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STUDENTS' HABITS AND IDENTIFICATION OF BACTERIA ON INANIMATE SURFACE OF EDUCATIONAL SETTING

Ari Udijono[1,3]*, Agus Subagio[2], Mateus Sakundarno Adi[3], Bagoes Widjanarko[4], Fauzi Muh[3], Nissa Kusariana[3], Dwi Sutiningsih[3]

[1]Doctor of Public Health Progam, Faculty of Public Health, Universitas Diponegoro, Semarang 50275, Indonesia
[2]Department of Physics, Faculty Science and Mathematics, Universitas Diponegoro, Semarang 50275, Indonesia
[3]Department of Epidemiology and Tropical Disease, Faculty of Public Health, Universitas Diponegoro, Semarang 50275, Indonesia
[4]Department of Health Promotion and Behavioral Science, Faculty of Public Health, Universitas Diponegoro, Semarang 50275, Indonesia

Corresponding Author:
*) ari.udijono@gmail.com

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Abstract

Introduction: *Diponegoro Clinic reported 29.1% of students seek infectious diseases treatment. Student habits in the classroom were thought to play roles in the presence of bacteria. Surfaces of inanimate objects in the classroom were potential source for bacteria. The research objectives were analyzing student's habits and identifying bacteria on the surface of inanimate objects in the classroom.* ***Methods:*** *Four types of samples, including the surfaces of tables, chairs, flips of air conditioners, and floors were taken from 13 faculties at Universitas Diponegoro. Plate count agar media were used to isolate bacterial colonies, and PCR analysis was performed for DNA extraction and amplification. DNA Sanger sequencing techniques were used for genetic bacterial identification. The online questionnaire was used to assess student habits in the classroom. Two hundred students responded.* ***Results and Discussion:*** *Acinetobacter baumannii and Staphylococcus aureus were found in classrooms. These bacteria were associated with respiratory tract infections. This study revealed that 86.5% were between the ages of 17-21, 60.81% were from outside Semarang City, and 88.33% lived in Semarang City. About 60.81% of respondents studied in health sciences. Furthermore, it was reported that 66.67% of respondents were sick in the last few weeks, attended class despite being sick (72.52%), and coughed and sneezed in class (40.99%).* ***Conclusion:*** *Bacteria associated with respiratory tract infection were found. Students' habits in the classroom were potentially caused by the presence of these bacteria. The use of antibacterial agents could reduce the presence of bacteria on inanimate object surfaces.*

INTRODUCTION

Nowadays many activities are carried out indoors. Indoors, almost every surface that exists has the potential to be overwhelming to engage with a wide variety of microorganisms. Regular contact with indoor bacteria can lead to many opportunities, such as the spread of disease and an interesting relationship with our own commensal microbiome (1). Schools typically implement various preventive measures to control potential infections by promoting hand hygiene, using personal protective equipment, and improving the frequency of vaccination (2). Contamination caused by pathogenic microorganisms in an environment or room can impact an individual's health in the space, generally public health. The extent to which microbiological contamination arises depends on a particular limit, for a specific environment (3). Disease transmission can occur directly or indirectly from one person to another or within a population due to specific conditions, such as exposure to pathogens. Direct transmission occurs through skin-to-skin contact. Human-to-human transmission occurs, most likely through contact with contaminated hands or surfaces but also droplets (4). Indirect transmission, however, can occur through less obvious media such

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as airborne particles, objects, or vectors (*vector-borne disease*). Furthermore, a variety of factors influence transmission, including the spread of the infectious microorganism from a surface to mucous membranes, the environment's rate at which infectivity declines, the presence of an infectious titer, and the immune system's state (5).

