

# Phenotypic Cluster Analysis of Acinetobacter Species Isolated From Ventilator Associated Pneumonia During the First Wave Of COVID 19 in Surabaya, Indonesia

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## Abstract

**Introduction** Coronavirus disease-2019 (COVID-19) has been reported as epidemic in December 2019 in Wuhan, Hubei Province, People Republic of China. Hospitalized COVID-19 patients, especially with worse clinical appearance and on ventilator, need thorough diagnostic tests as COVID-19 patients tend to mimic bacterial infections. Positive blood culture in COVID-19 patients is more likely to be contaminants and hospital associated infections than primary co-infections. This research aims to phenotypically group data of Acinetobacter isolates associated with Ventilator Associated Pneumonia (VAP) using hierarchical clustering.

**Method** This is a descriptive study presenting collection of Acinetobacter culture data performed by Microbiology laboratory of a COVID-19 referral hospital from 26 March 2020- 31 February 2021. Hierarchical clustering were performed using statistical software.

**Results** Hierarchical clustering were performed to 50 VAP associated Acinetobacter isolates. There are 4 cluster clades showed in biochemistry dendrogram. Two dominant clusters 1A and 3A were found and both similar MIC pattern.

**Conclusion** Acinetobacter phenotype cluster 1A and 3A dominates VAP associated isolates. Dominant clusters indicate isolate transmissions between hospital. This method can be applied to facilities with limited resource setting.

Keywords: COVID-19; Isolation Wards; Acinetobacter; Ventilator Associated Pneumonia

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## 1. Introduction

Coronavirus disease-2019 (COVID-19) has ruled every aspect of human life since being reported as epidemic in December 2019 in Wuhan, Hubei Province, People Republic of China. As per June 10th, 2021, 175 236 571 confirmed cases have been found and death toll have reached 3 778 706 (2.16%). While in Asia, India reported the most cases (29 183 121 confirmed cases). Indonesia has the highest number of confirmed cases in South-East Asia Region (1 885 942 confirmed cases) with death toll as high as 52 373 (2.8%). The death proportion in Indonesia is higher than world death proportion (WHO, 2021)

COVID-19 causes systemic inflammation that resulted in multi organ failure by acute inflammation and activation of fibroblasts (Tobin, 2020). Death by COVID-19 is not directly caused by the course of viral infection, but most likely due to multi-organ damages by severe cytokine storm and systemic fibrosis. The progress of fibrosis in respiratory system decreases brain ability to detect hypoxia and worsens the ability to take oxygen as source of energy (Tobin, 2020; Wilkerson, 2020).

Hospitalized COVID-19 patients, especially with worse clinical appearance, need thorough diagnostic tests as COVID-19 patients tend to mimic bacterial infections. First wave of COVID-19 makes establishment of necessary diagnostic protocols possible. Especially in cases of severe and critical COVID-19 where bacterial co-infections and healthcare associated infections can occur while the clinical progression of COVID-19 mimics bacterial infection (Rawson, 2020). Other earlier study regarding COVID-19 showed that bacterial co-infection and healthcare associated infections are few. Ventilator associated pneumonia (VAP) dominated bacterial secondary infection in healthcare associated infections (HAI). Positive culture is rare in early course of COVID-19 (Rawson, 2020; Dudoignon, 2020; Adler, 2020; Contou, 2020; Hughes, 2020; Sepulveda, 2020).

Other reports showed that secondary infection in COVID-19 patients occur in 50% of severe patients resulting in death (Zhou, 2020). The number of true bacteremia and VAP in severe and critical patients are 1,6% (Sepulveda, 2020) and 27% (Dudoignon, 2020). A systematic review towards 2183 COVID-19 patients showed that 7% of all patients present with co-infections and are hospitalized in intensive care units. Agents of co-infections in COVID-19 patients are *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae* (Lansbury, 2020). Most of the studies showed, high rate of positive bacterial culture in COVID-19 patients describes more of culture contaminations or invasive device colonization associated to HAI than primary co-infections rather than primary co-infections (Hughes, 2020).

Management of COVID-19 patients are performed in special isolation wards with negative pressure (Siegel, 2007; WHO, 2020). Highest level of personnel protective equipments are used in patient care thus limiting care giver sight and movements in delicate procedures. This contributes to high level of aerobic culture contamination. Positive blood culture can reach 42%-65%, very high compared to low number of true bacteremia previously stated (Hughes, 2020; Sepulveda, 2020).

