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Update on Non-Communicable Diseases: Global Perspective on Health Challenges and Innovation

Identification of Bacteria on Classroom Air Conditioner at Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia

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ABSTRACT

Background: Air conditioner became a significant factor in indoor air quality. Poorly maintained Air Conditioners can become breeding grounds for bacteria, leading to health problems and symptoms of Sick Building Syndrome, such as headaches, loss of concentration, dry throat, eye and skin irritation, and acute respiratory infections.

Aims: Identify bacteria types and count bacterial colonies on Air Conditioning units in the classrooms of buildings A and B at Faculty of Medicine, Universitas Swadaya Gunung Jati, Cirebon, Indonesia.

Methods: Cross-sectional study using 16 air conditioner samples from lecture rooms at Faculty of Medicine, Universitas Swadaya Gunung Jati. Direct swabs were taken from the air conditioner filters using cotton swabs, which were then identified using McConkey, Blood Agar, and Nutrient Agar media to count bacterial colonies followed by confirmatory bacterial tests using Triple Sugar Iron Agar (TSIA) and Indol, Methyl red, Vogues proskauer, Simmon Citrate (IMVIC) media.

Results: Bacterial growth identification tests identified *Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Coryneform*/*Diptheroid*, *Enterobacter sp.*, and *Stenotrophomonas sp.*. Highest colony count was 402 CFU/cm³ and lowest was 16 CFU/cm³.

Conclusion: Bacterial identification on classroom the Air Conditioner revealed that the microbial community in the system was predominantly composed of Gram-positive strains, which were detected more frequently than Gram-negative strains.

Keywords: *Air conditioner, Bacteria, Classroom, Confirmatory bacterial tests.*
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1. Introduction

People spent over 90% of their time indoors. In addition to indoor residential environments, people spent most of their time in offices, educational institutions, and other industrial buildings. Indoor air quality (IAQ) around structures and buildings was related to the health and comfort of building occupants. Indoor air quality (IAQ) was very important because it could affect the health of everyone, especially children, adults, and those suffering from asthma and heart disease.(Mannan and Al-Ghamdi 2021)

The impact of Air Conditioner maintenance on the development of bacteria indoors was significant. Research showed that poorly maintained air conditioners could become breeding grounds for microorganisms, including bacteria that could affect air quality and the health of occupants. Air conditioners that were rarely cleaned could accumulate dust, dirt, and moisture, creating an ideal environment for the growth of bacteria and mold. These conditions could result in health problems such as respiratory irritation and symptoms of Sick Building Syndrome (SBS), including headaches, loss of concentration, dry throat, eye and skin irritation, and Acute Respiratory Infections (ARI).(Azteria 2021)(Kesehatan and Indonesia 2011)

Based on the figures, nosocomial infections or HAIs (Hospital Acquired Infections) worldwide reached 9% or approximately 1.4 million patients. The transmission of nosocomial infections in hospitals occurred in various ways, one of which was through the air. A survey conducted by the WHO in 55 hospitals from four countries obtained an average of 8.7% of inpatients experiencing HAIs, while the highest rate in Southeast Asia was 11.8%. A similar study was conducted in Indonesia in 11 hospitals in DKI Jakarta, showing that approximately 9.8% of inpatients experienced nosocomial infections. The most common infections were primary bloodstream infections at 26.4%, pneumonia at 24.5%, surgical site infections (SSI) at 18.9%, and urinary tract infections (UTI) at 15.1%.(Susilawati, Ilham, and Guspianto 2021)

Microorganisms dispersed in a room were called bioaerosols. Bioaerosols could originate from the external environment or from internal contamination. Biologically, bioaerosols came from plants, animals, and marine ecosystems, containing viruses, bacteria, fungi, and pollen. Physical characteristics and environmental conditions were two categories that influenced bioaerosols. Physical factors included air moisture content, relative humidity, temperature, air exchange rate, and human activity. Droplet or particle form was another physical characteristic.(Ghosh, Lal, and Srivastava 2015)

Based on research conducted by Watanabe K, et al. in Japan on 17 household air conditioning units, the 10 most common bacteria found were *Pseudomonas*, *Staphylococcus*, *Paracoccus*, *Corynebacterium*, *Acinetobacter*, *Streptococcus*, *Methylobacterium*, *Enhydrobacter*, *Sphingomonas*, and *Actinotignum*, which were present on Air Conditioning filters, cooling coils, fans, and air outlet surfaces.(Watanabe et al. 2022)