Direct transmission is generally the main infection pathway despite indirect transmission, which is often not recognized. Pathogens could contaminate the soil or object indirectly, and infected individuals may transfer the microorganism to others through respiratory secretions or droplets. In order to operate as a reservoir of bacteria that may spread through direct touch, surfaces offer a key means of disease transmission (6). Small droplets and particles that are respirable either stay in the air or pass through other solid suspended pollutants. Meanwhile, a study is now being conducted on the airborne transmission route (7). Risk behaviors such as sneezing, coughing, or talking and inanimate objects in a room significantly contribute to greater contamination. The frequency of fomite contamination and exposure results in the perpetuation of surface transmission; the number of pathogens released by the host, the infectious agent's ability to infect a vulnerable person, the organism's pathogenicity, the immunocompetence of those in contact, and the preventative measures, such using disinfectants and practicing good personal hygiene, as well as additional variables. The likelihood that a pathogen may infect its host and cause disease is not always determined by the presence of the pathogen (8). Some studies have reported that indoor formites such as desks, chairs, walls, and floors are the most common contaminated surfaces. Research in classrooms at the University of Oregon identified several types of bacteria contaminants, including *Lactobacillus*, *Corynebacterium*, and *Staphylococcus* on chairs; *Streptococcus*, *Brevundimonas*, and *Candidatus phytoplasma* on desks; *Alicyclobacillus*, *Chroococcidiopsis*, and *Rhodopseudomonas* on walls; and *Roseomonas* and *Salmonella on* floors. These pathogenic bacteria causing mild to severe diseases include pneumonia, meningitis, and osteomyelitis (9).

Classrooms are potential indoor modes of transmission, especially for occupants who spend long periods in close proximity. Indoor places do not have open ventilation and use air conditioning (AC) (10). However, one technical way to improve the quality of indoor air is through the use of ventilation systems. Even though they are a secondary source of microbiological pollution, contemporary ventilation systems can reduce airborne contamination in addition to their health-related uses (11). In contrast, proper use of AC can reduce airborne pathogen exposure (12-13). In some cases, however poorly maintained AC systems increase the risk of pathogens, especially on indoor surfaces such as AC filters, transmission channels, handling units, and fans (14). Similarly, artificial lighting sources, contribute to higher risks of pathogen exposure through both the air and solid surfaces than direct sunlight. Since pathogenic bacteria invisibly exist (14) in significant quantities. Student behaviors such as touching the body organs (15), and eating food or drinking without proper hygiene may increase the risk of pathogen contamination in the classroom (15-16).

Data from the Diponegoro Pratama Clinic shows that around 21.9% of students come with complaints of infectious diseases. Generally, they suffer from respiratory and gastrointestinal tract infections. Diponegoro Pratama Clinic is the first refferal health service for students of Universitas Diponegoro (17).

Typically, classrooms pose a risk of bacteria transmission (18). Epidemiology Science has underlined that indoor spaces are much more risky contagions, compared to open spaces, due to the density. Therefore, it is suggested that buildings should have suitable ventilation to prevent contamination (7). A study observed the spatial layout of Universitas Diponegoro the majority of classrooms utilize air conditioning. The classrooms in 13 faculties at Universitas Diponegoro had desks, floors, and chairs, using flocked nylon swabs moistened with sterile buffer solution (0.9% NaCl). These fomites were investigated, and their surfaces were sampled. The samples were inoculated in the laboratory for bacterial growth and species identification.

Knowledge of the ecological processes that drive the assembly of microbial communities indoors, is also not yet understood, to what extent humans share microbes with indoor surfaces. Considering the classroom as a place for learning processes, as well as a closed ventilation with the potential for the growth of microorganisms, and the lack of data regarding bacteria in classrooms at Universitas Diponegoro, this research is conducted. Therefore, this study aimed to analyze the habits of students and identify bacteria on the inanimate surface in the classroom.

METHODS

Cross sectional study was used in this research. The population of the survey is all active students at Universitas Diponegoro. The online questionnaire was given to all students who have used selected classes. Two hundred students responded and filled out the questionnaire.

Sample Collection

Samples were taken from the most used classrooms in 13 faculties at Universitas Diponegoro. The selected classroom was the one that was most frequently used during one semester, with a minimum capacity of 40 students, and has been used for lectures at least once a week. Each faculty was assigned one classroom, resulting in a total of 13 classrooms. The surface of desks, floors, air conditioner's flips and chairs of each class were sampled following identical protocols (Classroom area: 9 m × 11.5 m). The spatial distribution of the samples in the classrooms is shown in Figure 1. A nylon-flocked swab dampened with sterile buffer solution (5 mL of 0.9% NaCl solution) was used to sample the surfaces. The swabbed samples were then taken into the laboratory for inoculation.

