molecular preliminar usando el análisis de número variable en tándem de repeticiones (VNTR) de cepas de *Acinetobacter baumannii* productoras de OXA-23 aisladas en hospitales de Rio de Janeiro, Brasil

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ABSTRACT

The Gram-negative multidrug-resistant (MDR) bacterium *Acinetobacter baumannii* constitutes a serious cause of nosocomial infections in Brazilian hospitals. A panel of 36 strains, belonging to five pre-determined pulsed-field gel electrophoresis (PFGE) genotypes from four different hospitals in the City of Rio de Janeiro, Rio de Janeiro State, Brazil, was submitted to simple agarose gel electrophoresis to determine the variation of nine variable number tandem repeats (VNTR) band profiles. Based on published data VNTR were classified in two categories, long (L) and short (S) repeats with different evolutionary implications. The results demonstrated the superior discrimination of VNTR over PFGE. The VNTR Abaum_3002 marker was the least discriminatory with only one allele, while VNTR10 and Abaum_2240 each presented four alleles. The use of a combination of the nine VNTR resulted in a refined genotyping tool that provides valuable epidemiological information.

Keywords: *Acinetobacter baumannii*; VNTR; Public Health; Epidemiology.

The Gram-negative bacterium, multidrug-resistant (MDR) *Acinetobacter baumannii*[1] constitutes a signficant cause of nosocomial infections in Brazilian hospitals. According to the MYSTIC Program Brazil[2] it features considerably among the total number of nosocomial bacterial isolates recorded in intensive care units. Moreover, the carbapenemases OXA-23 and OXA-143 continue to be observed in Brazilian hospitals[3]. Recently Zarrilli et al[4] reviewed the phenotypic and genotypic methods for typing of *A. baumannii*. In 1996, Dijkshoorn et al[5] reported the development of a rapid and simple detection method, based on analysis of variable number tandem repeats (VNTR), for clonal types of important nosocomial bacteria, that could potentially overcome the limitations and reduce the financial burden associated with pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). PFGE has a limited level of discrimination and is time consuming while MLST, although it is a portable technique, is a costly being restricted primarily to research laboratories[6]. VNTR have been found in the genomes of several bacteria including *Staphylococcus aureus*, *Bacillus anthracis*, *Bacillus cereus* and *Neisseria*[7,8,9,10,11]. Turton et al[12] and Pourcel et al[6] suggested the use of VNTR as a complement or even for substitution of the other methodologies. VNTR is a technique based on the PCR detection of specific repetitive sequence(s) active in different individuals, which results in an electrophoretic fingerprint. Multiple-locus VNTR analysis, also known as MLVA, presents the polymorphism among different tandemly repeated DNA sequences[12]. The division of VNTR markers into two groups is made according to their size and rate of evolution. Thus, Short repeats (S-repeats)

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are 6 to 9 bp in length and appear to have a high rate of evolution, while Long repeats (L-repeats) are 26 to 99 bp in length and show a slower rate of evolution[6]. This rationale was proposed to aid in phylogenetic studies, outbreak investigations and epidemiological surveillance[6].

The occurrence of different PFGE types among OXA-23 producing *A. baumannii* in different hospitals in Rio de Janeiro State, Brazil, was reported in 2011, and more recently Carvalho[13] studied some phenotypic characteristics such as, adherence to epithelial cells and biofilm formation in the same set of strains used in the present study. In addition, the strains were subjected to PCR and phenotypic detection of carbapenemases genes and characterization by MLST for population studies[3]. In light of the existing classification, we decided to investigate the potential utility of VNTR analysis using a small, but representative, panel of 36 strains of this collection of OXA-23-producing *A. baumannii* clinical isolates which were recovered from four different hospitals in Rio de Janeiro between January 2006 and September 2007 and that represented five PFGE types[3,14]. The 36 strains belong to the five previously defined PFGE genotypes (A, B, C, D and E) and S (non-producing OXA-23) (Table 1)[3]. The VNTR profile (string numbers) was obtained by simple agarose gel electrophoresis of PCR amplicons according to previously described methodology[6].

