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IDENTIFICATION OF AIRBORNE AEROBIC BACTERIA IN THE INTENSIVE CARE ROOM USING MALDI-TOF MS

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Abstract

Introduction: *Indoor air quality can affect the spread of airborne microorganisms which can lead to healthcare-associated infections (HAIs). The quality and quantity of airborne microorganisms are responsible for mortality and morbidity in infection-prone hosts such as patients admitted to the intensive care unit (ICU). The research aims to determine the quality of microorganisms in the air, identify the types of aerobic bacteria, and assess the physical parameters of the air in the ICU.* ***Methods:*** *This study was a cross-sectional study with a descriptive observational method. Air specimens were collected using the MAS-100 NT tool with blood agar plate solid culture media, which then incubated in an aerobic atmosphere for 24 hours at 37±2°C. Observation and measurement of air microbiological quality was by counting the number of microorganisms in CFU/m³ and identification of bacteria using MALDI-TOF MS.* ***Results and Discussion:*** *The maximum concentration of microorganisms in the air exceeds the standard value, and the average value of the concentration of microorganisms in the air is 736 CFU/m³. The most common types of aerobic bacteria in the air were Bacillus sp. (n=12), Coagulase-negative Staphylococci (n=5), and Staphylococcus aureus (n=5). There was an increase in physical parameters in the form of average temperature (26.24°C) and humidity (70%) with a ventilation system and air regulation using mechanical ventilation sourced from a split air conditioner with an exhaust fan without a high-efficiency particulate-absorbing (HEPA) filter.* ***Conclusion:*** *Low indoor air quality has the potential to increase the concentration of microorganisms and bacterial findings in the air.*

INTRODUCTION

According to the Republic of Indonesia's Minister of Health's Regulation, interactions within the hospital environment can cause a health problem characterized by a decrease in hospital environmental health quality indicators (1). The important concern is the quantity and quality of airborne microorganisms because the types of airborne microorganisms are responsible for morbidity and mortality in hosts that are susceptible to infection (2–4). The presence of airborne microorganisms in the work environment is called bioaerosol. Bioaerosols are defined as biological particles in aerosol form originating from all types of organisms and spreading into the air by various abiotic and biotic mechanisms. Bioaerosol or organic dust consists of live or dead bacteria that are pathogenic or non-pathogenic, fungi, viruses, allergens, bacterial endotoxins, mycotoxins, peptidoglycan, and others that are parts of the air environment (5).

Airborne microorganisms are considered to have pathogenicity that causes infections in humans such as HAIs. Poor environment and indoor air quality in hospital rooms can affect the spread of microorganisms in the air, which can cause HAIs (6-7). Risk factors for HAIs depend on the implementation of infection control in health facilities, the patient's immune status, and the prevalence of pathogens that cause infection in the community.

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Apart from that, several other risk factors such as long hospital stays, comorbid diseases, installation of mechanical ventilators and invasive procedures, and treatment in the ICU make patients vulnerable to pathogenic infections in the hospital environment. This pathogen can be acquired from hospitalized patients, staff, or healthcare facilities. Transmission of pathogens in healthcare environments is very complex and can occur through direct contact with health workers or contaminated surrounding environments (8). Even though the incidence of HAIs associated with air is only 20%, it cannot be underestimated because there are also bacteria that have been found to have resistance to antibiotics (9).

A journal article reported that the highest incidence of HAIS in intensive care settings were urinary tract infections related to catheter insertion, bloodstream infections, hospital infections due to *C. difficile* and pneumonia with the most common causative bacteria being *C. difficile*, *Klebsiella sp*., *Acinetobacter baumanii*, *Enterococcus* and *Pseudomonas aeruginosa* (10). Arca et al., in their study, found a significant positive correlation between the rate of hospital-acquired infections and the incidence density of hospital-acquired infections identified in the ICU during the study. Infection rates increase in proportion to the length of ICU stay. Likewise, the rate of infections identified in the ICUs increased as the number of colonies found in both ICUs increased. This study found gram-negative bacteria such as *Acinetobacter baumanii and Klebsiella pneumoniae* which are resistant to carbapenems and tigecycline and gram-positive bacteria such as *Staphylococcus aureus* were susceptible to colistin and tigecycline from the air (9)

The World Health Organization estimates that HAIs are the most common side effect of treatment. HAIs, especially those caused by pathogens that are resistant to antimicrobials, are of public health concern worldwide. Appropriate diagnosis, therapy, and prevention can reduce the length of stay in hospital, treatment costs, and suffering for patients (9,11–13). The research aims to determine the quality of microorganisms in the air, identify the types of aerobic bacteria, and assess the physical parameters of air in the intensive care unit (ICU).

