



STUDY ON BIOAEROSOL LEVEL IN THE INDOOR AIR OF SELECTED TEACHING LABORATORIES OF UNIVERSITY OF CYBERJAYA, MALAYSIA

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Abstract: High exposure to bioaerosols may promotes range of infection. Bioaerosols causes air pollutants in indoor or outdoor environment. Teaching laboratories, one the facility that commonly used by the student and staff designed to have a control indoor environment to minimise level of bioaerosol that may affect health and contaminate the experimental results. Hence, this study investigated the indoor air quality by measuring the bacterial and fungal bioaerosol in 4 different teaching laboratories in University of Cyberjaya. Settle plate and contact plate method were used for passive air and surface sampling. Colonies were counted after 48 hours incubation and identified based on morphology characteristic. Bacterial identifications were evaluated using streak plate and Gram-staining method. Results showed that Pharmacognosy Laboratory has the highest number of bacterial and fungal bioaerosols with 124.00 and 38.00 CFU/m³ respectively compared to Medical Science Laboratory 1 and Clean Room. The lowest number of bacterial and fungal bioaerosols measured in Medical Science Laboratory 2 with 12 CFU/m³ and 16 CFU/m³ respectively. Culturable bacterial bioaerosol showed a variety colour of colonies, with different morphological characteristic. Gram stain of the bacteria from all four laboratories revealed that 62.5% was gram positive cocci, 18.75% was gram negative cocci and 18.75% was gram negative coccobacilli. This study found the indoor air quality of laboratories of University of Cyberjaya is within the standard level and the bioaerosol is considerably low, hence it is safe to students and staffs. However, proper maintenance is needed to prevent bioaerosol emission.

Keywords: bioaerosols; bacterial bioaerosols; fungal bioaerosols

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INTRODUCTION

Bioaerosols is defined as a tiny, airborne particle that may consist of fungi such as moulds and yeasts, spores, hyphae, bacteria and bacterial spores, microbial toxins and pro-inflammatory components such as mycotoxins, allergenic pollens, arthropod allergens such as from mites and cockroaches, pet allergens, algae, amoebae viruses and even dust [17]. Their existence in the air is the result of dispersal from a site of colonization or growth [18]. Study reported that bioaerosols contributed to approximately 5–34% of the total indoor airborne contaminants [2]. Bad indoor air quality resulting from high levels of fungi and bacteria has become a critical issue in the world. Depending upon exposure time and

concentration of pollutants, both indoor and outdoor air pollutants are reported to be harmful to human health. Research found most people spend their time indoors which is about 90 percent exposed to many types of pathogens [1]. People have experienced adverse health effects because of their indoor environments such as infections, toxic reactions and hypersensitivity pneumonitis [1]. In normal conditions, many species of fungi and bacterial are not expected to cause any infection, but they are found to spread diseases in immunocompromised people. Although the World health organization (WHO) showed concern towards indoor biological agents and building moisture, the majority of countries in the world are having no clear regulations or proposed guidelines for acceptable concentrations of micro-organisms particularly in the indoor environments [11]. Thus, microbiological air quality is an important factor that must be taken into account when indoor workplaces are designed to provide a safe environment [6].

The exposure of bioaerosols in public buildings such as schools, universities and hospitals can cause serious health problems. Microbial air quality and ventilation in educational settings may affect health of students and consequently affect their learning performance. Some studies showed that high concentrations of airborne fungi and bacteria can cause persistent cough and wheeze which can be harmful to students [2]. In university, laboratories are one of the most frequently used by students, laboratories staff and lecturers for practical and research purposes. Bioaerosols quality in the indoor of laboratories in University of Cyberjaya is unknown. This may affect students' health if the air quality is not good and the students does not fulfill good laboratory practice while utilizing the laboratories.

Therefore, this study aims to identify the presence of bioserosols in air quality of laboratories in University of Cyberjaya to ensure that laboratories is a safe place for the students to use.

MATERIALS AND METHOD

Chemicals

Materials used in this study includes nutrient agar (Difco®), Sabouraud Dextrose Agar (SDA) (Oxoid®), 70% isopropyl alcohol, distilled water, tap water, Crystal Violet Stain (Oxoid®), Gram Iodine (Oxoid®), decolourizer (Difco®), Safranin Stain (Oxoid®) and marker pen (Faster®).

