MD627 101 Peer Review in Medical Microbiology Research I

Title: Proteomic and Genomic Characterization of Plasma Responsive *Acinetobacter calcoaceticus-baumannii* (ACB) Complex.

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1. Introduction

The *Acinetobacter calcoaceticus-baumannii* (ACB) complex includes six phenotypically related species: *A. calcoaceticus*, *A. baumannii, A. pittii*, *A. nosocomialis*, *A. seifertii*, and *A. dijkshoorniae*. Previously, conventional methods cannot identify ACB complex in species level (Mancilla-Rojano et al., 2020). Nowadays, this bacterial complex is clinically reported as "ACB complex" (Fitzpatrick et al., 2015). ACB complex is gram-negative bacteria causing nosocomial infection. Common clinical manifestations are pneumonia, bloodstream infection, skin infection, and urinary tract infection (Pogue et al., 2022). The major causes of nosocomial ACB complex infection are associated with ventilator and central-line intervention, both of which were mortality rate at around 52% (Dijkshoorn et al., 2007).

An important characteristic of *A. baumannii* is the drug resistance such as, carbapenems, multidrug resistance (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR). Therefore, the World Health Organization (WHO) has appointed as a critical priority pathogen. which poses a serious threat to public health (Jiang et al., 2021). Nevertheless, previous studies show the mortality rate of patients is not associated with MDR-AB infection (Nithichanon et al., 2022). *A. baumannii* presents several virulent factors to survive and evade host immune system when infection. Capsules are one of virulence factors of *A. baumannii* that facilitate resistance to complement-mediated bactericidal activity by preventing membrane attack complex (MAC) deposition and lysis (Magda et al., 2022). Recently, *A. baumannii* was discovered according to normal human sera complement resistant assay into three groups, including highly susceptible, intermediate susceptible, and resistant

(Kamuyu et al., 2022). However, the clinical significance of complement resistance of *A. baumannii* is still unknow.

The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a powerful technology for identification of pathogens nowadays. The principle is analysis of mass spectral fingerprints to identify unique signatures of each microorganism. Therefore, MALDI-TOF MS is suitable to apply for bacterial identification in routine laboratory with high accuracy, budget friendly, and short time-to-report (Croxatto et al., 2012). Furthermore, MALDI-TOF MS can be applied for analysis of all possible in resistance mechanisms, and it provides results that can be used for diagnosis (Hrabák et al., 2013).

Therefore, this study aims to 1) investigate the clinical significance of plasma resistant ACB complex along with whole genome analysis to identify possible virulence, 2) identify plasma resistant or susceptible ACB complex using MALDI-TOF MS with specific peak of MALDI-TOF MS fingerprints from plasma resistant or susceptible ACB complex, and optimization for the most effective combination strategy.

2. Hypothesis

1. Plasma resistance ACB complex is associated with mortality rate.

2. Plasma resistance ACB complex can be identified by MALDI-TOF MS. And the differentially expressed MS peak can be identified.

3. Objectives

1. To investigate for clinical significance of plasma resistant ACB complex by comparing between percentage of death and survival in patients.

2. To identify plasma resistant and susceptible ACB complex using MALDI-TOF MS compared to results from conventional method a reference

3. To identify specific peak of MALDI-TOF MS fingerprints from plasma resistant and susceptible ACB complex, and optimization for the most effective combination strategy using bioinformatics approaches

4. To compare genome of plasma resistant and susceptible ACB complex by using whole genome sequencing analysis

4. Material and Methods

4.1 Plasma susceptibility test.

Culture ACB complex on NB broth and incubated for 6 hours at 37°C, then 1 ml was collected and centrifuged at 14,000 rpm for 2 min, after which it was resuspended in 1 ml normal saline solution (NSS). Then mix well and centrifuge again (2 times). The number of cells will be counted as $5x10^4$ cell/ml, by adding NSS and measuring turbidity at 600 nm by spectroscopy. Follow Table 1 for loading ingredients in each condition, incubated at 37°C for 24 hours and measuring turbidity at 600 nm in every 2 hours by Varioskan lux.

Ingredient	Condition		
	Nutrient broth	**Heat inactivated plasma**	**Normal human plasma**
Bacteria (µl)	50	50	50
Nutrient broth (µl)	100	100	100
100% Heat inactivate plasma (µl)	-	50	-
100% Normal human plasma (µl)	-	-	50
Normal saline solution (NSS) (µl)	50	-	-

Table 1. Ingredients in each condition for Plasma susceptibility test.

4.2 MALDI-TOF MS

Direct colony method.

The ACB complex was cultured on NB agar and incubated for 18 hours at 37°C. Then, collect a single colony and smear it on the target plate of MALDI. After that, let it dry at room temperature. Subsequently, add 1 µL of matrix solution, a-Cyano-4-hydroxycinnamic acid (HCCA or CHCA), and let it dry.

