THE DISTRIBUTION AND GENETIC DIVERSITY EVALUATION OF *ACINETOBACTER BAUMANNII* STRAINS ISOLATED BY RAPD TECHNIQUE AT THANH NHAN HOSPITAL IN 2017 - 2018

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SUMMARY

Objectives: *To determine the distribution and genetic diversity of A. baumannii strains isolated from inpatients at Thanh Nhan Hospital from 10/2017 to 10/2018.* ***Subjects and methods:*** *A descriptive study and laboratory analysis on sixty-seven A. baumannii strains from isolated samples of inpatient at Thanh Nhan Hospital from 2017 to 2018.* ***Results:*** *The distribution of A. baumannii strain in A. baumannii infected male was higher than in female, 89.5% of the patients were over 50 years of age. The rate of A. baumannii was highest with 61% (41/67) that isolated from sputum samples. Patients infected with A. baumannii in intensive care unit was 91%. Analyzing the genetic relationship of 67 strains of A. baumannii at Thanh Nhan Hospital with 12 RAPD primers obtained 126 DNA segments showed that each allele on the primer ranged from 7 - 19 alleles, 100% of the primers were for segment polymorphism. The obtained PIC coefficients ranged from 0.770 to 0.945; the average PIC value was 0.831; 67 strains were divided into 5 main groups, with genetic similarity coefficients ranging from 0.62 to 0.97, indicating that the 67 strains had a relatively large genetic diversity.* ***Conclusions:*** *These strains can be spread through numerous pathways, especially through contact transmission. Therefore, standard precautions and compliance with hospital infection control measures are essential.*

** Keywords: A. baumannii strain; Capital technique.*

INTRODUCTION

Multidrug-resistant organisms are considered the biggest problem in the 21st century, especially in developing countries with burden of infectious diseases [3]. In Vietnam, according to the Global Antibiotic Resistance Association, the level of carbapenem-resistance of *A. baumannii* strains was nearly 50% at 6 hospitals in 2008 [2]. Report by 108 Military Central Hospital at the workshop "Sharing experiences in interdisciplinary coordination in monitoring and response to antibiotic resistance in Vietnam" demonstrates that the rate of carbapenem resistant *A. baumannii* in 2015 - 2016 was 32%.

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More than 60% of *Acinetobacter* strains isolated from several hospitals such as Bach Mai, Cho Ray and National Tropical Diseases are multi-resistant strains [2]. The figure for resistance to carbapenem and cephalosporin of *Acinetobacter* spp. isolation in pediatric pneumonia patients at Vietnam National Children's Hospital was very high level: 75 - 100% of resistant strains to Imipenem and MEM; 100% resistant strains to 3rd and 4th generation of cephalosporins [3]. Especially, in the past 15 years, many studies showed that *A. baumannii* had the ability to accumulate many antibiotic resistance genes leading to the development of multi-resistant strains [1]. Therefore, the rate of antibiotic-resistant *A. baumannii* was spreaded in Vietnam with increasing rate.

Although *A. baumannii* has become one of the most common organisms of nosocomial infections worldwide, knowledge about their genetic identity was inadequate. The genetic modification of *A. baumannii* associated with outbreaks was reported by Senok, et al (2015); Alnimr, et al (2020) through evaluation of genetic relationships using molecular markers [5, 6].

In Vietnam, up to now there was no report onthe genetic relationship of the *A. baumannii* by RAPD PCR. Therefore, we conducted this study: *To determine the distribution and origin subgroup of A. baumannii strains from different patients to fully understand about the genetic structure of A. baumannii strains at Thanh Nhan Hospital.*

BJECTS AND METHODS

1. Subjects

Sixty-seven *A. baumannii* strains from isolated samples of inpatient at Thanh Nhan Hospital from 2017 to 2018.

Table 1: List of RAPD primers used in research.

Primer name	The priming sequence (5'-3')	Primer name	The priming sequence (5'-3')
OPC6	GAACGGACTC	OPB13	TTCGCTCGCT
OPC02	GTGAGGCGTC	OPC20	ACTTCGCCAC
OPC05	GATGACCGCC	OPB6	TGCTCTGCCC
OPC09	CTCACCGTCC	OPC12	TGTCATCCCC
OPB20	GGACCCTTAC	OPC13	AAGCCTCGTC
OPB4	GGACTGGAGT	OPC14	TGCGTGCTTG

2. Methods

Descriptive research and laboratory analysis were used in this study.