Sampling sites

Four different laboratories of University of Cyberjaya that mostly used by the students for the teaching and research activities were selected. The selected laboratories were Medical Science Laboratory 1, Medical Science Laboratory 2, Clean Room and Pharmacognosy Laboratory.

Settle plate sampling location: The samples for settle plate method were taken at four different locations in each laboratory which were door, corner, table and sink [16]. One plate of nutrient agar and one plate of SDA agar were placed at each location. All the samples were taken at 12:00pm until 4:00pm.

Contact plate sampling location: The samples for contact plate method were taken at four different locations in each laboratory which were table, wall, stool and door handle [16]. One plate of nutrient agar and one plate of SDA agar were pressed at each location. All the samples were taken at 12:30pm.

Sampling Method

Settle Plate Method: Four plates of nutrient and sabouraud dextrose agar were prepared for each sample sites. The plates were examined for any contamination prior to use. Each plate was labelled and placed at the adjacent to the laboratory door, corner of the laboratory room, on the laboratory table and beside the laboratory sink. The lid of the plates was opened and placed at the respective place for 4 hours. After 4 hours, the agar was incubated for 2 days in the incubator at temperature of 20°C to 35°C. The plates were observed for any growth and presence

of any microorganisms was recorded based on morphological characteristic, colour and number of colonies [3].

Contact Plate Method: Four plates of nutrient and sabouraud dextrose agar were prepared for each sample sites. The plates were examined for any contamination prior to use. The agar on the labelled plate was pressed gently for 10 seconds by applying a constant force spread evenly over the whole contact plate at the surface of the laboratory table, stool, wall and door handle. Each surface was ensured dry before the sample was taken. Each surface was cleaned with a 70% isopropyl alcohol to remove any possible traces of residual agar. Then, the agar was incubated for 2 days in the incubator at 20°C to 35°C. Any growth of microorganisms was recorded based on morphological characteristic, colour and number of colonies [3].

Bacterial isolation

Streak Plate Method

The streak plate method was carried out to isolate the microorganisms that presence from settle plate and contact-plate. The working area was cleaned with 70% isopropyl alcohol to prevent contamination. The petri dish was labelled. The inoculating loop flamed to sterilize it and the lid of the plates containing the inoculum was lifted. The single colonies on the culture agar were scraped. The inoculum was smeared backwards and forwards across a small area of the medium. The loop was removed and the petri dish was closed. The petri dish were incubated for 2 days in the incubator at 20°C to 35°C [4].

Bacteria Identification

Gram Stain Method

The working area was cleaned with 70% isopropyl alcohol to prevent contamination. The microscope slides were labelled. Using a sterile inoculation loop, a drop of distilled water was added on the microscope slide. The colony of the bacteria was scraped from the agar and layered on the microscope slide. The slide was stand to dry for few minutes before proceed with Gram staining. A drop of crystal violet was added. After 1 minute, the slide was rinsed using tap water. The similar step was repeated for iodine, decolourizer with alcohol and safranin. Finally, the slide was dried and observed using microscope at 100X magnification. The shape and the colour of the bacteria was recorded [5].

RESULTS

acterial growth on nutrient agar

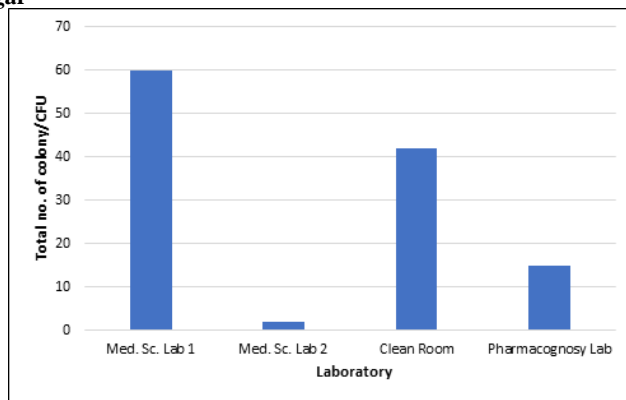


Figure 1. Total number of bacterial colony growth on nutrient agar at four different laboratories using settle plate

method.

Figure 1 showed the different total number of colony growth on nutrient agar in settle plate method among four laboratories in University of Cyberjaya.