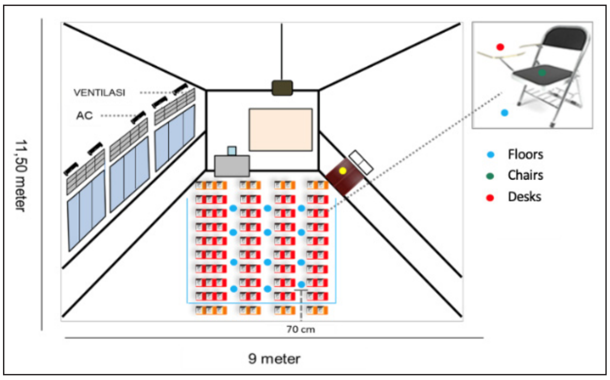

Figure 1. The Schematic Diagram of Sampling in Each Classroom

Bacterial Inoculation

The preparation of the plate count agar media (PCA, Merck USA) followed the guidelines provided by the manufacturer. The PCA media were cooled and the bacteria were inoculated using a pour-plate method. A total of 1 mL of bacteria solution was inoculated with around 5 mL of PCA solution and mixed well. The bacteria were grown at 350C for 24 hours.

Gram Staining Microscopy

A single bacterial colony was taken and spread on the clean microscope glass containing a drop of PBS PH 7.0. The bacterial mixture was air-dried. A solution of Gram stain was used to color the bacterial samples (BD Difco BBL™ ref. 212525 (Gram Crystal Violet), 212542 (Gram Iodine), 212527 (Gram Decolorizer), and 212531 (Gram Safranin). Gram reactions (Gram-positive or Gram-negative) were used to distinguish the different types of bacteria, the shape of cells, and their arrangement. The bacterial morphology was then visualized using an Olympus CX23 at 100x magnification.

DNA Extraction and Amplification

Genomic DNA was extracted using a simple Chelex protocol. Sixteen S rRNA was used to amplify DNA 27F 5-AGAGTTTGATCCTGGCTCAG-3 and 1492R 5- GGTTACCTTGTTACGACTT-3. PCR was run with the following steps: an initial denaturation at 95°C for 3 mins, followed by 35 cycles of denaturation step at 95°C for 45 seconds, an annealing step at 54°C for 1 minute, an extension at 72°C for 1.30 minutes, and with a final extension at 72°C for 10 minutes. All the PCR reactions were performed in 25 µl aliquots containing 12.5 µl of PCR Master Mix, one µl of forward primer, one µl of reverse primer (each primer of 10 pmol), one µl of 100 ng/µl DNA and RNase-free water to final volume of 25 µl. PCR amplification products were analyzed by gel electrophoresis in 1.2% (w/v) agarose gel with a molecular size marker of 100 bp.

Data Analysis

The sequencing was conducted in both forward and reverse directions using the Sanger Sequencing method. The result was aligned using MEGA11, and then the species identity was identified by comparing sequences to GenBank with a homology threshold of >95%. The 16S rDNA sequences were extracted from the GenBank and alignment was performed with MUSCLE and the molecular evolutionary genetics analysis (MEGA) 11 program. In the final process, phylogenetic trees were constructed using MEGA11 (19).

RESULTS

Bacterial Identification in Classrooms

This study involved samples taken from the floor (L), chairs (K), and desks (M) of classrooms in 13 faculties of Universitas Diponegoro. Sampling was held using flocked nylon swabs moistened with 5 mL of sterile buffer solution, which was 0.9% NaCl. The bacteria were then inoculated with 5 mL of PCA solution and allowed to grow. All chairs and desks from the included rows were swabbed using four previously moistened sterile cotton swabs. Floor samples were obtained from chairs and desks.

The bacterial samples collected from the classrooms were categorized into two groups: clusters from the health science faculties and clusters from the non-health science faculties.

Bacterial Cultures in the Health Science Faculties

This study discovered two types of bacterial colonies were found on AC swabs in classrooms at the

Faculty of Public Health and the Faculty of Medicine. In contrast, no bacterial colonies were found on agar media taken from classrooms at the Faculty of Psychology. On the floor swab, two colonies were found in the Faculty of Public Health, the Faculty of Medicine, while only one bacterial colony was found in the Faculty of Psychology. The chair swab results showed two bacterial colonies were discovered in the Faculty of Public Health, Faculty of Medicine, and the Faculty of Psychology. The table swab results presented two bacterial colonies in the Faculty of Public Health and the Faculty of Medicine, while no bacterial colonies were found in the Faculty of Psychology.

