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Active Sampling Procedure of Indoor Air Quality to Evaluate Airborne Fungi in Dental Building of Higher Education Institution

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Abstract

The study aimed to measure the concentration and composition of airborne fungi in a higher education institution. The temperature and relative humidity were recorded using TSI Q Trak Indoor Air Quality Monitor. The mean concentration of the indoor air fungi was in the range between 17.67–91.28 CFU/m³. The most abundant airborne fungi were *Aspergillus* (22%), *Fusarium* (17%), and *Penicillium* (15%). The highest mean range concentration of airborne fungi was in the evening followed by in the afternoon and in the morning with a value range between 74 to 148 CFU/m³, 18 to 148 CFU/m³, and 5 to 30 CFU/m³, respectively.

Keywords: active sampling; airborne fungi; indoor air quality; dental building.

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1.0 Introduction

Routine use of instruments during dental treatment such as slow-speed drilling into teeth, bones and tissues and the ultrasonic scaler generates hazardous aerosols (Sawhney *et al.* 2015). The aerosols composition varies based on the type of dental treatment procedure and oral flora of the patient (Jimson *et al.* 2015). The smaller particles of an aerosol (0.5 to 20 µm in diameter) have the potential to be suspended in the air and penetrate deep into the pulmonary alveoli and have the potential to cause infections (Dawson *et al.* 2016). Larger particles of a splatter (greater than 50 µm in diameter) settle easily onto environmental surfaces (Schmalz *et al.* 2018). Thus, the risk of acquiring infections by aerosols may be a hazard to patients as well as health care workers in dental settings.

During the dental procedure, surfaces and equipment such as chairs, spotlight, and dental equipment might also be contaminated by the aerosols. There were 14 species of fungi isolated from dental chairs, which have been identified belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Fusarium*, *Curvularia*, *Drechslera*, and *Paecilomyces* (De Almondes *et al.*, 2016). The internal air conditioning system also contributes to the proliferation and spreading of fungi in dental settings. The indoor environment of the dental building is strongly influenced by the fluctuations in its surroundings which makes the scheduled monitoring of the indoor air

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environment of this sensitive building essential (De Sousa & Fortuna, 2011). Exposure to airborne fungi can cause various diseases from allergic reactions, irritations, asthma, and pneumonia to toxic effects and infections (Kadaifciler & Cotuk 2014).

The external and internal causes such as environmental conditions, human activity, and ventilation efficiency influence fungal dispersion in dental clinic environments (Kadaifciler & Cotuk 2014). Furthermore, many main problems such as floods, water leaks, construction defects and poor ventilation are among the major causes of moisture accumulation in indoor materials which are known to initiate and sustain microbial growth (Sterflinger, 2010). Besides, prolonged moisture causes dampness and become a favourable growth condition for microorganisms such as available nutrients, leading to visible fungal growth on building material surfaces (Gock et al., 2003). The growth of fungi under these conditions over time results in different parts of the fungal growth. At any given time, fungal mycelium may exist in several discrete microsites.

There is a lack of studies regarding indoor airborne fungi and temporal variation in the dental building area particularly in the tropical climate seasons such as Southeast Asia (Husna *et al.*, 2011). Furthermore, the workers and occupants in the dental workplace usually spend on average 10 hours in the building for a day. As such, the Department of Occupational Safety and Health Malaysia has established the standard for the acceptable standard limit of indoor airborne fungi is below or not more than 1000 CFU/m³. This study provides the concentration and composition of the airborne fungi in the indoor air of different microenvironments in the dental building area. This information will be useful as a reference and a baseline to improve the indoor air quality of the airborne fungi in the dental building area. Monitoring and control of microbial aerosols are also of great importance in preventing infectious diseases related to the indoor environment.

2.0 Methodology

2.1 Sampling site

This study was conducted in the workplace areas at different microenvironments of the Faculty of Dentistry, Universiti Teknologi MARA. Samples were taken from Dental Clinic (DC) 1, Dental Clinic 2, Dental Clinic 3, Dental Clinic 4, Laboratory (Lab) 1, Laboratory 2, Preparation Room (CSSU), Lecturer Room (LR) 1, Lecturer Room 2 and Lecturer Room 3. The airborne fungi for DC 1, DC 2, DC 3, DC 4, and Laboratory 1 were sampled at three different periods (8:00 A.M., 1:00 P.M. and 4:00 P.M.) during working days. Those rooms were selected for sampling as the rooms were mainly used daily and having people coming in and out during the working hour.