METHODS

This study used a descriptive observational method with a cross-sectional study design and conducted observations, measurement of air microbiological quality, and identification of aerobic bacteria in the air in the ICU at Stella Maris Hospital Makassar on 5th- 12th March 2023.

Airborne bacteria samples were collected and measured using a "Microbiological Air Sample" tool (MAS-100 NT; PT. Merck Chemical and Life Sciences; Germany, Serial Number: 79385) with solid culture media blood agar plate placed in the air inlet. First, the area of the room was measured and the number of sampling points determined based on the second edition ISO 14644-1 International Standard guidelines regarding "Cleanroom and Associated Controlled Environments" Part 1 in 2015. The MAS-100 NT tool was placed at a distance of ± 1m from the floor and the wall, which lasted approximately 10 minutes at each specimen collection point (14).

Incubation was conducted in an incubator within an aerobic atmosphere for 24 hours at 37±2^{o}C and the number of growing microorganisms measured in CFU/m^{3}. The samples each in a petri dish were counted using a colony counter. The numbers obtained were then further processed using the Positive Hole Conversion Table MAS-100 from the value *of r*, where *r* is the number of colonies that appeared on the plate agar media after the incubation process.

Identification of aerobic bacteria from colonies on blood agar media was using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) at the Makassar Health Laboratory Center. Bacterial preparation was done by taking an isolated colony using a 1µl loop and then placed in the center of the spot position on the sliding target. The colony was then flattened using a loop and 1µl Vitek® MS-CHCA matrix added in the center of the spot position using a micropipette and allowed to dry. The Vitek®MS-DS slide target was then attached to the adapter which was placed on the carrier. The acquisition process is where the adapter will enter the instrument tool until the spectra display and the number of profiles are obtained.

Microorganism concentration standards were based on the Republic of Indonesia Health Minister Decree (4) and quality standards for temperature, humidity, and air pressure in the ICU based on Regulation of the Minister of Health of the Republic of Indonesia (1).

The data obtained were used to summarize the demographics of the room area and the number of sampling points, physical parameters (humidity, temperature, and air pressure), air source and air setting, the concentration of microorganisms in the air, and the results of aerobic bacteria identification using descriptive analysis.

RESULTS

Indoor Air Quality and Physical Parameters in the ICU

Table 1 provides an overview of the ICU consisting of five rooms with different room areas. Rooms A, B, and C have the same room area (35 m^2) with nine sampling points for each room. Room D has a room area (12 m^2) with five sampling points and room E has a room area (12 m^2) with five sampling points.

Table 1. Distribution of Location, Area and Number of Air Sampling Points

Location	Room Area	Number of Sample points (n)
Room A	35m^2	9
Room B	35m^2	9
Room C	35m^2	9
Room D	12m^2	5
Room E	12m^2	5

Table 2 shows the results of measuring the concentration of indoor air microorganisms; the microbiological air quality in the ICU exceeds the value of indoor air quality standards (200 CFU/m^3). In this study, the average concentration of microorganisms in the air in the ICUs is more than 200 CFU/m^3. The highest concentration in rooms B and C was 853 CFU/m^3.

Table 2. Distribution of Concentration of Air Microorganisms

Room	Concentration of Microorganisms in Air (CFU/m^3)	Standards for Maximum Concentrations in Air
Room A	752	200 CFU/m^3
Room B	853	200 CFU/m^3
Room C	853	200 CFU/m^3
Room D	677	200 CFU/m^3
Room E	547	200 CFU/m^3

The temperature and humidity in each room in the ICU exceed the quality standards. The average temperature in the ICU room is 26.2°C with 70% indoor humidity, and the air pressure in the room is positive (Table 3). Other data related to the air conditioning and air management system was using mechanical ventilation in the form of split air conditioners and exhaust fans without high-efficiency particulate absorbing (HEPA) filters (Table 4).