Species detection and antimicrobial susceptibility profiling in HAI are important in isolate tracing and intrahospital epidemiological study. Genotypic tracing has been done in many studies and have developed into predictive microbiology, but high-end instruments and trained professionals are not readily available in most facilities and routine tracing might be costly. There is no standard method for phenotypic tracing other than antibiogram. Thus, a simple and credible phenotypic tracing method is needed for isolate tracing in limited resource facilities. This study aims to describe *Acinetobacter* species isolated from ventilator associated infections from COVID-19 patients admitted to public hospital in Surabaya using hierarchical clustering.

## 2. Methods

This is a descriptive study presenting collection of *Acinetobacter* culture data performed by Microbiology laboratory of a COVID-19 referral hospital from 26<sup>th</sup> March 2020- 31<sup>st</sup> March 2021. Data collected using non-consecutive sampling method from patients using ventilators. VAP were defined using PNU-1 and PNU-2 criteria from National Healthcare Safety Network Patient Safety Manual (NHSN, 2021). *Acinetobacter* isolates that are analyzed were VAP associated isolates from endotracheal aspirate specimens or spontaneous and induced sputum during infection window period post ventilator use and Secondary Blood stream infection associated to VAP that were collected in secondary bloodstream attribution period of VAP. Hierarchical cluster analyses were performed to analyze similarity of isolates using biochemistry profile and Minimum Inhibitory Concentration (MIC) from automated identification system (BD Phoenix, Becton-Dickinson).

### 2.1 Ethical statement

This research has been exempted under review of dr. Soetomo public hospital Ethical committee (0457/LOE/301.4.2/V/2021).

## 2.2 Data collection method and analysis

Biochemistry and MIC data were gathered using BD Epicenter (Beckton-Dickinson) program and clinical information were collected using hospital information system. Hierarchical clustering were performed using statistical software, with Euclidian distance analysis, between group linkage. Binary data setting was used to analyze biochemistry profile.

## 3. Results

The top three ventilator associated pneumonia and secondary bloodstream infection caused by VAP agents were *Acinetobacter baumannii* (n=50), *Klebsiella pneumoniae* (n=19), and *Staphylococcus aureus* (n=8). Hierarchical cluster analyses were performed to isolates identified as *Acinetobacter baumannii*, *Acinetobacter baumannii-calcoaceticus* complex, and *Acinetobacter sp.*. Those isolates were analyzed together as *Acinetobacter* group 1, 2, 3, and 13 TU are closely related and hard to distinguish by biochemistry profile only. From 50 *Acinetobacter* isolates that are associated with VAP in the first wave of COVID-19: 36 (37.1%) isolates were pathogens of respiratory organ and 14 (14.4%) isolates were pathogens of secondary bloodstream infections. Most of the isolates (78%) were also associated with patient death by VAP and related secondary bloodstream infections (Table 1).

Table 1. Isolate cluster frequency in HAI related diagnosis

		HAI Diagnosis			Death (%)
		n (%)	VAP (%)	SBI (%)	
Isolate Cluster	<b>1A</b>	<b>25</b> (69)	<b>17</b> (47)	<b>8</b> (22)	<b>18</b> (50)
	<b>1B</b>	<b>4</b> (11)	<b>3</b> (8)	<b>1</b> (3)	<b>2</b> (6)
	<b>1C</b>	<b>1</b> (3)	<b>1</b> (3)	<b>0</b> (0)	<b>1</b> (3)
	<b>2A</b>	<b>5</b> (14)	<b>4</b> (11)	<b>1</b> (3)	<b>4</b> (11)
	<b>3A</b>	<b>11</b> (31)	<b>7</b> (19)	<b>4</b> (11)	<b>10</b> (28)
	<b>3B</b>	<b>2</b> (6)	<b>2</b> (6)	<b>0</b> (0)	<b>2</b> (6)
	<b>3C</b>	<b>1</b> (3)	<b>1</b> (3)	<b>0</b> (0)	<b>1</b> (3)
	<b>4A</b>	<b>1</b> (3)	<b>1</b> (3)	<b>0</b> (0)	<b>1</b> (3)
	<b>Total</b>	<b>50</b>	<b>36</b> (72)	<b>14</b> (28)	<b>39</b> (78)

HAI: Healthcare Associated Infection

SBI: Secondary Bloodstream Infection

VAP: Ventilator Associated Pneumonia

There are 4 cluster clades showed in biochemistry dendrogram (which are coded in numbers) and 3 cluster clades (which are coded in capital letters) showed in MIC dendrogram (Fig. 1). It is important to note that this grouping nomenclature only applies to this data. Different data analysis can result in different group category.