2.2 Sample collection of airborne fungi

The airborne fungi were collected at each sampling location point by using Andersen Cascade Impactor at stage 6 with the hole diameter size range from 0.65 to 1.1 μm . The Anderson Cascade Impactor was operating at an airflow rate of 28.3 L/min for 5 min (Kimmerle et al., 2012). The sampling was depending on the nature of sampling locations where the Anderson Cascade Impactor was set up at a height of 1.5 meters above the ground to represent the breathing zone of a standing person, or at a height of 1.0 meter above the ground to simulate the breathing zone of a seated person (Rajasekar & Subramaniam, 2011) (Fig.1). The fungi samples were collected using Malt Extract Agar (MEA) agar plates.

The samples were taken during working days in three different periods for each of the sampling locations. Each sample was taken in duplicate to avoid bias and to maintain the accuracy and precision of the results. The temperature and relative humidity were recorded at the mid-point of the air sampling duration. The temperature and relative humidity of the sampling location was measured by using the TSI Q-Trak IAQ Monitor.



Fig.1: The Anderson Cascade Impactor during air sampling

Plates containing fungal samples were incubated in an inverted position at 23°C for seven days which allow the growth of fungi on those plates. After the incubation, visible colonies formed on the plate were counted according to the standard conversion table for accounting of sterility and multiple deposited particles at single impaction sites (Classen et al., 2003). Counting was done daily along

the incubation process where the transformation of colony-forming units (CFU) appears to be similar to how the original CFU was counted. The counts were expressed in CFU/m³ which the value of the CFU/m³ was obtained by using the positive hole conversion table and by calculating the volume of the air sampled (Fradkin et al., 1987). The morphology of the fungi was determined by using the microscope.

3.0 Findings

Table 1 is showing the temperature, humidity and concentration of each sampling location. The concentration of fungi was depending on the size of the room. CSSU has a relatively smaller room size than DC whereas the DC 4 has a bigger size which can hold a bigger capacity of patient and treatment being conducted. This size of the room plays a vital role in the concentration of airborne fungi. For standard deviation, DC4 had the highest while CSSU had the lowest which the result was 60.75 and 6.21, respectively. This is due to the wide range of minimum concentration and maximum concentration of each sampling location. The minimum concentration in DC 4 was 7.07 CFU/m³ and the highest concentration was 310.95 CFU/m³. Meanwhile, the minimum concentration in CSSU was 14.31 CFU/m³ and the highest concentration was 56.54 CFU/m³.

Table 1: The temperature, humidity, minimum, maximum, and mean concentration, and also standard deviation in each sampling locations

Sampling location	Temperature (°C)	Humidity (%)	Minimum concentration (CFU/m ³)	Maximum concentration (CFU/m ³)	Mean concentration (CFU/m ³)	*SD
DC 1	22.20	60.98	14.13	99.00	38.87	7.42
DC 2	22.40	78.93	70.67	120.14	80.09	14.01
DC 3	21.58	67.06	21.20	127.21	32.98	21.77
DC 4	22.19	74.75	7.07	310.95	91.28	60.75
Lab 1	19.92	74.29	28.27	240.28	83.63	50.44
Lab 2	22.50	67.91	14.13	141.34	53.00	39.61
CSSU	22.89	62.76	14.13	56.54	24.74	6.21
LR 1	22.85	64.78	7.07	28.27	17.67	9.12
LR 2	25.63	64.80	21.20	84.81	49.47	33.15
LR 3	22.08	67.13	0	56.54	21.20	24.48

*SD = standard deviation

The mean concentration of all sampling rooms was in the range between 17.67 until 91.28 CFU/m³ as shown in Fig.2. The highest mean concentration of airborne fungi was found in DC 4 with 91.28 CFU/m³ meanwhile, the lowest mean concentration was found in LR 1 with 17.67 CFU/m³. The difference in mean concentration in each dental clinic, laboratory, preparation room, and lecturer room may result from different activities and environments conducted by each room. The airborne fungi in each dental room may have different origins such as from the dental procedure, the dental staff, or patients. It may also come from outside sources such as air, soils, and dust that penetrate the indoor air directly or indirectly (Rajasekar & Balasubramaniam, 2011). Previous studies have reported that bioaerosol concentration and composition varies from patient to patient, depending on the department and the type of procedure in the oral cavity (Choi et al. 2018).