Table 3. Distribution of Temperature, Humidity and Air Pressure Measurement Results

Room	Temperature (°C) (Quality Standard 22-23°C)	Humidity (%) (Quality Standard 40-60%)	Air Pressure Measurement	Air Pressure Measurement Quality Standard
Room A	26.5	70	Positive	Positive
Room B	27	70	Positive	Positive
Room C	25.5	68	Positive	Positive
Room D	26.3	74	Positive	Positive
Room E	25.8	68	Positive	Positive

Table 4. Distribution of Air Conditioning Systems and Air Regulation

Room	Air Conditioning and Control System			
	Mechanical Ventilation			HEPA Filter
	Air Conditioner		Exhaust Fan	
	Ducting (central)	Split		
Room A	-	Yes	Yes	No
Room B	-	Yes	Yes	No
Room C	-	Yes	Yes	No
Room D	-	Yes	Yes	No
Room E	-	Yes	Yes	No

Aerobic Bacteria in the Air in the ICU

The results of identifying the type of aerobic bacteria in the ICU are gram-positive bacteria, including *Bacillus sp.* [*Bacillus cereus group* (n=5), *Bacillus megaterium* (n=2), *Exiguobacterium acetylicum* (n=2), *Exiguobacterium auranticum* (n=1), *Bacillus flexus* (n=1) and *Bacillus firmus* (n=1)], followed by gram-negative bacteria *Acinetobacter baumanii* (n=2), *Micrococcus luteus* (n=1), Coagulase Negative Staphylococci [including *Staphylococcus arletae* (n=3) and *Staphylococcus haemolyticus* (n=2)], and *Staphylococcus aureus* (n=5). The *S. aureus* isolates in this study were sequenced and no genes encoding methicillin resistance were found.

Table 5. Results of Identification of Types of Aerobic Bacteria

Room	Aerob Bacteria
Room A	*Staphylococcus arletae* *Staphylococcus aureus* *Bacillus cereus grup* *Bacillus megaterium*
Room B	*Staphylococcus arletae* *Staphylococcus aureus* *Exiguobacterium acetylicum* *Bacillus cereus grup* *Acinetobacter baumaniii*
Room C	*Staphylococcus haemolyticus* *Staphylococcus aureus* *Exiguobacterium auranticum* *Bacillus cereus grup* *Acinetobacter baumanii*
Room D	*Staphylococcus haemolyticus* *Staphylococcus aureus* *Exiguobacterium acetylicum* *Bacillus cereus grup* *Bacillus megaterium*
Room E	*Staphylococcus arletae* *Staphylococcus aureus* *Micrococcus luteus* *Bacillus cereus grup* *Bacillus flexus* *Bacillus firmus*

DISCUSSION

HAIs are one of the causes of increased mortality and morbidity rates in hospitals and can become a new

health problem, both in developed and developing countries. Therefore, hospitals must be able to deliver high-quality care in compliance with established guidelines and must be applied by all health workers. In addition, due to the length of stay in HAIs patients who occur in the hospital, it can increase treatment costs (11). The spread of infectious diseases through the air is caused by the presence of droplets containing pathogenic microorganisms (15). Droplets containing these microorganisms survive in favorable conditions such as dry and cool atmospheres without direct exposure to sunlight. Indoor air quality in hospitals is very important because many related studies have statistically revealed and measured airborne microorganisms that can potentially cause HAIs (16-17).

The distribution of air microorganism concentrations in the ICU ward as shown in Table 2 finds a high index of the number of airborne germs in those wards (average value = 736 CFU/m^3). This is in line with a study in the international hospitals in South Sulawesi which reported that the risk of HAIs in the ICU was high with an average concentration of air microorganisms in the ICU room of 500 CFU/m^3 (18). The maximum concentration of air microorganisms in intensive care rooms in each hospital varies based on the standard value of the maximum concentration of microorganisms in the air in each country because each country uses different standard values such as WHO and European Commission standards on indoor air microbial load. This study is based on the regulation of the Minister of Health of the Republic of Indonesia (19).

The specimen collection technique used in this study is more effective than the passive method because the volume of air taken is measurable, specimen collection does not interfere with indoor activities and the collection time is shorter. Regarding the MAS-100 NT instrument, it does not produce significant noise. The main advantage of the active method is that all airborne suspended particles can be collected. This method requires a device for air sampling, so it is more expensive than the passive method, however the analysis of larger air volumes takes a shorter time (20).

Airborne bacteria originate from various external sources such as water, animals, plants, and soil, while the atmosphere itself is not usually considered a habitat for airborne bacteria. Bacteria released from various sources enter the atmosphere through an aerosolization process, which is then carried by air masses. Variations in meteorological factors significantly influence the initial aerosolization process and subsequent dispersal processes. Dry air and strong winds influence the spread of microbes in the air. Therefore, it is slightly difficult to explain and predict variations in bioaerosol characteristics precisely (21). A literature review stated that indoor air ventilation systems, humidity, and temperature, in hospital buildings influence various potential pathogenic organisms in the air such as fungi, viruses, and bacteria that affect hospitalized patients (22).