The study on air conditioners in classrooms at Faculty of Medicine, Universitas Swadaya Gunung Jati was very important because the air conditioning systems in classrooms played a crucial role in creating a comfortable and healthy learning environment for students and lecturers. Considering that classrooms were places where many people gathered for hours, indoor air quality could significantly affect the health and concentration of occupants. Air contaminated by bacteria or other microorganisms, especially if spread through poorly maintained air conditioners, could have increased the risk of health issues such as respiratory infections, allergies, and Sick Building Syndrome (SBS). Furthermore, understanding this condition specifically at Universitas Swadaya Gunung Jati would have helped the university take more appropriate preventive measures, such as implementing better air conditioner maintenance protocols, to ensure a safer and healthier learning environment.

Based on this background, the researchers wanted to find out the types of bacteria present in the Air Conditioners of the classrooms in buildings A and B of Faculty of Medicine, Universitas Swadaya Gunung Jati.

2. Methods

Study design/ Research procedures

This research was a Cross-sectional study employing total sampling, with 16 air conditioner samples collected from classrooms in buildings A and B of Faculty of Medicine, Universitas Swadaya Gunung Jati. The research was carried out in the classrooms of the Faculty of Medicine at Universitas Swadaya Gunung Jati, followed by microbiological testing at the faculty's Research Laboratory. This study was conducted in May 2024. Samples was obtained by directly swabbing air conditioner filters using sterile cotton swabs, using gloves and masks to prevent contamination, labeled, and stored in an ice box for transport to the laboratory.

a. Materials

The materials used in this study included 16 air conditioner filters for swabbing, sterile cotton swabs, gloves, masks, an ice box, MacConkey agar, Blood Agar, and Nutrient Agar media. Confirmation media for bacterial testing included Triple Sugar Iron Agar (TSIA) and Indole, Methyl Red, Voges-Proskauer, and Simmons Citrate Agar (IMVIC). Additional equipment used were inoculating loops, inoculating needles, a spirit lamp, petri dishes, an incubator, a colony counter, and an autoclave.

b. Sample Collection

A total of 16 air conditioner filters were used as samples, collected using the direct swab technique. The materials used for sample collection included sterile cotton swabs and gloves. Masks were used to prevent sample contamination from the researcher’s droplets, and an ice box was used to ensure the samples were not contaminated by outside air during transport to the laboratory.

c. Bacterial Culture and Identification

MacConkey was used to differentiate Gram-positive and Gram-negative bacteria based on their glucose metabolism. Blood Agar was used as a growth medium for various bacteria, both Gram-positive and Gram-negative, using the streaking method. Nutrient Agar was used as a basic medium to count the colonization of growing bacteria using the pour plate method. The media that had been inoculated were incubated in an incubator at 37°C for 24 hours after being sealed and packaged to analyze the growth of Gram-positive and Gram-negative bacterial cultures. The bacterial colonies that had grown were counted using a colony counter.

d. Biochemical Test for Bacterial Identification

Bacterial confirmation tests were performed on several bacteria using Triple Sugar Iron Agar (TSIA) was used to confirm whether the bacteria found belong to the group of bacteria that can ferment certain types of sugar to form acid and base with stab and streak method, The TSIA results indicated that none of the tested bacteria were able to ferment any of the three sugars (glucose, lactose, or sucrose) and produce acid or gas. Indole, Methyl Red, Voges Proskauer, and Simmons Citrate Agar (IMVIC), Indole test aimed to confirm that the bacteria detected had the ability to break down amino acids into indole compounds with stab inoculation, Methyl Red test sought to confirm that the identified bacteria could produce and maintain end products in the form of acid through the oxidation of glucose with broth inoculation, Voges Proskauer was used to detect the presence of acetyl methyl carbonil with broth inoculation , and Simmons Citrate Agar was employed to confirm bacteria that utilized citrate as a carbon source, resulting in a basic environment with streaking slant. The IMVIC results indicated increased pH of the medium from green to blue, indicated a positive Simmons Citrate test. This was due to production of alkaline compounds by the bacteria. Both of these tests were chosen because they were able to provide specific and comprehensive information about the biochemical properties of the bacteria, which was crucial for accurate identification.