Bacterial Cultures in the Non-Health Science Faculties

Six faculties (Faculty of Law, Faculty of Cultural Sciences, Faculty of Animal Science and Agriculture, Faculty of Science and Mathematics, Faculty of Engineering, and Vocational School) were identified as having two bacterial colonies from the AC swabs. Meanwhile, one bacterial colony was found in only three faculties: the Faculty of Social and Political Sciences, the Faculty of Fisheries and Marine Science, and the Postgraduate School. No bacterial colonies were found in the Faculty of Economics and Business. From the seat swabs, this study could mention that there were two bacterial colonies in 8 faculties: the Faculty of Economics and Business, Faculty of Law, Faculty of Social and Political Sciences, Faculty of Fisheries and Marine Science, Faculty of Animal Science and Agriculture, Faculty of Science and Mathematics, Faculty of Vocational Study, and Postgraduate School. Only two faculties (the Faculty of Humanities and the Faculty of Engineering) had one bacterial colony each. Furthermore, the floor swab results showed two bacterial colonies in all faculties. According to the table swab results, two bacterial colonies were found in eight faculties, and only one colony was identified at the Faculty of Engineering.

Morphology of Identified Bacteria

This study discovered two gram-positive and one gram-negative bacteria in the examined samples. The bacteria sampled from the seats showed gram-negative features with a coccus shape and pink color (Figure 2A). The bacteria on the floor were identified as gram-positive bacteria with a rod shape and blue color (Figure 2B). Meanwhile, the table surface was detected as having bacteria with a streptobacillus shape with gram-negative staining (Figure 2C). Based on the identification results, the pathogenic bacteria affecting the respiratory tract were *Acinetobacter baumannii* and *Staphylococcus aureus*, the bacteria most frequently detected in the samples.

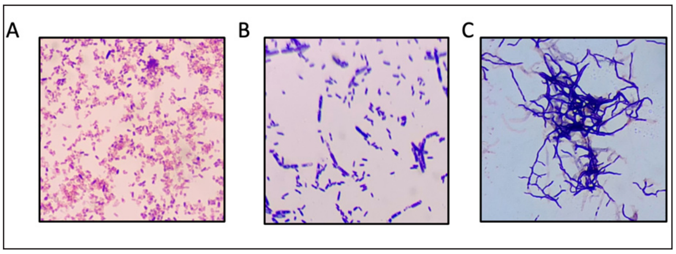

Figure 2. Gram-stained Microscopic Illustrations of the Samples. (A) Stool sample (gram negative); (B) Floor sample (gram positive); (C) Table sample (gram positive)

Results of Risk Factor Analysis of Bacterial Presence in Classrooms

The respondents' demographic information is displayed in Table 1. Twenty years old is the average age of the respondents. The ages of the respondents range from 17 to 21 years old, with 17 being the youngest. The majority of the respondents in this study originally came from other cities (88.29%), while most of them resided in Semarang City (83.33%) during the study.

Table 1. Distribution of Respondents by Age, Registered Address, and Address at the Period of the Study

Respondents' Characteristics	n = 222	%
Age		
17 - 21 years old	192	86.50
22 - 26 years old	19	8.60
27 - 31 years old	3	1.40
32 - 36 years old	3	1.40
37 - 41 years old	3	1.40
≥42 years old	2	0.90
Address as per ID card		
Semarang City	26	11.71
Outside Semarang City	196	88.29
Address at the period of the study		
Semarang City	185	83.33
Outside Semarang City	37	16.67
Total	**222**	**100.00**

In addition to the previous information, Table 2 displays the origins of respondents. This study suggested that 60.81% of the respondents were students from the Faculty of Health (Faculty of Medicine, Faculty of Public Health, and Faculty of Psychology).

Table 2. Distribution of Faculties Among Respondents

Faculty	n = 222	%
Health science faculty	135	60.81
Non-health science faculty	87	39.19
Total	**222**	**100.00**

The majority of respondents in this study did not experience any respiratory in the past few weeks (66.67%). However, 33.33% of the respondents got ill in the past few weeks but did not require hospitalization (100%).

Since behaviors also contributed to the extent to which the virus spreads, this study found that 72.52% of students were able to attend lectures despite getting influenza. Additionally, 31.98% of the ill students sneezed and coughed in the classroom, and 40.99% of the respondents sometimes did risky behaviors. Furthermore, 86.49% of students observed their peers attending lectures, and 38.29% of students saw their lecturers in the classrooms while they had flu. Additionally, 41.44% of students saw their lecturers sneezing and coughing in the classrooms. Regarding self-hygiene in the classrooms, 36.49% of students did not use masks, and 58.56% did not use hand sanitizer. Additionally, 63.96% of students sometimes ate food and drank in the classroom, and 54.05% of students thought that they often drank in the classroom.