VAP associated *Acinetobacter* isolates are divided into eight cluster combinations (Table 1), dominated by cluster 1A (69%) and followed by Cluster 3A (31%). Cluster 1A and 3A are also associated with death by HAI in significant proportion (50% and 28%). Cluster 1A put together acetate, adonitol, dextrose, gamma-glutamyl, galactose, phenylalanine, glycine, and arginine utilizing isolates, while Cluster 3A put together metabolic inactive *Acinetobacter* (Garrity, 2019). Both clusters are mostly susceptible to trimethoprim-sulphamethoxazole; resistant to imipenem, meropenem, and ciprofloxacin; intermediate to amikacin,

gentamycin, ampicillin-sulbactam, piperacillin tazobactam, ciprofloxacin, and colistin according to CLSI document M-100 (CLSI, 2021; Magiorakos, 2012).

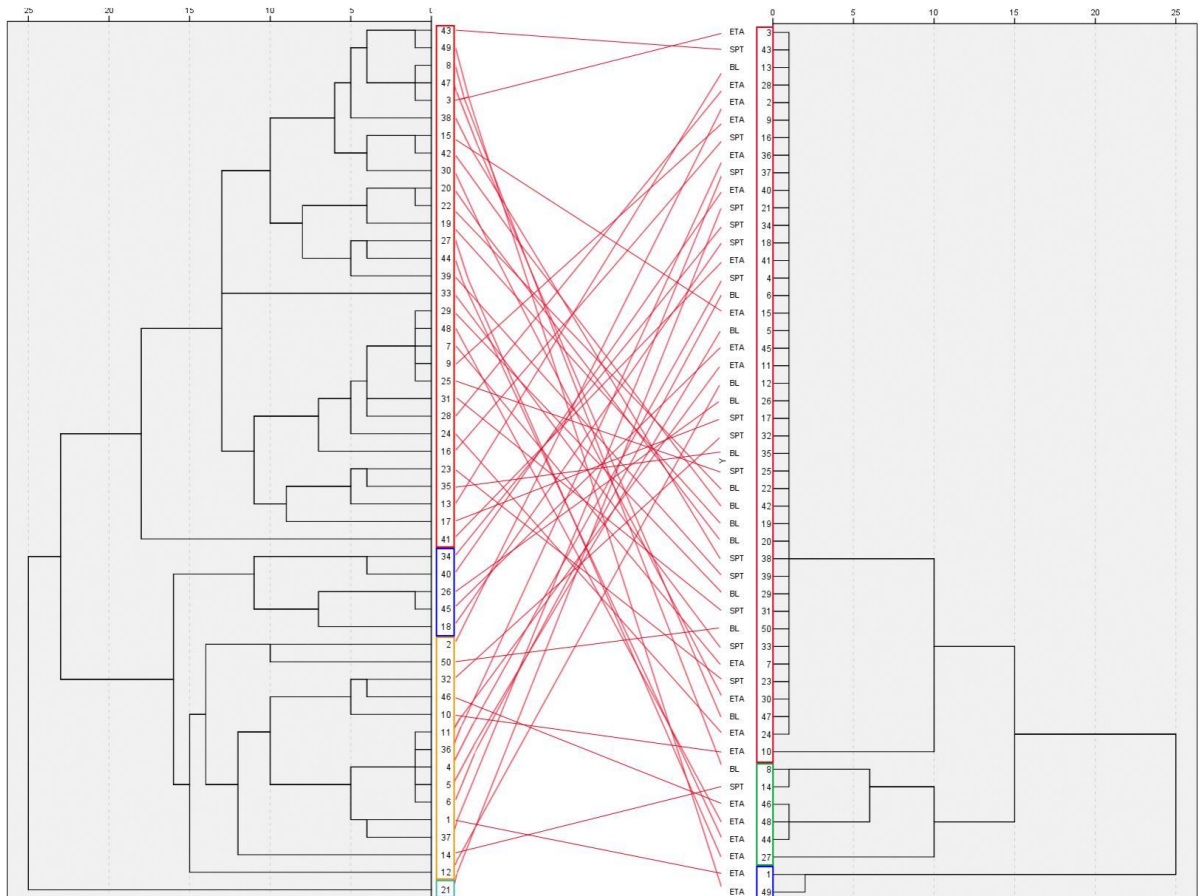


Fig. 1. Dendrogram based on hierarchical analyses of *Acinetobacter* isolates. Left dendrogram shows biochemistry profile grouping between isolates. Each cluster is marked with colored rectangles. Right dendrogram shows MIC grouping between isolates. Each clade is marked with rectangles. BL: Blood specimen; ETA: Endotracheal Aspirate specimen; SPT: Induced or spontaneous sputum specimen collected in infection window period after being weaned off a ventilator.

#### 4. Discussion

Severe and critical COVID-19 patients using ventilators are more susceptible to VAP than non-COVID-19 patients. This is due to disruption of mucociliary clearance by SARS-CoV-2 and hyaline membrane production prolongs recovery (Zhou, 2020; Maes, 2021; Kumar, 2021). Immunoparesis in COVID-19 also take part in severe and critical patients who also experience cytokine storm and sepsis (Morris, 2018). The clinical course and susceptibility of respiratory infections is worsened by immobility and suppression of cough reflexes

(Zhou, 2020). Pulmonary dysbiosis due to antibiotic use also plays significant part in HAI. Pulmonary flora is dominated by Firmicutes (*Enterococcus spp.* and *Staphylococcus spp.*) (Morris, 2020).

Endotracheal aspirate is the recommended specimen in diagnosis in VAP because it pictures: the clinical increase of tracheal secretions and suctioning needs, endotracheal tube intra luminal colonization (not oral flora) (NHSN, 2021; Kalil, 2016; Joseph, 2010; Leber, 2016). More invasive but accurate specimen is broncho-alveolar lavage and bronchial brushing. As this method can obtain more specimen and less oral contamination (NHSN, 2021; Kalil, 2016; Leber, 2016). The more minimal oral contamination, the fewer number of bacteria obtained that can be interpreted as true pathogen. Endotracheal aspirate specimens usually contain only few types of microorganism (Kalil, 2016; Leber, 2016; Papazian, 2020). This increases the meaning of each isolate grown in primary plating and minimizes the probability of analyzing non-pathogenic or contaminants (Papazian, 2020).

Acinetobacter genus are members of Moraxellaceae that are resistant to dehydration and can survive for a long period in environment. This genus is also associated with HAI are significant pathogen in device-related infections (Garrity, 2019). This descriptive result pictures closely related isolates by phenotypic grouping. Isolate group 1A is the most common phenotype found as VAP isolates and related secondary bloodstream infections. Hierarchical clustering is based on high internal homogeneity and high external heterogeneity. If these isolates are from the same clone, this phenotype had been circulating among the hospital for a period of time that is carried into the COVID-19 isolation room. On the other hand, if the isolates are not from the same clone, knowing that phenotype might be triggered or suppressed due to biological, chemical, or physical stressor experienced by the bacteria, these bacteria have undergone similar stressor that elicits the same phenotype, especially when the MIC is grouped into the same cluster (Cluster A). Acinetobacter is also an intrinsically multi resistant bacteria and might acquire other resistant abilities via mobile genetic elements (de Oliveira, 2020). Molecular tracing of resistance genes might not be available vastly. In this method, using MIC instead of susceptibility test interpretation reduces. Continuous data gathering and analysis is needed to make this simple analysis applicable.

## 5. Conclusion

Acinetobacter species phenotype cluster 1A and 3A dominates VAP associated isolates. This method can be used to trace common source of bacterial outbreak or be used as proof for isolate tracing by phenotypic similarity especially in limited resource settings. The use of this method can set reference for managerial interventions, thus preventing HAI.

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## Competing Interest

The author(s) declare that they have no competing interest.

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