The concentration of airborne fungi and relative humidity were compared to find the relationship between those variables (Fig.3). The relative humidity is the moisture in the air that is captured as a percentage of the total moisture contained at the current temperature (Osmani et al., 2016). We found that the concentration of airborne fungi in CFU/m³ increases when the percentage of humidity increases. The lowest humidity of indoor air (53.1%) was found in DC 1 which correlates with the lowest average concentration of indoor airborne fungi (14 CFU/m³). The highest humidity (89.1%) was found in DC 2 where the concentration of indoor fungi at 120 CFU/m³. The high relative humidity is required for fungi to be able to grow in the absence of free water. Maximum growth for fungi occurs at a relative humidity between 95 to 100%. However, the growth may decline in relative humidity between 80 to 85% and some fungi grow at a relative humidity at as low as 65%. Higher environmental relative humidity favours the microbial growth for fungus and it is also an important factor causing the seasonal difference in the microbial concentrations (Ren et al., 1999). An increase in humidity also can affect the growth of the fungi (Jones, 1999). Furthermore, in high humidity, the moisture may trigger bioaerosol emissions such as fungi due to droplet splash vibrations which can increase the microbial concentrations by the convective lifting of the microbial into the ambient air.

The most dominant airborne fungi in every microenvironment are represented in Table 2. *Aspergillus* sp. was found to be the most genus that existed in every room except for CSSU rooms. *Penicillium* sp. was also found in most rooms except for DC 1, Lab 2, LR 2, and LR 3. Meanwhile, *Fusarium* sp. mainly exists in every room except for CSSU rooms, similar to *Aspergillus* sp. *Aspergillus* sp. was found to be the most abundant airborne fungi that can be detected in each of the different microenvironments. This was due to the species of *Aspergillus* was usually identified from the recovered isolates (Asif et al., 2018). The presence of *Aspergillus* species in the indoor air of hospitals was considered a risk factor for patients due to their ability to cause nosocomial infections and allergies (Verde et al., 2015).

Penicillium is also one of the major airborne fungi that can be found in the indoor air environment. However, *Penicillium* indicates the inverse dependency of the highest concentrations observed at high relative humidity levels (Sadys *et al.*, 2015) since air temperature and relative humidity, irrespective of the geographic region and climate conditions, are important influential parameters for most dry spores found in the air (Ilanovici, 2016).

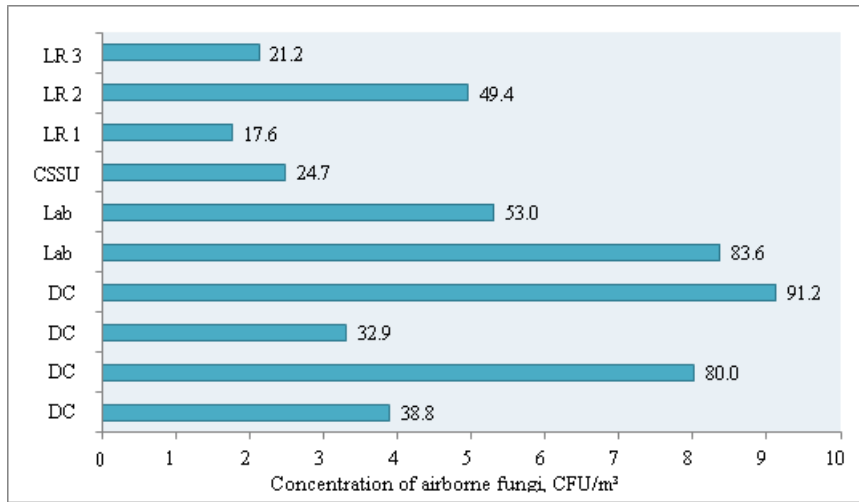


Fig.2: The mean concentrations of indoor airborne fungi at each sampling locations

