Indo Global Journal of Pharmaceutical Sciences, 2022; 12: 216-219

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

Carbapenemase Detection Among Carbapenem-Resistant *Acinetobacter baumannii* Clinical Isolates Using a Modified Blue-Carba Test

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Received: 01.02.2022
Accepted: 20.04.2022
Published: 08.08.2022
Keywords Acinetobacter baumannii, Blue-Carba Test (BCT), carbapenem-resistant A. baumannii (CRAB), antibiotic selection.

ABSTRACT: **Objective**: *Acinetobacter baumannii* is a Gram-negative opportunistic bacteria with carbapenemase-associated antibiotic resistance accounting for a majority of nosocomial infections globally. We sought to detect carbapenemase producers among carbapenem-resistant *A. baumannii* (CRAB) clinical isolates using Blue-Carba Test (BCT). **Methods**: This study was an observational descriptive study. At first, *A. baumannii* clinical isolates (142) were collected from January 2014 to September 2017 and stored at the Microbiology Laboratory of the Faculty of Medicine, Atma Jaya Hospital. Next step involved the identification of the carbapenem-resistant isolates using WHONET 5.3 software (https://whonet.org/software.html). Here, Imipenem and Meropenem served as markers. Finally, the identified carbapenem-resistant isolates were subjected to Blue-Carba Test. **Results**: Among 142 *A. baumannii* isolates, 51.4% (73/142) were identified as CRAB. Of these, 36 isolates were revived and all were found to be carbapenemase positive. **Conclusion**: Conclusively, a cent percent (36/36) prevalence of carbapenemase producers was observed in the CRAB group, which is an important clinical finding for antibiotic selection against *A. baumannii* infection.

Cite this article as: Tjoa, E.; Joon, S.; Angeline, D.; Lieputra, A.A.; Edbert, D.; Moehario, L.H. Carbapenemase detection among carbapenem-resistant Acinetobacter baumannii clinical isolates using a modified Blue-Carba Test. Indo Global J. Pharm. Sci., 2022; 12: 216-219. **DOI**: http://doi.org/10.35652/IGJPS.2022.12027

INTRODUCTION

Acinetobacter baumannii is Gram-negative bacteria frequently found in hospitals and accounts for opportunistic nosocomial infection [1, 2]. Reports from various parts of the world have suggested that the incidence of *A. baumannii infection* has *significantly increased accompanied by antibiotic resistance* [2, 3]. This resistance is mainly caused by the production of the carbapenemase enzyme [3-5]. One of the phenotypic tests for the Carbapenemase enzyme detection is Blue Carba Test (BCT) [6]. It relies on the alterations in pH values indicated by a change in color. Here, Bromothymol blue serves as an indicator. Further, the acid produced by the hydrolysis of Imipenem antibiotic is detected because of the Carbapenemase enzyme activity. BCT is an advantageous biochemical test as it rapidy detects the carbapenemase synthesis in Gram-negative bacilli from their respective culture directly (in less than 2 hours). Reportedly, the sensitivity and specificity of BCT are 100% for the *Pseudomonas*, *Enterobacteriaceae*, and *Acinetobacter* species [6, 7]. A standard BCT protocol requires an incubator shaker in the incubation step. Unfortunately, a majority of the diagnostic laboratories in Indonesia are not equipped with this instrument. Hence, a modified BCT that eliminates the usage of this instrument is desirable in developing countries like Indonesia. Therefore, in this study, we sought to detect carbapenemase producers among carbapenem-resistant *A. baumannii* (CRAB) clinical isolates using a modified BCT. This unsophisticated, inexpensive, and rapid test was

performed using a static incubator (without shaking movement) instead of a shaker incubator.

MATERIALS AND METHODS

Study outline and specimen

CRAB isolates in this observational descriptive study were procured from clinical specimens in Atma Jaya Hospital collected from January 2014 to September 2017, and stored at Microbiology Laboratory, Faculty of Medicine, the Atma Jaya Hospital. The carbapenem-resistant strains were sorted using WHONET 5.3 software (https://www.whonet.org/). The retrieved clinical isolates and their processing from -20 were assisted by 4BANKING™. The workflow of the study is described in **Figure 1**.

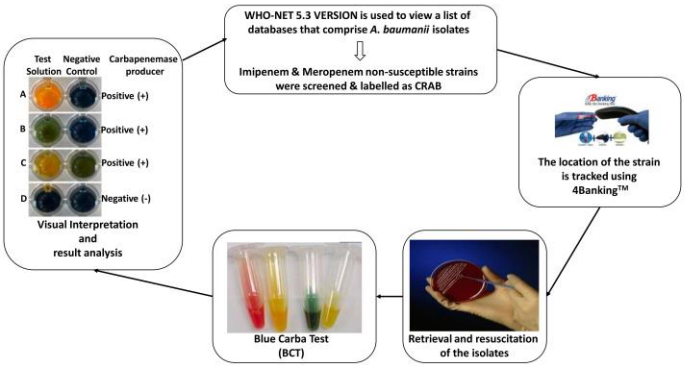

Figure 1. Workflow of the study.

Research ethics and a patient's consent

The clinical specimens were obtained with the prior informed consent of the patient. The present study was performed according to the standard guidelines and approval of the Departmental Ethical Committee of the School of Medicine and Health Science, Atma Jaya Catholic University of Indonesia, North Jakarta, Indonesia (Ethical approval number: 11/05/KEP-FKUAJ/2017).

Modified Blue-Carba Test (BCT)

A schematic representation of the Modified Blue-Carba Test (BCT) is shown in **Figure 2**. The Modified BCT was performed in triplicates in two steps as mentioned below.