Table 1 – Characteristics of *A. baumannii* used in this study and the genetic diversity of PFGE group and MLVA profile

Strain	PFGE group*	Sex	Source	Year	Origin	Ab 2240†	Ab 3002†	Ab 1988†	A 3530†	Ab 3468†	Ab 0826†	Ab 0845†	VNTR 1‡	VNTR 10‡
15	A	F	Blood	2006	H1	2	1	1	2	3	2	1	2	2
56	A	M	Liquor	2006	H3	4	1	1	2	1	2	1	3	2
65	A	F	Liquor	2006	H3	3	1	1	2	2	1	1	2	2
130	A	M	WS	2007	H1	2	1	1	2	3	3	1	3	2
167	A	F	TS	2007	H1	2	1	1	2	3	3	1	2	–
175	A	F	BAL	2007	H1	2	1	1	2	3	3	1	2	2
261	A	F	Catheter	2007	H3	2	1	1	2	3	2	1	3	2
305	A	F	Blood	2007	H1	–	1	1	2	1	2	1	1/3	2
317	A	M	Catheter	2007	H4	2	1	1	2	1	3	1	3	2
325	A	M	Catheter	2007	H1	2	1	1	2	3	1	1	3	–
350	A	M	Blood	2007	H4	–	1	–	2	1	2	1	3	2
379	A	M	TS	2007	H2	2	1	1	2	2	1	1	3	2
380	A	F	Sputum	2007	H2	2	1	1	2	2	3	1	3	2
382	A	M	BAL	2007	H2	2	1	1	2	2	3	1	3	2
384	A	M	Blood	2007	H2	2	1	1	2	1	3	1	3	1
401	A	M	C	2007	H2	2	1	1	2	2	3	1	3	2
405	A	F	Liquor	2007	H1	2	1	1	2	1	3	1	3	2
436	A	M	TS	2007	H4	–	1	1	–	3	3	1	3	1
501	A	F	TS	2007	H4	2	1	1	2	2	3	1	3	2
295	B	F	TS	2007	H4	1	–	2	2	1	3	1	–	–
356	B	M	BAL	2007	H3	3	1	2	2	1	2	1	1	3
375	B	M	Blood	2007	H2	1	1	2	2	1	2	1	1	4
386	B	M	TS	2006	H2	3	1	2	2	1	2	2	1	3
387	B	M	BAL	2007	H1	3	1	2	2	1	2	2	1	2/3
394	B	F	TS	2006	H2	3	1	2	2	1	2	2	1	4
396	B	F	TS	2006	H2	3	1	2	2	1	2	2	1	3
403	B	M	AS	2007	H1	3	1	2	1	3	1	2	1	1
470	B	F	Blood	2007	H3	3	1	2	1	3	2	1	–	1
473	B	M	BAL	2007	H3	3	1	2	1	3	3	1	–	1
480	B	M	TS	2007	H3	3	1	2	1	3	3	1	–	1
535	C	M	WS	2007	H1	–	1	2	2	1	2	1	1	3
539	C	M	BAL	2007	H3	1	1	2	2	1	2	1	1	2/3
542	C	M	Blood	2007	H3	1	1	2	2	2	3	1	–	3
71	D	M	An Swab	2007	H4	3	1	1	2	3	2	1	2	2
508	E	M	Blood	2007	H1	2	1	–	–	2	3	2	3	2
549	S	F	Urine	2007	H3	1	–	2	–	1	–	1	1	1

WS: wound secretion; TS: traqueal secretion; BAL: bronchoalveolar lavage; An swab: anal swab; AS: abdominal secretion; C: culture; *Grosso et al[3]; †Pourcel et al[6]; ‡Turton et al[12]; (–) null allele.

As reported by Grosso et al[3], strains from PFGE type A, B, C and D were classified as ST131, ST132, ST133 and ST134 in accordance with the MLST-OD scheme (associated with the Oxford Database) and ST79, ST15 and two new allelic profiles within the MLST-IP scheme (developed by the Institute Pasteur). The genotypes E and S were not submitted to multilocus sequence typing (MLST) schemes.