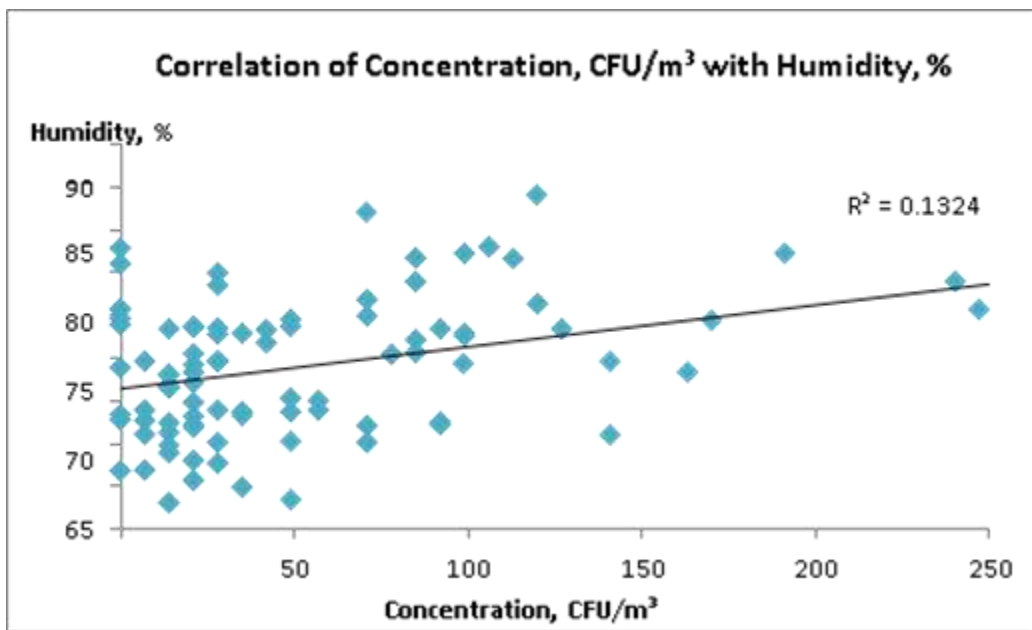


Fig.3: The correlation of concentration of airborne fungi with humidity

Aspergillus, *Penicillium*, and *Cladosporium* produce a high number of small and light spores (Schwab & Straus, 2004) which become dominant in the airborne environment. Furthermore, *Aspergillus* sp. is also known to be the main causative agent of fungal sinusitis which some people are very sensitive to these biological pollutants after having repeated or high levels of exposure.

The total percentage composition of the airborne fungi in different microenvironments was represented in Fig.4. The most dominant indoor airborne fungi found in all the different microenvironments were *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. Kuan and Hwan, (2006) also found that *Aspergillus* would mainly grow indoors. Other indoor fungi are *Alternaria*, *Cladosporium*, and *Gliocladium* which *Alternaria* and *Cladosporium* are the most important indoor airborne fungal allergens which same as *Penicillium* and *Aspergillus*. *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp., and *Aspergillus* sp., were recognized as opportunistic pathogens for humans and often associated with clinical manifestations of allergy, rhinitis, asthma, and conjunctivitis (Samuel and Abayneh, 2014). All those airborne fungi were also considered potential candidates involved in the establishment of sick building syndromes (Schwab & Straus, 2004).

Table 2: The composition of airborne fungi in each different environment

Fungi species	TR 1	TR 2	TR 3	TR 4	Lab 1	Lab 2	CSSU	LR 1	LR 2	LR 3	Total	Composition (%) of airborne fungi
<i>Penicillium</i> sp.	-	6	3	14	9	-	10	3	-	-	45	15
<i>Aspergillus</i> sp.	7	16	3	28	4	3	-	3	1	1	66	22
<i>Cladosporium</i> sp.	1	8	2	6	10	1	3	-	2	1	34	12
<i>Alternaria</i> sp.	-	1	-	2	-	1	-	1	-	1	6	2
<i>Fusarium</i> sp.	1	15	2	3	11	11	-	1	5	1	50	17
<i>Rhizopus</i> sp.	-	1	-	-	-	1	-	-	-	-	2	1
<i>Curvularia</i> sp.	-	3	-	-	-	-	-	-	-	-	3	1
<i>Candida</i> sp.	3	-	1	24	-	-	1	-	-	-	29	10
<i>Rhodotorula</i> sp.	10	1	4	5	1	-	3	-	-	-	24	8
Others	11	2	-	3	5	14	1	-	-	-	36	12
Total	33	53	15	85	40	30	7	8	9	4	295	