Statistical techniques

The data were entered into Excel and then analyzed using the SPSS application to determine the prevalence of bacteria across all air conditioner units. This study only aimed to identify the types of bacteria present in the air conditioners. The colony count was expressed as Colony Forming Units per square centimeter (CFU/cm²), a unit

used to quantify the number of bacteria or microorganisms capable of forming colonies on a specific surface, calculated per unit area. WHO Guidelines for Indoor Air Quality: Dampness and Mould 2009, typically ranged between 100 and 500 CFU/m^3. According to the Indonesian Ministry of Health's number 1077/MENKES/PER/V/2011 guidelines on indoor air quality, the maximum allowable level for airborne pathogenic bacteria was 0 CFU/m^3.(Kesehatan and Indonesia 2011)(WHO 2009)

Ethical Clearance

This research was conducted in accordance with the research procedure, following the approval of the research permit which was obtained after passing thesis proposal examination and ethical review by Faculty of Medicine, Universitas Swadaya Gunung Jati Ethics Committee, with the number 7/EC/FKUGJ/IV/2024.

3. Results

The results of bacterial identification and colony counting from bacterial growth on the media were as follows.

Table 1. The distribution of bacteria and colony counts in the subjected air conditioners

Sample Code	Colony Forming Units	Genus	Observations
A C 1 AC 1	44 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	
A C 1 AC 2	16 CFU/cm^2	*Bacillus sp.* *Staphylococcus sp.* *Mold*	
A C 2 AC 1	104 CFU/cm^2	*Bacillus sp.* *Staphylococcus sp.* *Micrococcus sp.*	
A C 2 AC 2	72 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	
A C 3 AC 1	76 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	
A C 3 AC 2	78 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	
A C 4 AC 1	104 CFU/cm^2	*Bacillus sp.* *Staphylococcus sp.* *Streptococcus sp.*	
A C 4 AC 2	21 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.* *Stenotrophomonas sp.*	 TSIA and IMVIC test
B C 1 AC 1	156 CFU/cm^2	*Bacillus sp.* *Staphylococcus sp.* *Enterobacter sp.*	 TSIA and IMVIC test
B C 1 AC 2	212 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	
B C 2 AC 1	210 CFU/cm^2	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*	

B C 2 AC 2	208 CFU/cm²	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*
B C 3 AC 1	402 CFU/cm²	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.* *Streptococcus sp.* *Coryneform/difteroid*
B C 3 AC 2	186 CFU/cm²	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.*
B C 4 AC 1	212 CFU/cm²	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.* *Streptococcus sp.*
B C 4 AC 2	108 CFU/cm²	*Bacillus sp.* *Micrococcus sp.* *Staphylococcus sp.* *Coryneform/* *Diptheroid*

Observations:
A : Building A
B : Building B
C : Classroom
AC : Air Conditioner
TSIA : Triple Sugar Iron Agar
IMVIC : Indole, Methyl Red, Voges-Proskauer, and Simmon Citrate

Based on table 1, Bacterial growth identification tests identified *Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Coryneform*/*Diptheroid*, *Enterobacter sp.*, and *Stenotrophomonas sp.*. The presence of bacteria such as *Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Coryneform*/*Diptheroid*, *Enterobacter sp.*, and *Stenotrophomonas sp.* on air conditioners (AC) in classrooms could have posed various serious health risks. *Bacillus sp.* could have caused skin infections or mild to moderate respiratory problems through the inhalation of spores. *Staphylococcus sp.* and *Streptococcus sp.* had the potential to cause skin and respiratory infections that could have spread within the classroom environment. *Micrococcus sp.* and *Coryneform*/*Diptheroid*, although generally non-pathogenic, could have been a cause of infection in individuals with weakened immune systems. Meanwhile, *Enterobacter sp.* and *Stenotrophomonas sp.* could have triggered respiratory, urinary, and wound infections, especially in immunocompromised individuals.

Highest colony count was 402 CFU/cm² and lowest was 16 CFU/cm². The health implications for building occupants, especially students, included an increased risk of respiratory infections, allergies, and skin irritation due to exposure to bacteria such as Bacillus sp. and Staphylococcus sp., which were commonly found in air conditioners. Repeated exposure to these bacteria could have led to respiratory disorders like asthma or bronchitis, as well as skin infections, particularly in immunocompromised individuals. Additionally, a contaminated learning environment could have adversely affected students' concentration and comfort, ultimately impacting their health. The maximum acceptable level for the number of bacterial colonies in indoor air, according to the WHO Guidelines for Indoor Air Quality: Dampness and Mould 2009, typically ranged between 100 and 500 CFU/m³, depending on the type of bacteria and environmental conditions. According to the Indonesian Ministry of Health's number 1077/MENKES/PER/V/2011 guidelines on indoor air quality, the maximum allowable level for airborne pathogenic bacteria was 0 CFU/m³. (Kesehatan and Indonesia 2011)(WHO 2009).