In the present study we included nine VNTR primer pairs; Abaum_2240, Abaum_3002, Abaum_1988, Abaum_3530 (long-repeats), Abaum_3468, Abaum_0845, Abaum_0826, VNTR1 and VNTR10 (short-repeats). The amplification conditions used were as described by Pourcel et al[6]. The PCR amplicons were submitted to electrophoresis in 3.2% (S-repeats) and 2% (L-repeats) agarose gels. Following electrophoresis the molecular weight (MW) of each band was estimated using the LabImage 1D gel analysis program (Loccus Biotecnologia, Brazil). In the present study a dendrogram based on VNTR profiles (Figure 1) was created and comparison of the banding patterns was accomplished employing the unweighted pair-group method with arithmetic averages using the Dice similarity coefficient. Computer-assisted analysis was performed using BioNumerics v.4.0 (Applied Maths, Sint-Martens-Latem, Belgium).

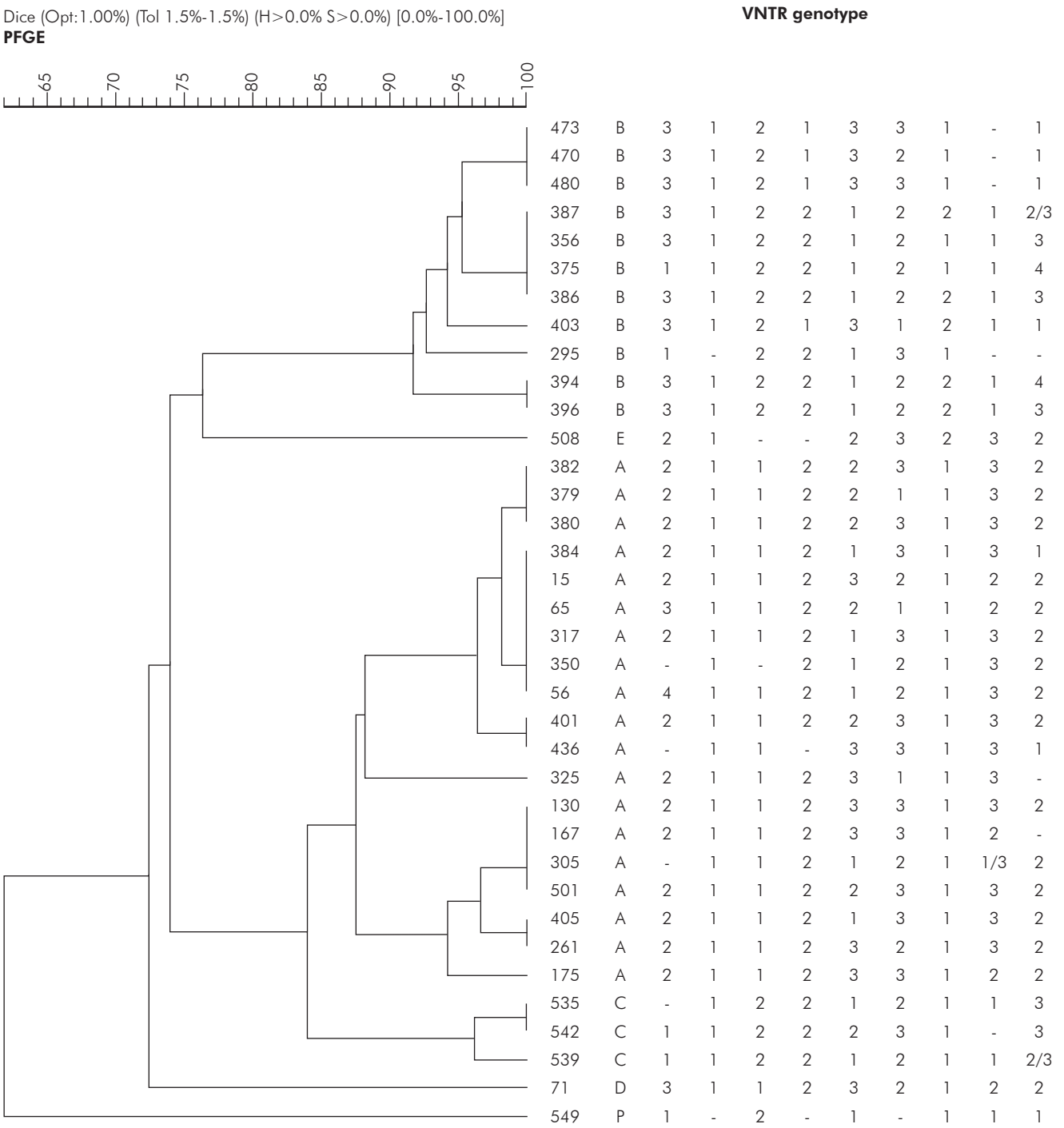

Figure 1 – VNTR profile: fingerprint concordance between MLVA-9 and PFGE typing. Dendrogram resulting from computer analysis of the PFGE profiles of *A. baumannii* isolated from four hospitals. The order of the profile (string number) of each VNTR is the same as in table 1

Table 1 shows the VNTR alleles for each VNTR maker used in the study. The analysis of the 36 *A. baumannii* strains, detected 30 different VNTR profiles, each represented by string of numbers (Figure1). The superior discrimination of VNTR relative to PFGE is clearly demonstrated in figure 1 where strains belonging to the same PFGE type presented variation in VNTR, with the exception of the strains LGB380, LGB382, LGB401, and LGB501 (genotype A), LGB317-LGB405 (genotype A), LGB386-LGB396 (genotype B) and LGB473-LGB480 (genotype B). As a specific example, the strains LGB130 and LGB305, both classified as PFGE type A isolated in the same year from the same hospital at the same time, demonstrated differences in four VNTR (VNTR1, Abaum_3468, Abaum_0826 and Abaum_2240).

Top et al[15] studying *Enterococccus faecium* demonstrated the discriminatory power of six VNTR-MLVA. More recently strains of *A. baumannii*, producers of CHDL belonging to ICL-II, were typed by MLVA-8[16]. In addition, Hauck et al[17] utilized an automated VNTR protocol in the investigation of *A. calcoaceticus-A. baumannii* complex strains from different hospitals. Moreover, Hu et al[18] compared a VNTR based analysis with PFGE typing of *A. baumannii* in China and observed a remarkable congruence between MLVA-7 and PFGE-based strain clustering.