The most common airborne aerobic bacteria found in ICU in this study were *Bacillus sp., Staphylococcus aureus, Coagulase Negative Staphylococci* (CoNS), *Micrococcus luteus* and *Acinetobacter baumanii*. Some studies related to aerobic bacteria most commonly found in the air in ICUs including *Cronobacter sakazaki, Staphylococcus gallinarum, Geobacillus sterothermophilus, Acinetobacter baumannii, Spingomonas paucimobilis, Bacillus mycoides/thuringirns/cereus, Bacillus megaterium,* and *Staphylococcus haemolyticus* (18, 23–24).

The most common airborne aerobic bacteria found in this study were *Bacillus sp.* The findings of *Bacillus sp*. in this study indicate quite high contamination of such bacteria in the air in this intensive care unit. Aerobic bacteria in the air identified as *Bacillus sp.* are bacteria that are commonly found in the environment such as air, water, soil, and plants and they can be a pathogen causing infection in immunocompromised patients in the intensive care unit (25–27). *Bacillus sp*. was found from the results of airborne bacterial cultures that have the potential to become infectious pathogens, especially when the level of contamination in the air is high. In the case of outbreaks due to *Bacillus sp.* infection, preventive measures that can be taken such as the installation of barriers or barriers in building construction work areas in the hospital environment as well as the application of standard precautions and transmission are reported to be able to reduce the incidence rate (26).

Aerobic bacteria found in the air in the study having the potential to cause clinical infections in hospitals are a gram-positive *Staphylococcus aureus* which is a commensal bacterium in humans and its main habitat is in the anterior squamous nares epithelium and oropharynx. *S. aureus* usually does not cause infection on healthy skin, but this species can cause various potentially serious infections when it enters the bloodstream or the internal tissues, especially the emergence of multi-drug resistant strains such as MRSA (28). In this study, we did not find *S. aureus* that was resistant to methicillin. *Staphylococcus aureus* is the most common bacteria associated with the incidence of infections in hospital and community settings, MRSA strains whose incidence is increasing (29–30).

Staphylococci that do not produce coagulase

are called coagulase-negative *Staphylococci* (CoNS) a normal flora on the mucosa and skin but can be caused the infections such as bacteremia, eye infections, and urinary tract infections which are associated with infections in the hospital environment, especially in patients with central line installation in the ICU ward or hemodialysis patient. Some of the species from this group that are most frequently found and have clinical significance include *Staphylococcus lugdunensis, Staphylococcus haemolyticus Staphylococcus saprophyticus*, and *Staphylococcus epidermidis* which are known to be significant pathogens. *Staphylococcus epidermidis* is known to be closely associated with various infections acquired in the hospital environment, while *Staphylococcus saprophyticus* is mainly associated with urinary tract infections (UTI), especially in young women. *Staphylococcus haemolyticus* is a CoNS that is sometimes found in wounds, septicemia, UTI and infectious endocarditis and also has a high level of resistance to antimicrobial such as methicillin-resistant *Staphylococcus haemolyticus* (MRSH). *Staphylococcus lugdunensis* is sometimes difficult to distinguish from *Staphylococcus aureus*, especially in the conventional catalase test using plasma. *Staphylococcus lugdunensis* can be an aggressive infectious pathogen, especially in cases of bacteremia associated with catheter use and endocarditis (31).

There is a lack of data on reports on the incidence of infection due to *Acinetobacter bauminii* in patients treated in intensive care in this study. However, it needs to be a concern for working staff that the finding of this bacteria in the air in the intensive care room needs special attention because several reports state that the finding of *Acinetobacter baumanii,* which is a bacterium that lives in moist environmental conditions and is an opportunistic bacterium that is commonly found in hospital or healthcare settings, is included in particular concern, especially when it is found in the community because of its natural resistance to antibiotics and its ability to experience high antibiotic resistance. Resistance to this bacterium is associated with a low immune system of infected patients or in ICUs with cases such as ventilator-associated pneumonia (VAP) or hospital-acquired pneumonia (HAP), skin and soft tissue infections, surgical site infection (SSI), bloodstream infections and CAUTI in patients treated in the ICU (32–34). Some studies reported that one of the most drug-resistant and clinically significant microorganisms in ICUs worldwide, antimicrobial-resistant *Acinetobacter baumannii*, was found in dust on air conditioning vents, especially β-lactamase and carbapenem-producing strains in ICUs. The presence of *Acinetobacter baumanii* in dust on air conditioning vents may increase *Acinetobacter baumanii* contamination in hospital treatment wards providing a new proposal for monitoring and assessing *Acinetobacter baumanii* contamination in the hospital environment (35).