Table 3. Distribution of Frequency of Respondents' Illness History in The Past Few Weeks

Illness History	n = 222	%
Experienced illness in the past few weeks		
Yes	74	33.33
No	148	66.67
Had to be hospitalized due to illness (n = 74)		
Yes	0	0.00
No	74	100.00
Total	**222**	**100.00**

Table 4. Distribution of Risky Behavior for Contamination in The Classroom

Risky Behavior for Contamination	n = 222	%
Attending lectures in the classroom while being ill		
Yes	161	72.52
No	61	27.48
Have ever sneezed or coughed in the classroom while being ill		
Yes	71	31.98
No	60	27.03
Sometimes	91	40.99
Have ever seen an ill classmate attending lectures in the classroom		
Yes	192	86.49
No	30	13.51
Have ever seen an ill classmate sneezing and coughing in the classroom		
Yes	193	86.94
No	29	13.06
Lecturer gave lectures in the classroom while being ill		
Yes	85	38.29
No	137	61.71
Have ever seen an ill lecturer sneezing and coughing in the classroom		
Yes	92	41.44
No	130	58.56
Use mask in the classroom		
Yes	141	63.51
No	81	36.49
Use hand sanitizer in the classroom		
Yes	92	41.44
No	130	58.56
Consuming food and drinks in the classroom		
Never	71	31.98
Sometimes	142	63.96
Often	9	4.05
Frequency of drinking in the classroom		
Never	14	6.31
Sometimes	88	39.64
Often	120	54.05
Total	**222**	**100.00**

DISCUSSION

Description of Bacteria Identified in the Classroom

There are both gram-positive and gram-negative bacteria in the samples. Health and the immune system can be significantly and persistently impacted by the millions of bacteria, microbial fragments, and microbial metabolites that are absorbed into the human respiratory tract every hour from indoor air (20). Among the identified bacteria, some bacteria such as *Staphylococcus aureus* and *Acinetobacter baumannii* may cause respiratory disease and pneumonia (21). *A harmful gram-positive bacteria is called Staphylococcus aureus* (22) which can affect the upper and lower respiratory tract (21-22). Millions of bacteria are present in indoor air in the human nose and oral cavities. Certain species of bacteria, such as *Mycoplasma, Streptococcus*, and *Staphylococcus*, increase the proportion of infected persons by causing respiratory illnesses, allergies, and skin-related ailments (23). *S. Aureus* clones in the front portion of the nasal cavity. *S. aureus* has the ability to spread throughout nasal polyps' epithelial cells, infiltrate the mucosa, and release chemical mediators that may cause inflammation (24). *Staphylococcus aureus* has the ability to survive on dry surfaces and can form biofilms (25), a thin layer of bacteria that adheres to surfaces and provides additional protection against drying and disinfectants. Bacteria grouped and embedded in a self-made matrix, akin to a biofilm, are affixed to surfaces and/or to one another. Bacteria in biofilms can use a variety of survival techniques in addition to the matrix's protection to sidestep the host's defense mechanisms (26)

Acinetobacter baumannii can infect moist and soft tissue such as mucous membranes and wounds. *Acinetobacter baumannii* which causes pneumonia can form colonies in the lower respiratory tract (24). It is known for its high resistance to dryness and extreme temperatures. This bacterium can survive in less clean environments and persist on hard surfaces for days to weeks. Its capacity to create biofilms, withstand drying out, and be cleaned with chemicals (24) allows it to thrive within hospital settings. Strains of *A. baumannii* that are more susceptible to desiccation are those that lack the ability to produce biofilms or only do so very little (27). The lack of known host-damaging toxins in *A. baumannii's* genome, antibiotic resistance, persistence in the environment, and persistence all point to the possibility that the potential to infect this organism is predicated on a "persist and resist" approach. Additionally resistant to oxidative stress and complement-mediated death, this bacterium (28). Therefore, if both bacteria spread within the classroom, they will have the potential to infect, particularly students with weakened immune systems (29). Infections caused by *Staphylococcus aureus* and *Acinetobacter baumannii* with poor prognoses can lead to death. *Additionally, this* bacterium is resistant to oxidative stress and complement-mediated death. Multidrug-resistant *A. baumannii* (MDRAB) and carbapenem-resistant *A. baumannii* (CRAB) strains of the bacteria have made treating A. baumannii infections more challenging (30). *Staphylococcus aureus* and *Acinetobacter baumannii* are known for their resistance to the environment and can persist on frequently touched surfaces such as desks, chairs (20), and door handles. Since bacteria can act as a reservoir for an indefinite amount of time through progressive cross-transmission of infections and subsequent interaction with patients and healthcare workers during disease management, bacterial contamination of inanimate surfaces and equipment is challenging to eradicate (31). As antimicrobial-resistant bacteria have arisen in community settings and may represent a danger of direct transmission to humans by contact with surfaces, objects, or other similar interactions, the fact is that human-environment relationships are considerably closer to these environments than previously thought (32). Although microbial infections are often mild, the infected body parts can become breeding grounds for the bacteria, leading to complications without proper management.