Protein extraction method.

The ACB complex was cultured on NB agar and incubated for 18 hours at 37°C. Then, collect a full loop of a single colony and dissolve the sample in 300 µL of sterile water in a sterile microcentrifuge tube. After thawing the sample, and then adding 900 µL of cold absolute ethanol the sample was centrifuged at 13,000 rpm, 4°c for 2 min, and discarded ethanol. The sample has been centrifuged and discarded ethanol again. After that, let it dry at room temperature for 5 minutes. Then add 80 µL of 70% Formic acid into the sample and mix by pipette. Then add 100 µL of 100% Acetonitrile into the sample and mix by pipette. The sample was centrifuged at 13,000 rpm for 2 minutes. And collect 1 µL supernatant was added to the MALDI target plate and then the supernatant was allowed to dry at room temperature. Subsequently, add 1 µL of matrix solution, a-Cyano-4-hydroxycinnamic acid (HCCA or CHCA), and let it dry.

5. Results

5.1 Plasma susceptibility test

All isolates were able to grow in nutrient broth (NB), heat-inactivated (HI) human plasma, or normal (N) human plasma, but some isolates failed to grow or grew slowly in N-plasma. This includes AB-ATCC, 20-012, and 20-059. When comparing the growth rates of heat-inactivated plasma and normal human plasma and calculating the generation time ratio. The value appears as shown in Figure 1.

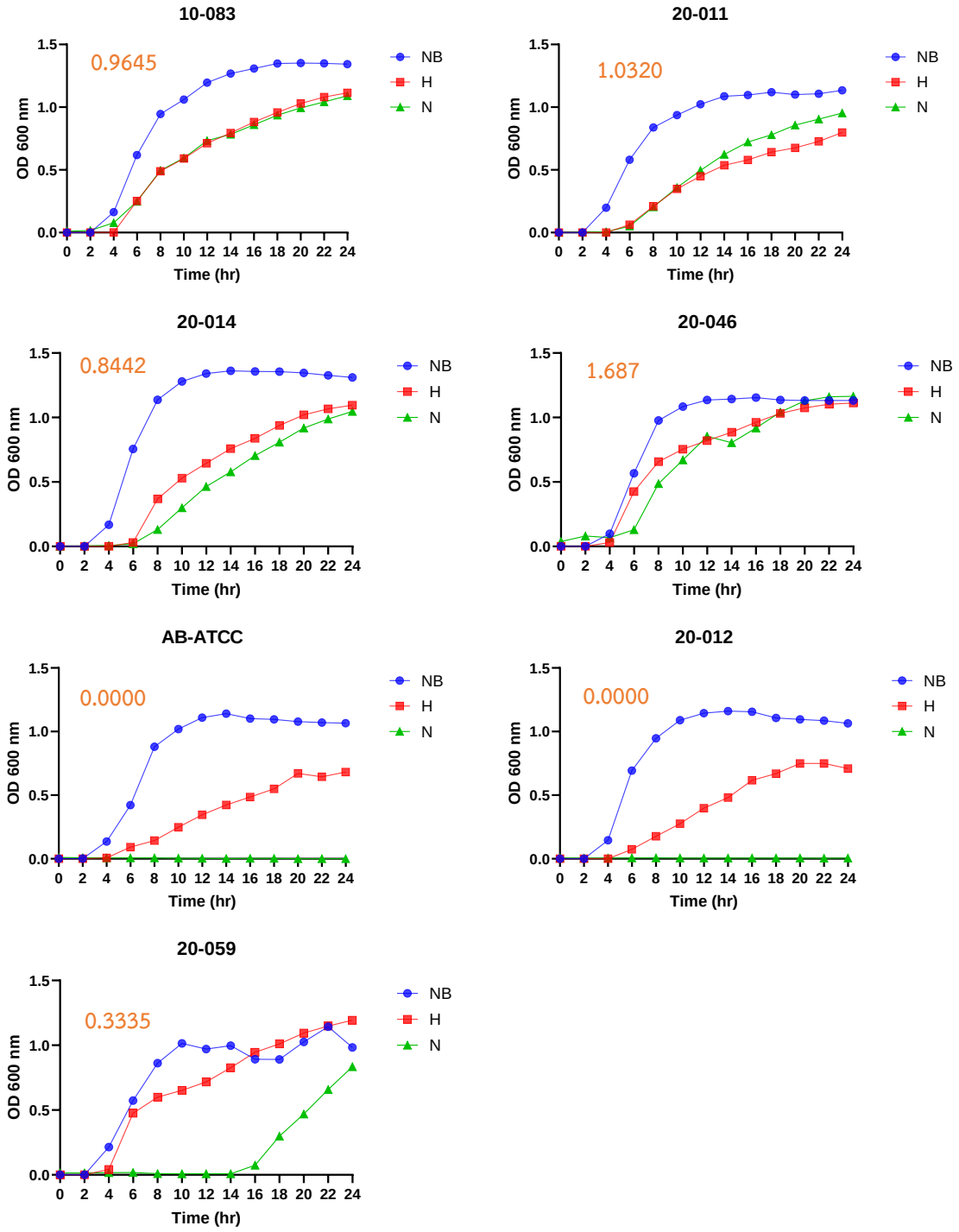

Figure 1. Growth rate of ACB complex for 24 hours in each condition including Nutrient broth (NB), Heat-inactivated (HI) human plasma, and Normal (N) human plasma. Generation time ratio calculated by growth rate in N- plasma device by growth rate in HI-plasma.