Table 2 demonstrated that three air conditioner samples yielded negative results on MacConkey agar, signifying the absence of bacterial growth. Conversely, positive results were observed for bacterial growth on MacConkey agar, blood agar, and nutrient agar.

Table 2. Bacterial colony growth on MacConkey, Blood, and Nutrient agar plates was observed.

Sample Code	MacConkey	Blood Agar	Nutrient Agar
A C 1 AC 1	-	+	+
A C 1 AC 2	-	+	+
A C 2 AC 1	-	+	+
A C 2 AC 2	-	+	+
A C 3 AC 1	+	+	+
A C 3 AC 2	+	+	+
A C 4 AC 1	+	+	+
A C 4 AC 2	+	+	+
B C 1 AC 1	+	+	+
B C 1 AC 2	+	+	+
B C 2 AC 1	+	+	+
B C 2 AC 2	+	+	+
B C 3 AC 1	+	+	+
B C 3 AC 2	+	+	+
B C 4 AC 1	+	+	+
B C 4 AC 2	+	+	+

Observations:
A : Building A
B : Building B
C : Classroom
AC : Air Conditioner

Based on table 3, The TSIA results indicated that none of the tested bacteria were able to ferment any of the three sugars (glucose, lactose, or sucrose) and produce acid or gas. Increased pH of the medium from green to blue, indicated a positive Simmons Citrate test. This was due to production of alkaline compounds by the bacteria.

Table 3. Bacterial confirmation tests

Sample Code	TSIA	Indole	Methyl Red	Voges Proskauer	Simmon Citrate
A C 4 AC 2	K/K	-	-	-	+
B C 1 AC 1	K/K	-	-	-	+

Observations:
A : Building A
B : Building B
C : Classroom
AC : Air Conditioner
TSIA : Triple Sugar Iron Agar
IMVIC : Indole, Methyl Red, Voges-Proskauer, and Simmon Citrate
K/K : Red slant and red butt (Red/Red or Alkaline (K)/ Alkaline (K)

4. Discussion

The study results showed that various types of bacteria were successfully isolated from air conditioner samples, including *Bacillus sp.*, *Micrococcus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Coryneform*/*Diptheroid*, *Enterobacter sp.*, and *Stenotrophomonas sp.*. *Bacillus sp.* and *Staphylococcus sp.* were the most frequently isolated bacterial genus, with isolation rates of *Bacillus sp.* at 100% (16 isolates), followed by *Staphylococcus sp.* at 100% (16 isolates), *Micrococcus sp.* 81% (13 isolates), *Streptococcus sp.* 12% (2 isolates), *Coryneform/Diptheroid* 12% (2 isolates), *Enterobacter sp.* 6% (1 isolate), and *Stenotrophomonas sp.* 6% (1 isolate). A significant variation in colony count was found, with the highest colony reaching 402 CFU/cm³ and the lowest at 16 CFU/cm³.

The latest research results showed that the air conditioners in the classrooms of the Faculty of Medicine, Muhammadiyah Sumatera Utara University had bacterial growth, with *Bacillus subtilis*, a Gram-positive bacterium, found in 10 samples (66.7%), with *Penicillium sp.* for half of the identified fungal growth, found in 7 samples (50%).(Dharma 2020) The research conducted by Watanabe K, Yunagi U, Shiraishi Y, Harada K, and

Ogino F in 2022 on *Bacterial Communities in Various Parts of Air Conditioning Units in 17 Japanese Houses* found the following types of bacteria: *Pseudomonas sp.*, *Staphylococcus sp.*, *Paracoccus*, *Corynebacterium*, *Acinetobacter*, *Streptococcus sp.*, *Methylobacterium*, *Enhydrobacter*, *Sphingomonas*, and *Actinotignum*. The filter surface was primarily populated by six genera, all of which were Gram-negative bacteria.(Watanabe et al. 2022)