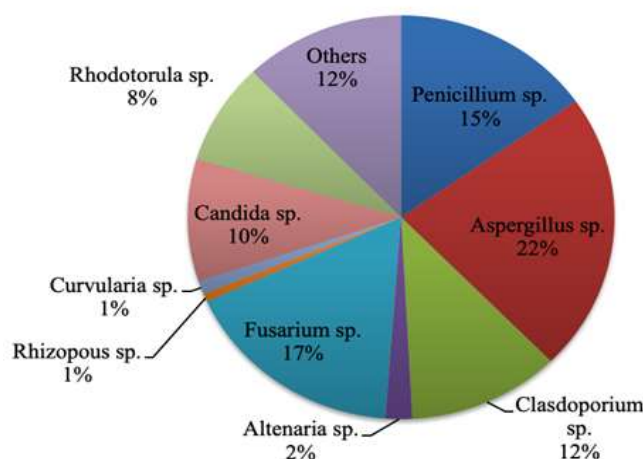


Fig.4: Total percentage composition of airborne fungi

In a comparison of the temporal for different time sampling times, the high range concentrations of airborne fungi were found in the evening with a concentration of 74 to 148 CFU/m³ (Fig.5). The range concentration of airborne fungi in the afternoon was 18 to 148 CFU/m³. Meanwhile, the lowest concentration of the airborne fungi was found in the morning at 5 to 30 CFU/m³. This shows that in the overall mean concentration of airborne fungi, the higher average concentration was detected in the evening followed by the afternoon. Meanwhile, the lowest average concentration was detected in the morning.

The concentration of the airborne fungi at different sampling locations was significantly different in each of the microenvironments. The concentration of the airborne fungi was high in the morning which may indicate that the airborne fungi that may be presented in the air during that time were not all human-borne (Soto *et al.*, 2009). The building design, cleaning practices, and the ventilation of each room contribute to the number of airborne fungi. The concentration of airborne fungi in the afternoon was in the middle between morning and evening readings. Through observation, the dental staffs, doctors, and dental students applied their practices of cleaning the desks, chairs, and all the dental equipment before having their daybreak. These practices may slightly affect the concentration of the airborne fungi that might be presented during the sampling process.

Meanwhile, the highest concentration of airborne fungi (148 CFU/m³) was recorded in the evening which was in Lab 1. The concentration obtained during evening sampling has the highest average concentration among all different sampling times. This scenario may be due to the accumulation of airborne fungi in the evening that results in high concentration. This may also be attributed to the difference in the number of patients seen in treatment rooms every day. This pattern is also seen in Lab an as a high number of students

conducted lab works in the laboratory during the assessment day. The number of occupants that visited a room may increase the activity conducted in the rooms and trigger microbial airborne fungi growth (Samuel & Abayneh, 2014).