Taken as a whole our results are in congruence with data presented in the literature and support the use of VNTR analysis for discrimination of isolates of *A. baumannii*. According to table 1 it can be inferred that the similarity of MLVA is greater within PFGE profiles than between them. As such the use of VNTR does not undermine to PFGE data, but it does show a greater power for detecting genetic variation in clonal groups than can be achieved by PFGE. It was noted that the marker Abaum_1988, except in the case of strain LGB350 (which seems to have a null allele for this VNTR), was conserved among strains of PFGE type A. Nevertheless, more strains should be analyzed in order to confirm if these *loci* could be employed as a molecular marker of Brazilian PFGE type A. Interestingly, no genetic variation was observed among our samples using the VNTR Abaum_3002, which is in contrast with data reported by Pourcel et al[6] which showed that this marker generated three numbers of alleles or types.

It was suggested previously that determination of the number of repeats in VNTR1 and VNTR10 could be valuable in cross-infection studies, with VNTR1 providing the greatest level of discrimination[12], at least among the isolates of OXA-23 clone 1 that were included in that study. It is clear that the discriminatory power of this technique depends on the choice of *locus*. In this context, our data based on the number of alleles showed that VNTR10 was the most polymorphic among the S-repeats while Abaum_2240 were the most polymorphic among the L-repeats, both produced four alleles each (bands) among our strains. Moreover, within the same PFGE group, the VNTR10 and Abaum_2240 markers revealed more differences, an observation which may prove useful in studies that need to be simple, faster and cheaper. In addition, strains with the same PFGE type from different hospitals but from the same hospital chain, e.g. strains LGB382 and LGB130 or strains LGB386 and LGB387 presented a single *loci* difference: Abaum_3468 and VNTR 10, respectively, S-repeat (Table 1), suggesting that the traffic of employees and material between hospitals may serve to promote dissemination of this bacterium. In this context, the analysis of a group of isolates from the same hospital and belonging to the same PFGE group showed higher rates of evolution in the S-repeat in comparison to L-repeat markers. Indeed, with the exception of Abaum_2240 the pattern of L-repeats was maintained among PFGE-types, while the pattern of S-repeats was highly polymorphic.