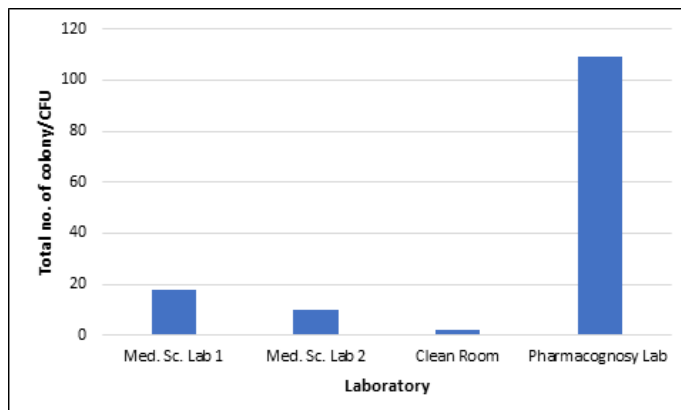


Figure 2. Total number of bacterial colony growth on nutrient agar at four different laboratories using contact plate method.

Figure 2 showed the different total number of colony growth on nutrient agar in contact plate method among four laboratories in University of Cyberjaya

Pharmacognosy Laboratory has the highest reading among the others with the total number of 124 CFU/m³. Meanwhile,

Medical Science Laboratory 2 has the lowest reading with the total number of 12 CFU/m³. Medical Science Laboratory 1 showed the second highest with 78 CFU followed by Clean Room with 44 CFU.

Table 1. The colony morphological characteristic of bacterial bioaerosol observed in all selected laboratories.

Location	Morphological characteristic								Colour of colonies
	Settle Plate				Contact Plate				
	Door	Corner	Table	Sink	Table	Wall	Stool	Door handle	
Medical Science Lab 1	Smooth, wrinkled, circular, rhizoid, convex, entire, filamentous.	Smooth, circular, convex, entire.	Smooth, circular, convex, entire.	Wrinkled filamentous, rhizoid, flat.	Smooth, wrinkled, circular, rhizoid, convex, pulvinate, filamentous, entire, lobate.	-	Smooth, wrinkled, circular, filamentous, umbonate, raised, entire.	Smooth, irregular, pinpoint, raised, entire.	Yellow, white.
Medical Science Lab 2	Wrinkled filamentous, flat.	Wrinkled, filamentous, flat.	-	-	Smooth, irregular, raised, entire.	Radiated, filamentous, umbonate.	Contoured, irregular, raised, undulate.	Radiated, irregular, umbonate, filamentous, entire.	White.
Clean room	Smooth, wrinkled, circular, filamentous, convex, entire.	Smooth, wrinkled, circular, filamentous, convex, entire.	Smooth, filamentous, convex.	Smooth, wrinkled, circular, rhizoid, convex, entire, filamentous.	Smooth, irregular, raised, entire.	-	-	-	White, yellow, pink, orange
Pharmacognosy Lab	Radiated, contoured, irregular, circular, raised, lobate.	-	Wrinkled, filamentous, flat, pulvinate.	Wrinkled, rhizoid, filamentous.	Smooth, irregular, raised, entire.	-	Smooth, concentric, irregular umbonate, convex, entire.	Smooth, circular, irregular, raised, entire.	White, yellow, pink, orange.

Table 1 showed the colonial morphological characteristic of bacterial bioaerosol observed in all four laboratories. The varieties of morphology was observed includes colonial surface, colonial edge, colonial elevations and colonial margins. The colonies majority smooth in surface, circular in shape, convex in elevation and entire in edge. The majority of the colonies showed white in colour.

Fungal growth on Sabouraud Dextrose Agar

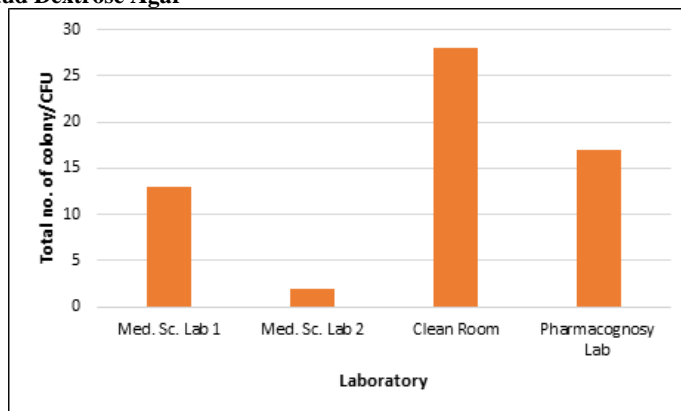


Figure 3. Total number of fungal colony growth on SDA agar at four different laboratories using settle plate method.