These strains were identified as *A. baumannii* by the routine techniques at the Department of Microbiology. Twelve random primers used in RAPD reaction belong to 2 groups of OPB and OPC supplied by Operon, USA, including: OPC6, OPC02, OPC05, OPC09, OPB20, OPB4, OPB13, OPC20, OPB6, OPC12, OPC13, OPC14 (*table 1*).

a. Bacteria culture and isolation techniques

Collected strains will be isolated according to standard hospital procedures. The strains were then re-identified by Malditoff machine (Germany) at the National Institute of Hygiene and Epidemiology.

b. Total DNA extraction method

Use the QIAamp DNA Mini Kit commercial kit (Qiagen, Germany). DNA extraction procedure follows the manufacturer's instructions with the kit.

c. RAPD-PCR analytical techniques

Twelve random primers of 10 nucleotides length were used for genetic diversity analysis of 67 *A. baumannii* strains. The PCR reaction with 15 µl total volume, including 10x PCR buffer and final concentration of other ingredients was: $MgCl_2$ 2.0 mM; dNTPs 0.2 mM; primer 0.5 µM; 0.2 units of Taq polymerase and 20 ng of mold DNA. The reaction was carried out according to the 95°C program in 5 minutes, 35 cycles with 3 main steps: denaturing the mold DNA in 50 seconds at 95°C, priming in 1 minute at 34°C and stretching the chain at 72°C in 1 minute 30 seconds; repeat 35 cycles; lasting 72°C for 7 minutes; store at 10°C. Electrophoresis of PCR reacted products on 1.5% agarose agar and stained with Red safe. Observe and analyze the results on the gel imaging system.

**Data analysis*: Based on the RAPD product electrophoresis images, the appearance of the electrophoresis bands was estimated the size and statistics of the electrophoresis bands with each primer in each sample. The presence or absence of the electrophoretic bands was gathered to analyze the data according to the principle: Number 1: Presence of DNA fragment and number zero: No DNA segmentation. The polymorphic information content (PIC) of each RAPD indicator is determined by the formula: $PICi = 1 - \sum(Pij)^2$ (where Pij is the j allele frequency of genotype i tested). Data were processed using NTSYSpc version 2.1 program (Exeter Software, New York, NY, USA).

RESULTS AND DISCUSSION

1. The distribution of *A. baumannii* by age, gender, sample and department

In this study, we used the *A. baumannii* samples collected in the project named: *"Evaluation of the antibiotic resistance status of bacteria in Vietnam, identification of the genetic structure characteristics and the related factors of common drug-resistant bacteria in Vietnam".*

** Characteristics of age and gender:*

Table 2: Some age and gender characteristics of study subjects.

Characteristics		Number of patients	Percentage %
Age	< 30	3	4.5
	30 - 50	4	6.0
	> 50	60	89.5
Gender	Male	50	74.6
	Female	17	25.4

Among 67 *A. baumannii* strains isolated at Thanh Nhan Hospital, 50 strains were detected in male, accounting for 74.6% and 17 strains detected in female accounting for 25.4% (Odds ratio = 2.94).

Therefore, it showed that the rate of male patients infected with *A. baumannii* was higher. Regarding the age, the majority was over 50 years (89.5%).

** Distribution of isolated A. baumannii by samples:*

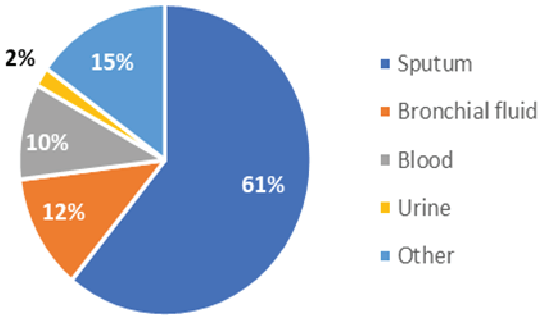

Figure 1: Distribution of isolated *A. baumannii* by samples.

67 strains of *A. baumannii* during 2017 - 2018 were collected from a variety of specimens such as blood, sputum, bronchial fluid, pleural fluid, catheter insertion site, urine, etc, of which the highest rate was present in the sputum samples (61%), followed by bronchial fluid 12% (8/67), blood 10% (7/67), urine 2% (1/67), the other samples were 15% (10/67). Thanh Nhan Hospital is a general hospital, samples were collected from the variety of sources, therefore their types were diverse. The rate of *A. baumannii* strains in sputum and bronchial fluid were highest. The reasons may be that *A. baumannii* is most commonly seen in lower respiratory tract than the other sites.