The dissemination of *A. baumannii* in Rio de Janeiro is reflected by the detection of the same VNTR genotype in different hospitals. This observation calls for improvements in the procedures for prevention of hospital infection and prudence in the use of carbapenems. Improved recognition and early detection of potentially epidemic isolates that show a propensity to emerge in hospital wards can be determined by MLST, and also, based on our data, by VNTR. In light of these data we contend that VNTR should be considered as an important tool for infection control.

Although a VNTR data bank (http://mlva.u-psud.fr/)[9] exists, we did not use this resource since our intention was to facilitate the routine of laboratories which receive samples from hospitals for screening. In conclusion, our results corroborate the impressive discriminatory power of VNTR, as reported by numerous authors and importantly, provide the first report of the applicability of this technique in Brazil. In light of the fact that both MLST and PFGE are technically demanding, expensive and time consuming, the use of VNTR, specifically VNTR10 (due the fact of being a short repeat marker) may be a candidate for further studies on the characterization of strains that belong to an outbreak and to the same PFGE genotype.

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Estudo epidemiológico molecular preliminar usando a análise de número variável de repetições em tandem (VNTR) de cepas de *Acinetobacter baumannii* produtoras de OXA-23 isoladas em hospitais no Rio de Janeiro, Brasil

RESUMO

A bactéria Gram-negativa *Acinetobacter baumannii* multirresistente (*Ab*MR) constitui uma séria causa de infecções nosocominais em hospitais brasileiros. Um painel de 36 cepas, pertencentes a cinco genótipos pré-determinados por eletroforese em gel de campo pulsado (PFGE) de quatro diferentes hospitais na Cidade do Rio de Janeiro, Estado do Rio de Janeiro, Brasil, foi submetido à eletroforese em gel de agarose simples para determinar a variação de nove perfis de número variável de repetições em tandem (VNTR). Com base em dados publicados, os VNTR foram classificados em duas categorias, longa (L) e curta (C) repetições com diferentes implicações evolucionárias. Os resultados demonstraram a discriminação superior de VNTR sobre PFGE. O marcador VNTR Abaum_3002 era o menos discriminatório com apenas um alelo, enquanto VNTR10 e Abaum_2240, cada um, apresentaram quatro alelos. O uso da combinação de nove VNTR resultou em uma aperfeiçoada ferramenta de genotipagem que fornece relevantes informações epidemiológicas.

Palavras-chave: *Acinetobacter baumannii*; VNTR; Saúde Pública; Epidemiologia.

Estudio epidemiológico molecular preliminar usando el análisis de número variable en tándem de repeticiones (VNTR) de cepas de *Acinetobacter baumannii* productoras de OXA-23 aisladas en hospitales de Rio de Janeiro, Brasil

RESUMEN

La bacteria Gram negativa *Acinetobacter baumannii* multirresistente (*Ab*MR) es una seria causa de infecciones nosocomiales en hospitales brasileños. Un panel de 36 cepas, pertenecientes a cinco genotipos predeterminados por electroforesis en gel de campo pulsado (PFGE) de cuatro diferentes hospitales en la Ciudad de Rio de Janeiro, Estado de Rio de Janeiro, Brasil, fue sometido a electroforesis en gel de agarosa simple para determinar la variación de nueve perfiles de número variable de repeticiones en tándem (VNTR). Con base en datos publicados, los VNTR fueron clasificados en dos categorías, larga (L) y corta (C) repeticiones con distintas implicaciones evolutivas. Los resultados demostraron la discriminación superior de VNTR sobre PFGE. El marcador VNTR Abaum_3002 era el menos discriminatorio con un solo alelo apenas, mientras que VNTR10 y Abaum_2240, cada uno, presentaron cuatro alelos. El uso de la combinación de nueve VNTR resultó en una herramienta de genotipado perfeccionada que suministra relevantes informaciones epidemiológicas.

Palabras clave: *Acinetobacter baumannii*; VNTR; Salud Pública; Epidemiología.

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