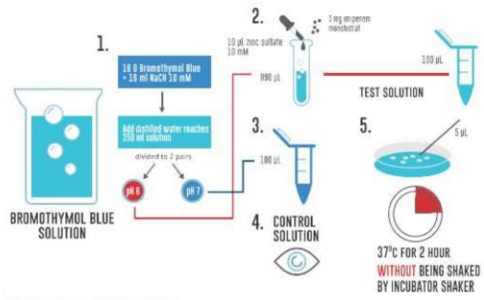

Figure 2. The modified Blue-Carba Test (BCT).

Optimization of incubation time

Firstly, the incubation time was optimized and the incubator shaker was not used as with the routine protocols. Therefore, the optimization of incubation time should be done before reading the result. *Pseudomonas aeruginosa* blaVIM-2 (+) was procured from the Microbiology FKUI collection [8] and taken as the positive control while *Escherichia coli* ATCC 25922 was the negative control. These control strains were reconfirmed using a polymerase chain reaction (PCR) as shown in **Figure 3**.

Figure 3. Bla Vim positive (+) Carbapenemase producer visualization. Band generated on 390kb was considered as Bla Vim (+). Marker, 100bp; Negative, *Escherichia coli* ATCC 25922; Positive, *Pseudomonas aeruginosa*.

Interpretation of Modified BCT test results

The interpretation of test results is described in **Table 1**.

Table 1. Blue Carba Test (BCT) interpretation guidelines.

Test tubes	Negative control	Carbapenemase producer
Yellow	Blue	+
Yellow	Green	+
Green	Azure	+
Green	Green	-
Blue	Blue	-

RESULTS AND DISCUSSION

Incubation time optimization

It was noted that the Incubation time was consistent after 2 hours. Results for each hour of incubation remained unchanged within 24 hours. Accordingly, the incubation time was optimized at two hours for this study.

Detection of Carbapenemase producer *A. baumannii* using the BCT method

Figure 4. (a) The positive control was MBL confirmed *Pseudomonas aeruginosa* (bla VIM (+)). Result: A change in the colour was noted, BCT positive. (b) The negative control was *Escherichia coli* ATCC 25922. Result: No change in the colour was noted, BCT negative.

Table 2. Prevalence of carbapenemase producer among *Acinetobacter baumannii* using Blue Carba Test (BCT).

	Number of isolates (N=36)	**Percentage**
Carbapenemase producer	36	100%
Non-Carbapenemase producer	0	0%

A total of 142 *A. baumannii* isolates were reviewed and 51.4% (73/142) identified as CRAB. Half of those were successfully revived (n=36). These 36 CRAB isolates were subjected to BCT and all (100%) tested positive for carbapenemase (**Table 2**). The results were reported in comparison to the quality control strains that were taken for each isolate (**Figures 4** and **5**).

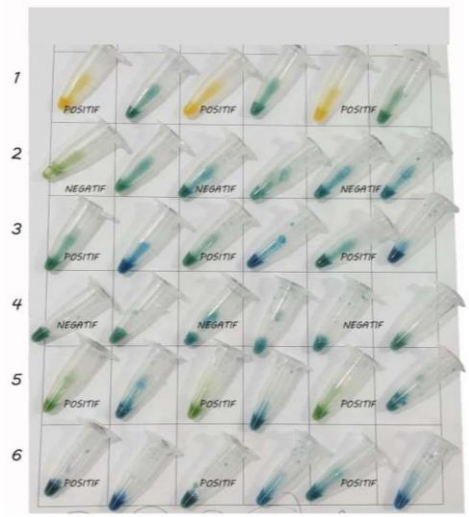

Figure 5. Blue-Carba Test (BCT) results. Isolate 1, positive control; Isolate 2, negative control; Isolates 3-6: Test isolates.

The Blue Carba Optimization test conducted in this study aims to get the right incubation time using an ordinary incubator. Based on the results of the optimization, the Blue Carba Test interpretation results were unchanged from observation at the 2nd hour until the 24th hour. Hence, the optimization time was 2 hours.

The results of this optimization indicated that the Blue Carba Test could be done without a shaker incubator with the same results reading time of 2 hours. The prevalence of carbapenemase in carbapenem-resistant *A. baumannii* in this study was 100% (36/36). A similar prevalence was also found in studies in several other countries. Rolain *et al*. observed that all carbapenem-resistant *A. baumannii* isolates had carbapenemase enzyme mediated by the OXA23 gene13 [9]. Further, Fouad *et al*. showed that all carbapenem-resistant *A. baumannii* isolates carried both OXA-23 and OXA-51 genes [10]. Furthermore, Xiao *et al*. demonstrated that the prevalence of carbapenemase in Imipenem-resistant *A. baumannii* was 100% (46/46), all carried the OXA-51 gene, 96% (44 / 46) carried the OXA-23 gene, and 2% (1/46) carried the NDM gene [11].

CONCLUSION

The prevalence of carbapenemase producer among carbapenem-resistant *Acinetobacter baumannii* were 100% (36/36). This finding might assist clinicians in selecting antibiotic against Carbanepamase producer bacterial strains when the carbapenemase producer test is included in the microbiology work-up and offer a rapid result.

AUTHOR'S CONTRIBUTION

ET and SJ: Conceptualization, visualization, methodology, validation, and data curation. SJ: Formal analysis, data curation, original draft preparation, review, and editing. AD, LAA, SA, and ED: Methodology, data curation, and formal analysis. LHM: Supervision. All authors contributed significantly to the study and have approved the final manuscript.

FUNDING SOURCE

This study did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

DATA AVAILABILITY

All relevant data is present in the manuscript, and the same is accessible for the peer-review process.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript; journal and Publisher will not be responsible for any copyright infringement and plagiarism issue.

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