** Distribution of A. baumannii strains by departments:*

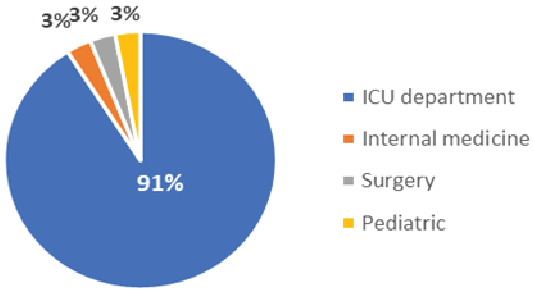

Figure 2: Distribution of *A. baumannii* strains by specialists.

A. baumannii is one of the leading pathogens of nosocomial diseases infection. That explains why *A. baumannii* is mainly distributed in the ICU (Intensive Care Unit) where high-risk patients including long hospitalization stay and mechanical ventilation. Thus, the rate of healthcare-associated infections including *A. baumannii* in these population was quite high. On the other hand, the patients undergoing surgery are also taken to the ICU. At that time, the patient's immune system was easily vulnerable to be infected by nosocomial organisms.

2. DNA polymorphism analysis by RAPD technique

Twelve RAPD primers cloned a total of 126 DNA segments with sizes ranging from 200 - 4,000 bp. On average of 10.5 alleles per locus ranged from 7 - 19 alleles, with the highest being OPC02 primer (19 alleles) and the lowest being OPC14 primers (7 alleles).

Table 3: PIC values and polymorphic segment rate of 67 strains of *A. baumannii* with 12 RAPD indicators.

No	Indicator	Segment size (bp)	Polymorphic information content (PIC)	Polymorphic segment	Total segment	% polymorphic segment
1	OPC06	250 - 3,000	0.817	8	8	100
2	OPC02	400 - 2,500	0.945	19	19	100
3	OPC05	600 - 2,500	0.837	12	12	100
4	OPC09	550 - 3,000	0.832	13	13	100
5	OPB20	200 - 5,000	0.815	11	11	100
6	OPB4	200 - 3,000	0.886	11	11	100
7	OPB13	300 - 2,600	0.792	9	9	100
8	OPC20	650 - 1,700	0.811	8	8	100
9	OPB66	350 - 3,500	0.800	9	9	100
10	OPC12	300 - 3,000	0.797	9	9	100
11	OPC13	300 - 4,000	0.865	10	10	100
12	OPC14	600 - 4,000	0.770	7	7	100
Total		200 - 5,000	-	126	126	-
Average		-	0.831	10.5	126	100

100% primers in our study were for polymorphic segments, the polymorphism was shown in the presence or absence of DNA segments as compared to strains of bacteria together in the same primer (*figure 1*). This result reveals that *A. baumannii* strains have quite high genetic diversity.

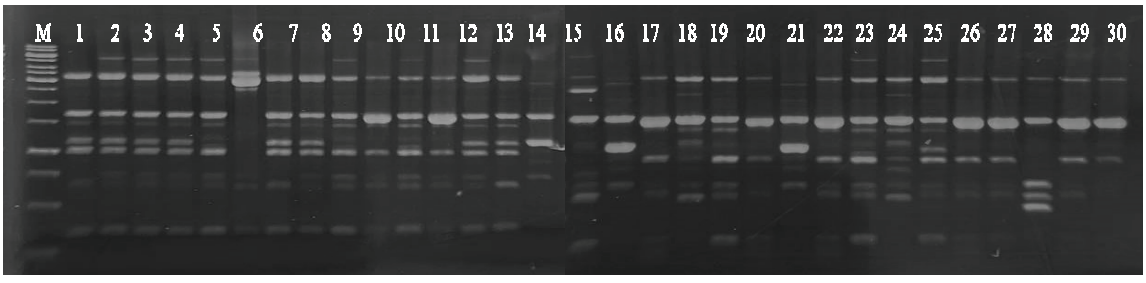

Figure 3: The electrophoresis of RAPD-PCR products results of 30 strains of *A. baumannii* with OPB20 primer. (M: Marker Fermentas 1kb, wells 1 - 30: Corresponding to the number of studied strains from 1 to 30).