Figure 3 showed the different total number of colony growth on Sabouraud Dextrose Agar in settle plate method among four laboratories in University of Cyberjaya.

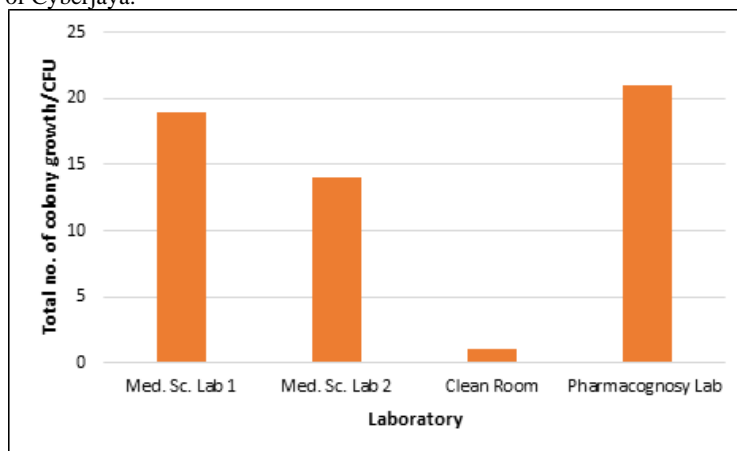


Figure 4. Total number of fungal colony growth on SDA agar at four different laboratories using contact plate method.

Figure 4 showed the different total number of colony growth on Sabouraud Dextrose Agar in contact plate method among four laboratories in University of Cyberjaya.

Total number of colony growth showed that Pharmacognosy Laboratory has the highest reading among the others with the

total number of 38 CFU. Meanwhile, Medical Science Laboratory 2 has the lowest reading with the total number of 16 CFU. Medical Science Laboratory 1 showed the second highest with 32 CFU followed by Clean Room with 29 CFU.

Table 2. The colony morphological characteristic of fungal bioaerosol observed in all selected laboratories.

Location	Morphological characteristic								Colour of colonies
	Settle Plate				Contact Plate				
	Door	Corner	Table	Sink	Table	Wall	Stool	Door handle	
Medical Science Lab 1	Wrinkled, circular, convex, filamentous.	Smooth, circular, pulvinate, entire.	Smooth, circular, pulvinate, entire.	Radiated, wrinkled, rhizoid, raised, filamentous.	Wrinkled, irregular, umbonate, filamentous.	Contoured, irregular, raised, entire.	Wrinkled, circular, irregular, pulvinate, raised, filamentous.	-	White

Medical Science Lab 2	-	Wrinkled, circular, raised, filamentous, white.	-	-	Wrinkled, circular, convex, filamentous.	Wrinkled, circular, umbonate, filamentous.	Smooth, circular, convex, filamentous.	Wrinkled, irregular, umbonate, filamentous.	White, green
Clean room	Smooth, wrinkled, circular, filamentous, convex, entire.	Smooth, wrinkled, circular, filamentous, convex, entire.	-	Smooth, wrinkled, circular, rhizoid, convex, entire, filamentous.	Wrinkled, circular, pulvinate, entire.	-	-	-	White, yellow, green.
Pharmacognosy Lab	Wrinkled, circular, convex, filamentous.	Wrinkled, filamentous, raised.	Wrinkled, rhizoid, raised, filamentous.	Wrinkled, irregular, raised, filamentous.	Contoured, irregular, pulvinate, raised, filamentous, entire.	Wrinkled, filamentous, convex.	Wrinkled, Circular, filamentous, Umbonate, undulate.	-	White, green.

Table 2 showed the colonial morphological characteristic of fungal bioerosal observed in all four laboratories. The colonies majority wrinkled in surface, circular in shape, convex in elevation and filamentous in edge. The majority of the colonies showed white in colour.