Classroom Behaviors for Transmission Risks

The air quality in the classroom is an important factor affecting it (1). High activity levels and students' spending hours nearby create an ideal environment (33) for pathogenic bacteria to spread. Pathogens can spread from one location to a vast area by contact with soiled objects like doors, tables, and toilets. For instance, coming into contact with a doorknob or other unsanitary surfaces, like a toilet, might spread bacteria to people who use it, including teachers, staff, and students (27). Consequently, the spread of an unanticipated, unknown pathogenic infection will be accelerated by repeated contact with infected surfaces or items (34). Furthermore, risky activities like chatting, singing, and yelling in a crowded area with inadequate ventilation are linked indirectly to significant epidemics brought on by droplet and aerosol transmissions (13). This study found that ill students and lecturers attending class exposed healthy people in the same room to the risk of droplet and aerosol transmission (29,35). In addition to the control measures, some students did not use masks, and most students did not use hand sanitizer (32). The bacteria can spread through the air over greater distances thanks to aerosol transmission. Technically, masks are the best tool to prevent infections in a large population (36). In order to maintain cleanliness, hand sanitizers (HS) and alcohol-based hand rubs are often used as disinfectants. These exogenous disinfectants have been shown in clinical trials to inhibit the growth of a number of germs on hands (37). Disinfectants must be used to sanitize inanimate items and bodily surfaces in order to eradicate harmful bacteria (37). Consequently, pathogenic bacteria in the air or on objects can easily infect people as they often touch the surfaces of objects (5). Besides being less likely to use hand sanitizer and masks, most students also ate food and drank in class. This behavior can lead to airborne or hand-transferred pathogens (4).

Transmission of Pathogenic Bacteria in the Classroom

Bacteria contamination happens when individuals touch objects carrying pathogens (34). Bacteria may transfer from one to another through airborne transfer or direct contact with objects in the classrooms (5). Students infected with pathogenic bacteria spread these pathogens through droplets, physical contact, contact with surfaces, (32) and inhaling the infected air (37). Additionally, pathogens can persist in the air and adhere to objects for a certain period (38). *Acinetobacter baumannii* can survive on various surfaces for more than 30 days (39). Meanwhile, *Staphylococcus aureus* can persist for at least one week on all surfaces (18). The incubation period of pathogenic bacteria in the body allows for the bacteria to spread and cause symptomatic or unsymptomatic conditions (40).

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AUTHORS' CONTRIBUTIONS

AU and FM: Conceptualization ; AU, FM and NK: funding; AU, FM, NK, and DS : writing original draft; AU, FM, and NK : data curation; AU, FM, NK, and DS:

original draft preparation; FM and NK: visualization; AS, MSA, BW and AU: validation and reviewing. Each author has reviewed the published version of the manuscript and has given their approval.

CONCLUSION

This study was carried out in a classroom, and samples were taken from inanimated surfaces from chairs, tables, flip of air conditioners, and floors. The most common bacteria were *Staphylococcus aureus* and *Acinetobacter baumannii*. Additionally, the analysis of students' habits reveals that several activities in the classroom increase the risk of pathogenic transmission. Some students and lecturers went to class although they were ill. Some students and lecturers coughed and sneezed in the classroom. Some students did not use masks and hand sanitizer, and some also had meals and drinks in the classrooms. Efforts were needed to control the presence of these bacteria. Anti-bacterial substance can be used to reduce the presence of bacteria on the surface of inanimate objects in the classroom.

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