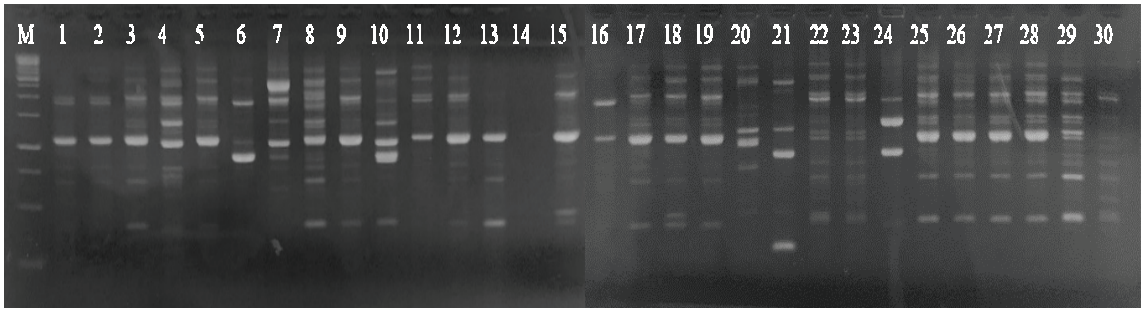

Figure 4: The electrophoresis of RAPD-PCR products results of 30 strains of *A. Baumannii* with primer B4. (M: Marker Fermentas 1kb, wells 1 - 30: Corresponding to the number of studied strains from 1 to 30)

According to Botstein et al, (1980), if the PIC index is over 0.5, the primer is used for high polymorphism, whereas the PIC index is in the range 0.25 ≤ PIC ≤ 0.5 for average polymorphism and with PIC index ≤ 0.25 for low polymorphism [7]. According to table 3, PIC values of 67 strains of *A. baumannii* ranged from 0.770 to 0.945, average PIC values of RAPD primers were 0.831. This was a high index indicating high polymorphism among strains. Through this, it can be seen that using the primers in this study were highly significant in evaluating the genetic diversity of *A. baumannii* strains.

3. The results of analyzing genetic relationships of *A. baumannii strains*

Genetic tree of 67 *A. baumannii strains* was designed based on the genetic similarity between coefficient Jaccard and UPGMA algorithm (Unweighted Pair Group Method).

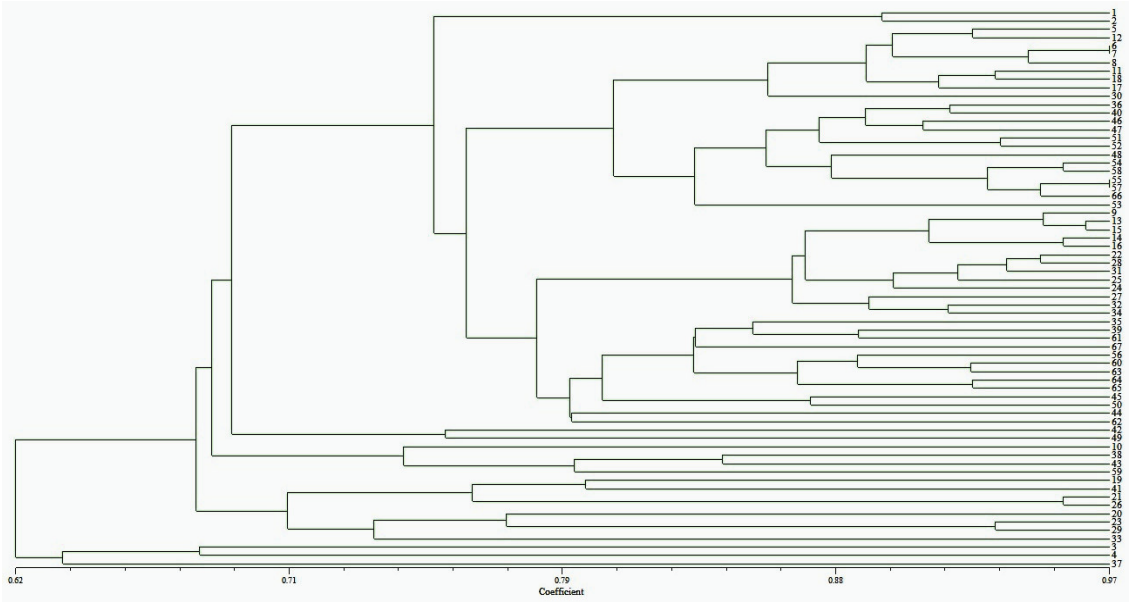

Figure 5: Genealogical tree of 67 strains of *A. baumannii* isolated at Thanh Nhan Hospital.