5.2 Interpretation of species identified by MALDI-TOF MS.

Using the direct colony approach, there were 80% high-confidence identifications, 11% low-confidence identifications, and 9% identifications for which no organism could be

identified. The protein extraction method, on the additional, provides 69% high-confidence identifications and 23% no organism identification at all (Fig. 2).

Figure 2. The percentage interpretation of species identified ACB complex by 2 methods of direct colony and protein extraction, no organism identification possible is red bar, low-confidence identification is yellow bar, and high-confidence identification is green bar.

5.3 Compare species identify of MALDI-TOF MS with Whole Genome Sequencing.

Compared to whole genome sequencing results, the identification results obtained with the direct colony method indicate 78% correct identification and 12% incorrect identification. 35% of the protein extraction method's identifications are incorrect whereas 65% of them are correct. (Figure 3)

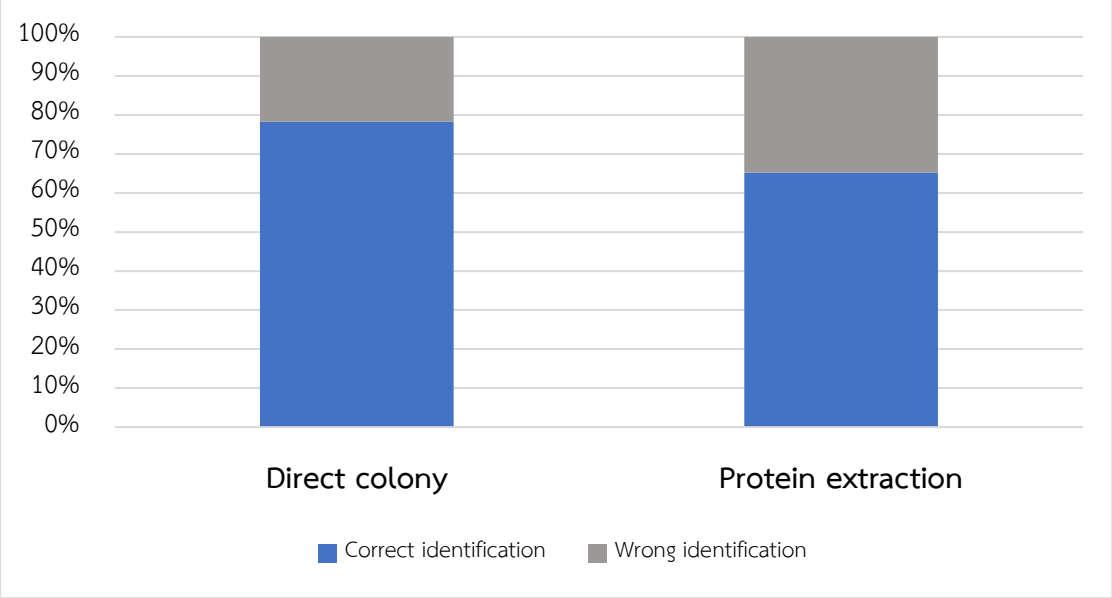

Figure 3. Percentage identification of ACB complex by 2 methods of direct colony and protein extraction, correct identification is blue bar and wrong identification is gray bar.

6. Conclusions

The generation time ratio can be easily used for classification groups of bacteria based on growth rate in normal human plasma and heat-inactivated human plasma. The cut-off used to group characteristics on plasma can be classified into two groups. In this study, the cut-off is 0.5000, the generation time ratio is more than 0.5001, this group is plasma resistant, and less than 0.5000 is plasma susceptible.

The direct colony method is more reliable in providing higher confidence identification and correct identification compared to whole genome sequencing results than the protein extraction method.

7. Thesis plan

Activity	1				2			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Course work								
Literature review and planning								
Objective 1								
• Comparing between death and survival patients								
Objective 2								
• Prepare sample								
• Using MALDI-TOF (Direct colony and Extract protein)								
• Compare peak (S/R)								
Objective 3								
• Identify specific peak								
• Combination								
• Sen/Spec/PPN/NPV								
Objective 4								
• Comparing genome of plasma resistant or susceptible								
Proposal								
Manuscript, preparation, and submit								
Publish research and defense								

8. Reference

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