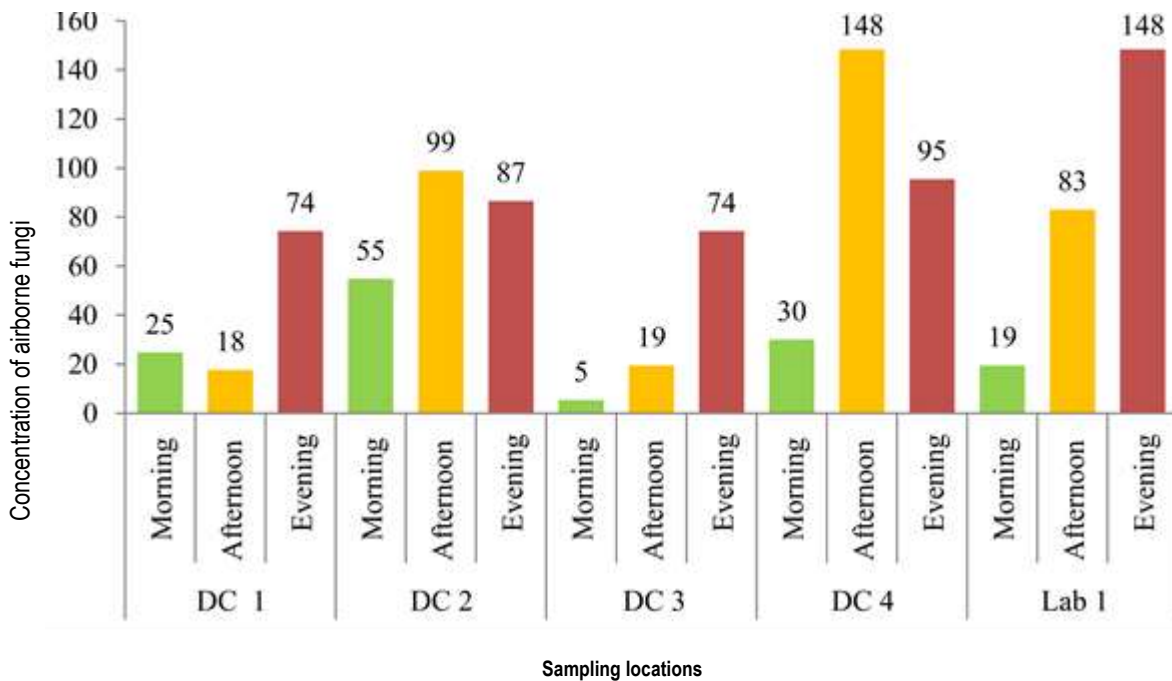


Fig.5: The temporal comparison of the concentration of the airborne fungi at the different microenvironment

Dental clinics and laboratory 1 were used to measure temporal comparison were the rooms that mainly used almost every day in 8 hours of working time. The dental clinics were attended by daily walk-in patients and the workplace where the dentist and students perform the treatment towards the patient. Each of the treatment rooms conducted different types of treatment.

First-hand treatment was done in DC 1 where the patient was examined before being handed over for further or specific treatment in other treatment rooms. DC 2 was handled by the postgraduates of Periodontology for special case teeth treatment such as periodontitis. DC 3 handled oral surgery treatment while polyclinic DC 4 was occupied by third-year dentistry student for regular treatment such as dental scaling. Meanwhile, an open laboratory Lab was used by all dentistry students doing prosthetic devices such as dentures, implants and therapeutic devices including orthodontic devices. These showed that different laboratory occupancy correlates with the concentration of the airborne fungi.

The concentrations of fungi measured in all the sampling locations have a variation to each other. These can be primarily explained by the difference in source for each sampling location that contributes to a broad range of airborne fungi concentrations (Hussin *et al.*, 2011) as different activities were performed by each room in every sampling location. In every treatment room, the airborne fungi may have different sources, such as from dental procedures, dental workers, or patients, and can be from outside sources such as air, soil, and dust. As such, airborne fungi may be transferred to dental workers or patients. Environmental factors such as variation of ventilation conditions may also enhance microbial growth and multiplication in the indoor atmosphere (Wamedo *et al.*, 2012). The World Health Organization also stated that dampness situations inside the building must be considered as a risk indicator for the health risks of biological contaminants of indoor air (WHO, 2010).

4.0 Conclusion & Recommendations

The concentration of airborne fungi may be affected by the activities and the number of occupants in a different room. The mean concentration of airborne fungi in the selected treatment room was below the recommended guideline standard hence it was an acceptable limit. It was found that the highest concentration of airborne fungi was found in the evening where a high number of dental students conducted lab works in the laboratory during the assessment day. The number of occupants that visited a room may increase the activity conducted in the rooms and trigger microbial airborne fungi growth. Generally, the activities related to dental treatment have a significant impact on the abundance of airborne opportunistic microorganisms. Thus, we suggested that frequent inspection and surveillance of microbial air as a useful tool for assessing the quality of indoor air and identifying critical circumstances that require corrective action to prevent or mitigate infections associated with health care.

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