It can be seen from the genetic relationship, the genetic similarity of coefficients of 67 strains ranged from 0.62 to 0.97 and were divided into 5 groups. At the genetic similarity level, 62% of the three strains number 3, 4 and 37 (Group I) had the furthest genetic relationship compared to the remaining 64 strains. Group II consisting of 8 strains were 19, 41, 21, 26, 20, 23, 2, 33, in which, two strains 21 and 26 had the closest genetic relationship at the genetic similarity level about 95.3%; strain number 33 has the furthest genetic relationship with the remaining 7 strains. Group III including 4 strains were 10, 38, 43, and 59 with the genetic similarity level 74.2%. Strain 10 had the furthest genetic relationship compared to 4 strains. 2 strains (38 and 43) had the closest genetic relationship at genetic similarity level of 84.4%. Group IV comprising 2 strains number 49 and 42, in which these 2 strains had genetic similarity level of 75.7%. Group V consisted of the rest 50 strains. Of them, the two strains number 1 and 2 had the most genetic relationship in the group with the genetic similarity level of 75%. Morever, in this group, bacteria strains number 6 and number 7; bacteria strains 55 and 57 had the closest genetic relationship at genetic similarity level of 97%, which is also the highest genetic similarity.

When analyzing the genetic relationship diagram of 67 *A. baumannii* strains, we found that they had a high genetic diversity level. When grouping genetic relationships, the genetic relationship diagram was divided into 5 groups without the relationship about collecting time. This result is similar to the study by Alnimr et al (2020) when studying the genetic diversity of 80 imipenem resistant *A. baumannii* strains at Intensive Care Unit in Saudi Arabia [6]. These results also indicate that these strains can be spread through a numerous pathways, especially contact transmission. Therefore, standard precautions and compliance with hospital infection control measures are essential.

CONCLUSION

Study on the distribution of *A. baumannii* strain presented that the rate *A. baumannii* strains in male was higher than in female. 89.5% of the patients were over 50 years old. The rate of *A. baumannii* strains isolated from sputum was the highest with 61% (41/67). Patients infected with *A. baumannii* in the Intensive Care Unit was 91%.

Analysis of the genotypic relationship of 67 *A. baumannii* strains collected at Thanh Nhan Hospital with 12 RAPD primers obtained 126 DNA segments showed that each allele on the primer ranged from 7 - 19 alleles, 100% of the study primers were for polymorphic segments. The PIC coefficients ranged from 0.770 to 0.945, average PIC was 0.831. The genetic relationship of 67 *A. baumannii* strains was divided into 5 groups, with genetic similarity coefficients ranging from 0.62 to 0.97, indicating that 67 strains had genetic diversity.

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REFERENCES

1. Le Xuan Ngoc, Tran Huy Hoang, Le Thanh Hai, Tran Nhu Duong. Resistant situation to carbapenem and cephalosporin of *Acinetobacter* spp. carrying the oxacillinase gene in pediatric pneumonia related mechanical ventilation outside of infancy at the National Hospital of Pediatrics, 2014 - 2015. Journal of Preventive Medicine 2016; 26(13):103-108.

2. Ana Maria Gonzalez-Villoria, Veronica Valverde-Garduno. Antibiotic-resistant *acinetobacter baumannii* increasing success remains a challenge as a nosocomial pathogen. Journal of Pathogens 2016:1-10.

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3. GARP. Global antibiotic resistance partnership (GARP) working group Vietnam. Situation analysis on antibiotic use and resistance in Vietnam 2010, Hanoi, Vietnam.

4. Laurent Poirel, Patrice Nordmann. *Acinetobacter baumannii*: Mechanisms of resistance, multiple ß-lactamases. Acinetobacter biology and pathogenesis. Springer. Temple University School of Medicine Philadelphia 2008:129-144.

5. Senok A, Garaween G, et al. Genetic relatedness of clinical and environmental Acinetobacter baumanii isolates from an intensive care unit outbreak. The Journal of Infection in Developing Countries 2015; 9(06):665-669. https://doi.org/10.3855/jidc.6726.

6...ADDIN Mendeley Bibliography CSL_BIBLIOGRAPHY Alnimr A, Alamri A, Alsultan A. Genetic diversity of imipenem-resistant *Acinetobacter baumannii* infections at an Intensive Care Unit. Critical Care Research and Practice 2020:1-6. https://doi.org/10.1155/2020/3290316.

7. Botstein D, White RL, et al. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetic 1980; 32(3):314-331.