Airborne diseases have been linked to poorly functioning ventilation, heating, and air conditioning (HVAC) systems such as the humidity, temperature, ventilation, and filter systems used. Some studies have shown the correlation of patient outcomes is dependent on the indoor environment of a hospital building. Most viruses, fungi, and bacteria survive longer at higher humidity (>70%) and infectivity is reduced at humidity between 40%-70%. Indoor air humidity in this study was above the established standard value (>60%), which of course affects the ability of bacteria to survive in the air. For a majority of gram-negative bacteria (*Klebsiella pneumonia* and *Escherichia coli*,), higher humidity can be detrimental to their survival, except for a few (*Salmonella Seftenberg*, *Klebsiella spp*., *Enterobacter spp*., and *Pseudomonas spp*.). Likewise, higher humidity is recommended to control gram-positive bacteria in the air (22,36). In this study we can see that both gram-negative bacteria (n = 2), especially gram-positive bacteria (n = 23, can survive in the air at high humidity.

Several studies related to the findings of airborne pathogenic bacteria or microorganisms in the HVAC system have been widely reported, such as the findings of burn infection due to MRSA associated with the findings of these bacteria in the air in the burn care unit. Other findings such as carbapenem resistance Acinetobacter sp. were also found on curtain supports in patient care units and ICU (36). In this study, we do not find an MRSA and carbapenem resistance Acinetobacter sp. in the air.

The HVCA system used in the intensive care room in this study does not fully comply with applicable standards because each room in the ICU does not have HEPA filters. Different ventilation, air pressure control, and air filtration strategies may subsidize the spread of airborne infectious diseases in hospitals. Along with the use of proper ventilation strategies, HEPA filtration systems can effectively reduce contamination loads. However, because polluted air from nearby spaces may restrict the effectiveness of HEPA filters in ducts in controlling airborne nosocomial infections, it is crucial to maintain a space pressure differential. Therefore, it is necessary to ensure that contamination does not occur in the air in critical care rooms in hospitals such as surgical treatment rooms, operating rooms, and ICUs (22).

Air health requirements depend on maintaining hospital indoor air quality that ensures clean, odorless,

and abscessed dust-free air. The lighting source of the room must have a good light intensity according to its function. Air conditioning and regulation in the room must pay attention to room ventilation maintained with positive pressure, air supply and exhaust should be driven mechanically and exhaust fans need to be at the end of the ventilation system and equipped with HEPA filters. The use of cooling systems such as split air conditioners should be maintained and operated according to the guidebook in producing good temperature, air flow, and humidity (1).

Currently, the trend of using methods of identifying microorganisms by rapid automation is more of an option in laboratory practice. The fastest and newest method currently widely used in clinical microbiology laboratories around the world is MALDI-TOF MS (37). One of the main advantages of using MALDI-TOF MS is time-saving, as bacterial identification can be done in less than an hour compared to other identification tools that take 24-48 hours (38). In this study we used MALDI-TOF MS in identifying bacterial isolates due to simpler sample preparation procedures, relatively lower costs, and faster analysis with better sensitivity of results. Identification of aerobic bacteria using MALDI-TOF MS is very good, especially in identifying gram-negative bacteria. The weakness is that using this method cannot provide antibiotic sensitivity test results for bacteria.

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CONCLUSION

Low indoor air quality has the potential to increase the concentration of microorganisms and the finding of airborne bacteria that can be pathogenic in the air is the cause of infection in patients in intensive care. The presence of microorganisms in the air such as *Bacillus sp., Sthaphylococcus aureus*, CONS, and *Acinetobacter baumanii* have the potential to cause infection, especially in the hospital environment.

The technique of taking specimens using the MAS-100 NT tool is very helpful in this study, especially in calculating the number of colonies because the bacterial growth pattern formed on the media makes it easy to count the number of bacterial colonies that grow. Identification using Maldi TOF MS is an identification method that is appropriate, time-efficient, and cheaper. Therefore, this identification method is considered more efficient in identifying aerobic bacteria in the air, especially in identifying colonies that are quite often found on agar plate media.

Physical parameters in the form of temperature, humidity, and pressure in the room as well as the air conditioning system and indoor air regulation are considered to affect indoor air quality in hospitals. This is the importance of monitoring and assessing air quality and measuring and identifying bacteria in the air that have the potential to cause disease. Air conditioning and regulation in the ICU room must pay attention to room ventilation maintained with positive pressure, air supply and exhaust should be driven mechanically and exhaust fans need to be at the end of the ventilation system and equipped with HEPA filters. The use of cooling systems such as split air conditioners should be maintained and operated according to the instruction book to produce good temperature, airflow, and humidity.

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