Gram Stain result

A total of 16 plates were collected from streak plate method and undergoes gram stain method to identify the type of bacteria. Figure 5 below shows the total number of gram positive and gram negative bacteria found in all four laboratories in University of Cyberjaya.

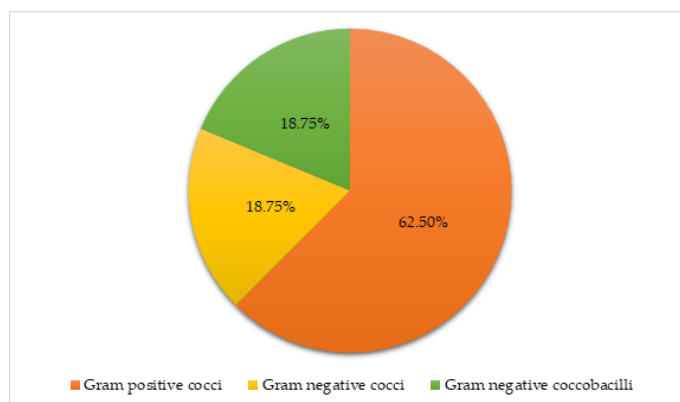


Figure 5. Total number of gram positive and gram negative bacteria in all four laboratories

DISCUSSION

The total number of colony growth in both settle plate and contact plate method revealed that Pharmacognosy Laboratory was the highest among the other laboratories which was 124 CFU. The factors that may affect the variation of bacterial aerosol in respective location in the laboratory may be due to high number of occupants. The occupants which mostly students may performed several activities such as talking, sneezing, coughing and walking that can enhancing the concentration of bacterial bioaerosols in the laboratory. Increase number of occupants also may increase the shedding of bacteria and agitation of air [6]. For Medical Science Laboratory 2, the results from both method showed the least among the other laboratories with the total of only 12 CFU. This may be due to low number of occupant during sampling time and good ventilation condition of the laboratory [6]. Small number of students may affect the concentration of bacterial aerosol because low density of occupant can cause the reduction number of bacterial aerosol. Moreover, this laboratory seems to

have good ventilation condition because air conditioning system in the laboratory was operating during the students' worktime. Air conditioning system was essential to produce good ventilation condition and it is an important method to decrease the levels of bacterial aerosol in indoor air [7].

Medical Science Laboratory 1 showed the second highest among the other laboratories. The surrounding outdoor air may be the major source of bacterial aerosol due to the location of the laboratory was near to the construction site. Human activities such as construction of buildings can lead to production of particulates in the form of fine particles of solid or liquid suspended in a gas [8]. The number of colony growth on nutrient agar in Clean Room showed the third highest among the other laboratories with the total of 44 CFU. This may be due to ventilation conditions relative to size of the laboratory. Clean Room has not been used by the students and lecturers for a certain duration of time since pandemic. This may cause improper ventilation due to minimum air turnover inside the room that can lead to the surge of airborne microbial

contamination [9]. The design of the buildings or rooms also plays an important role in accumulating the indoor bioaerosols. The size of the Clean Room is smaller than the other laboratories. When the room was not being used for a long time, poor ventilation system will occurs and enhancing the accumulation of bacterial bioaerosols in the confined space [10].

From the study, each laboratory showed a different fungal concentration. This may be due to several factors such as indoor moisture conditions, cleaning frequency and ventilation conditions in particular area [11]. Results from both methods showed that Pharmacognosy laboratory has the highest concentration of fungal bioaerosols. The main factor that contributed this result may be due to moisture conditions of the laboratory. Pharmacognosy laboratory was located in front of opened area which was strategic place of wind flow. During raining season, the moisture content seems to be higher and cause dampness. The outdoor moisture content may affect the indoor dampness especially when there is movement of students and laboratory assistant in and out of the laboratory. Dampness condition may enhance fungal growth and its multiplication in the indoor atmosphere of laboratories. It was mentioned by WHO that dampness situation needs to be considered as the risk factor of health problems. Different with concentration of fungal bioaerosols in Clean Room, the results from both methods showed that it has the lowest concentration of fungal bioaerosols. This may be because of location of the Clean Room which was in enclosed space of university building. The moisture content from outdoor environment may not affect the indoor condition resulting low dampness and reduce fungal growth and its multiplication [6].