Bacillus sp. may cause skin infections or mild to moderate respiratory problems through the inhalation of spores. *Staphylococcus sp.* and *Streptococcus sp.* had the potential to cause skin and respiratory infections that could have spread within the classroom environment. *Micrococcus sp.* and *Coryneform*/*Diptheroid*, although generally non-pathogenic, could have been a cause of infection in individuals with weakened immune systems. Meanwhile, *Enterobacter sp.* and *Stenotrophomonas sp.* could have triggered respiratory, urinary, and wound infections, especially in immunocompromised individuals.(Ryan, K. J., & Ray 2010)

Based on the research results, this did not meet the indoor air quality requirements according to the Regulation of the Minister of Health of the Republic of Indonesia Number 1077/MENKES/PER/V/2011 concerning Guidelines for Indoor Air Quality, which states that the maximum allowable level of pathogenic bacteria is 0 CFU/cm².(Kesehatan and Indonesia 2011) The impact of air conditioner (AC) maintenance on the growth of bacteria indoors was found to be significant. Studies revealed that poorly maintained AC units can become breeding grounds for microorganisms, including bacteria, which can adversely affect indoor air quality and the health of occupants. Infrequently cleaned Air Conditioner can accumulate dust, dirt, and moisture, creating an ideal environment for the growth of bacteria and mold. Research indicates that this condition can lead to health problems such as respiratory irritation and symptoms of Sick Building Syndrome (SBS).(Azteria 2021) A humidity level of 30%-50% is generally recommended for homes, and improving ventilation systems and maintaining a clean environment can help reduce bacterial counts and enhance indoor air quality.(Heseltine and Rosen 2009)

The health effects of pathogenic bacteria indoors include the development of infectious diseases such as nosocomial infections, flu, hypersensitivity (asthma or allergies), and toxicosis, where toxins in contaminated indoor air cause symptoms of Sick Building Syndrome (SBS). SBS symptoms include headaches, loss of concentration, dry throat, eye and skin irritation, and Acute Respiratory Infections (ARIs).(Kesehatan and Indonesia 2011) Sick Building Syndrome (SBS) was a condition in which building occupants experienced a variety of health symptoms, such as respiratory problems, headaches, and eye irritation, that appeared to be linked to the time spent inside the building. These symptoms were often attributed to poor indoor air quality, inadequate ventilation, high humidity, and exposure to chemicals from furnishings or cleaning products.(Shakoor, A., & Shah 2022)

The presence of these bacteria in air conditioning systems could serve as a source of indoor air contamination and pose potential health risks to occupants, particularly individuals with weakened immune systems. The diversity of bacterial species found underscored the importance of regular maintenance and cleaning of air conditioners to prevent microbial growth and ensure indoor air quality. This could have been affected by multiple influences such as heat level, air moisture, inadequate aeration, room usage intensity, room interior, and the characteristics and activities of individuals.(Hayleeyesus and Manaye 2014)(Nasri et al. 2022)(Xie, Y., Li, X., & Zhao 2023)

One of the limitations of this study was that only aerobic bacteria were identified. The detection of fastidious and anaerobic bacteria had proven to be challenging using the swab method, as these types of bacteria required different approaches or specialized media for successful isolation and identification. As a result, the methodology used may not have captured the full spectrum of bacterial species present, particularly those that thrived in low-oxygen environments or had specific growth requirements. In this study, the researchers did not measure the potential impact of variations in environmental conditions, such as temperature and humidity on bacterial growth. Recommendations for future research suggested scheduling regular cleaning and maintenance for air conditioner units to reduce bacterial contamination. It also explored seasonal variations in bacterial contamination, specific mitigation strategies, and the impact of different types of air conditioner units on bacterial presence.

5. Conclusion

Bacterial identification during the Air Conditioner swab test revealed a predominance of Gram-positive bacteria. This finding indicated that the microbial community present in the Air Conditioner system was largely composed of Gram-positive strains, which were more frequently detected than their Gram-negative counterparts. The dominance of Gram-positive bacteria, such as Bacillus subtilis and Staphylococcus spp., suggested that these microorganisms had a higher prevalence in the Air Conditioner environment, potentially due to their ability to survive and thrive under the specific conditions found in air conditioning systems. The importance of regular maintenance and cleaning of air conditioning systems as a proactive measure to reduce the risk of bacterial contamination and improve indoor air quality, thereby promoting the health and comfort of building occupants.

Conflict of Interest

There is no conflict of interest. Nothing to disclosure.

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