Other factors that may affect different concentration of fungal bioaerosols was cleaning frequency as well as ventilation condition of the laboratories. When the laboratories were being left not being used during pandemic, cleaning frequency became rarely done. Reduce cleaning frequency can cause accumulation of dirt and dust in the laboratories. Dirt and dust can serve as nutrients for enhancing fungal growth [12]. Moreover, distribution of fungal bioaerosols was detected presumably due to undisturbed air movement [13]. When, the laboratories not being used, there will be limited air movement due to unused of air conditioning system. Air conditioning system was essential to produce good ventilation condition and it is an important method to decrease the levels of fungal aerosol in indoor air [7]. It can helps in removing source of dirt and dust and can lead to reducing fungal concentration [13].

Gram positive cocci was the highest bacterial concentration found in all four laboratories. The bacteria showed purple in colour which indicates gram positive. Furthermore, the bacteria was sphere in shape which indicates cocci [5]. There are two types of medically important genera of gram positive cocci which was *Staphylococcus* and *Streptococcus*. Both are non-motile and do not form spore. From the microscopic observation, most of the gram positive cocci appear in clusters. Thus the bacteria may be indicated as *Staphylococci*. Human act as reservoir for staphylococci. The main site of colonization of staphylococci was human's nose. This type of bacteria may spread and floats in the indoor air of the laboratories during the students' and other occupants' activities such as talking, sneezing and coughing. Moreover, most gram positive bacteria in the laboratories were normal flora and non-pathogenic. However, in person with low immune system, it may cause

harmful effect. Various pyogenic infections such as endocarditis and food poisoning in immunosuppressed people are the reported effect due to gram positive infection [14]. The exact species of the gram positive cocci bacteria in this study have not been identified. Further study needs to be done to identify the species of the gram positive bacteria.

Gram negative cocci and coccobacilli also found in this study. The bacteria observed under microscope showed pink in colour which indicates gram negative bacteria. Two types of bacterial shape found which was sphere and oval shape. Sphere shape of bacteria indicates that the bacteria was cocci in shape and oval shape indicates coccobacilli in shape [5]. Medically important genera of gram negative cocci was *Neisseria*. It was a non-motile gram negative cocci that resemble paired kidney beans. It was a part of human respiratory tract. It also may spread and floating in the indoor air of laboratories during occupants' activities such as talking, sneezing and coughing. Similar to gram positive bacteria, this bacteria was non-pathogenic but in person with low immune system, it can be harmful as it contains virulence factors which were pili, capsule and IgA protease. It may cause disease such as meningitis and gonorrhoea [14]. The exact species of gram negative cocci in coccobacilli in this study was not been identified. Further study needs to be done to identify the species of the gram negative bacteria. The study of bacterial bioaerosol in the indoor air of teaching laboratories of was important to ensure a safe environment to the students and lecturers [6]. The bioaerosols quality of laboratories can be improve by increasing cleaning frequency of the laboratories to reduce accumulation of dirt and dust that serve as nutrients for enhancing bacterial and fungal growth [12]. Besides, to ensure ventilation condition of the laboratories is well functioning to filter and reduce the bioaerosols in the air [7]. Moreover, fumigation and disinfection also can be done to improve bioaerosols quality of the laboratories.[15].

CONCLUSION

As conclusion, four selected teaching laboratories of University of Cyberjaya showed different bacterial and fungal bioaerosols concentration. Pharmacognosy Laboratory has the highest concentration of bacterial and fungal bioaerosols while Medical Science Laboratory 2 has the lowest concentration of bacterial and fungal bioaerosols. Laboratories in University of Cyberjaya is safe to be used by students and lectures as it fulfill the requirement set by World Health Organization (WHO) to provide a safe indoor atmosphere which not exceed 750 to 1000 CFU/m³ for bacterial bioaerosols and not exceed 500 CFU/m³ for fungal bioaerosols. Few factors have been considered causing different number of bioaerosols in each laboratory which includes ventilation conditions, number of occupants, outdoor air pollution and reduce cleaning frequency of the laboratories. Gram positive cocci was the highest type of bacteria in the laboratories. Further study needs to be done to identify the exact species of the bacteria.

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