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Variations in the Microbial Contamination of Disinfectant Bottles Established During COVID-19 Based on Their Location and Operation Mode

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ABSTRACT

Disinfectant alcohol bottles have been installed at the entrances of various commercial facilities and schools in Japan as a precautionary measure against the coronavirus disease-2019. As they are touched by many people, we hypothesized that the disinfectant bottles are a source of microbial contamination. To verify this, in this study, we aimed to examine and compare the degree of microbial contamination of disinfectant bottles based on their location and mode of operation (hand- and foot-operated).

Keywords: COVID-19 measures, installation of disinfectant bottles, microbial contamination status

1. INTRODUCTION

Infectious diseases are caused by pathogens, such as viruses and bacteria, that invade and multiply in the body, exerting various harmful effects. The main routes of infectious disease transmission are direct contact, droplets, and air. The novel coronavirus infection, which has recently spread worldwide, is primarily transmitted via droplets and contact. Many preventive measures, including regular hand washing and gargling, have been implemented to prevent the spread of this infection. Disinfection is crucial in situations where immediate hand washing is not possible[1),2)]. Notably, 70% (v/v) ethanol and benzalkonium chloride are effective disinfectants against coronaviruses, including the novel severe acute respiratory syndrome-coronavirus-2[3),4)]. Following the declaration of a state of emergency, disinfectants were installed in medical facilities, restaurants, commercial establishments, and schools as priority measures to prevent coronavirus transmission. At our university, disinfectants were installed at the entrance and within each classroom. On May 8, 2023, the coronavirus disease-19 has been downgraded to a category V infectious disease (the least infectious and severe among categories I–V) in Japan. However, even in July, 2023, hand disinfectant stations were maintained at the entrances of many facilities, but their number gradually decreased till April, 2025.

Operation modes of disinfectants have expanded to include various non-contact modes in addition to the hand-operated (pump) mode, which was the standard before the novel coronavirus outbreak in 2020. These include foot-operated dispensers, which spray disinfectants without the need to touch the bottle, and sensor-type dispensers, which automatically release disinfectants when hands are waved over them. Importantly, disinfection cannot always be performed without contact, and although disinfectant use is mandatory, no strict specifications are provided regarding their mode of use, which can lead to instances of contact with the disinfectant bottle[5),6)]. In this study, we investigated the microbial contamination of disinfectant liquid bottles in **A** and **B** buildings, considering factors such as installation location, temperature, and humidity, to improve the public health. Additionally, we conducted a survey of general bacteria and fungi, *Staphylococcus aureus*, *Escherichia coli*, and coliform bacteria at all tested locations.

2. METHODS

2-1 Survey location overview

The survey locations included the front of the office on the first floor, front of the security guard office, front of the bicycle parking lot, and front of the computer room on the second floor in building **A** (**A1**–**4**, respectively) and front of the shop on the first floor,

cafeteria on the first basement floor, front of the health room on the second floor, and above the disinfectant bottles in the computer room in building **B** (**B5–8**, respectively; Table 1). The hand-operated dispensers were placed at **A1**, **A4**, **B6**, and **B8**, whereas foot-operated dispensers were placed at **A2**, **A3**, **B5**, and **B7**. The area of the top of the disinfectant bottle was 3.8 cm^2 for the hand-operated dispensers and 20.8 cm^2 for the foot-operated dispensers. Additionally, a survey was conducted of 100 cm^2 of the floor surface near the disinfectant installation locations **A1**, **A3**, **B6**, and **B7**.

Average temperature and humidity on the survey days were recorded based on the weather data published by Kochi city[7]. As the survey was conducted approximately every five weeks, average temperature and humidity before the first measurement were taken as the average values for the five weeks before the measurement (Table 2). Number of people using computer rooms in buildings **A** and **B** every week was considered as the number of people registered for classes (Table 3). As many students used other rooms, it possibly caused a negative error in the actual number of users. The number of cafeteria users was determined by asking the cafeteria staff about the number of daily transactions, and considering this as the number of daily users, total number of users during the survey period was estimated (Table 4). The amount of disinfectant to be replenished was determined by checking with the Finance and Facilities Division that has jurisdiction over campus facilities, viewing the prescribed record sheet, and determining the amount used during the survey period (Table 1).

2-2 Equipment, devices, culture medium, and water

The following items were used in the study: Sterile cotton swabs (Shimadzu Diagnostics Co., Ltd., Tokyo, Japan), sterile frames (Wiping Frames; Shimadzu Diagnostics Co., Ltd.), a thermohydrometer (TOA-CO2MG-001; Toa Sangyo, Tokyo, Japan), an ultrasonic cleaner (DG-1; Iuchi Seiei-do, Osaka, Japan), a clean bench (ADS161SHUG; Yamato Scientific, Tokyo, Japan), a vortex mixer (Genie 2l MS Equipment, Osaka, Japan), and a filter tip (RPT-5E-SF; Ina Optika, Osaka, Japan). The equipment used in the study included an incubator (MIR-154; SANYO Electric, Osaka, Japan), a pure water production system (Autostill WS200; Yamato Scientific), an ultrapure water production system (RFU414BA; Advantec Toyo, Tokyo, Japan), an automatic high-pressure steam sterilizer (MLS-3020-; Sanyo Electric Biomedica, Tokyo, Japan), sterile 15-mL centrifuge tubes (TR2001; Nippon Genetics, Tokyo, Japan), and a simple culture medium (Compact Dry; Shimadzu Diagnostics Co., Ltd.). Four medium types were used: One for general bacteria, one for yeast and mold (rapid type), one for *S. aureus*, and one for *E. coli* and coliform bacteria measurements. Yeast and mold measurement media were used for fungi, according to the manufacturer's instructions. Sterile water was prepared by turning pure water obtained from a water purifier into ultrapure water with resistivity ≥ 18 MΩ·cm using an ultrapure water production system and sterilizing it in a sterilizer at 121°C for 15 min, after which 6 mL was aseptically dispensed into centrifuge tubes and used for the survey.

2-3 Measurement method

On June 26, July 24, August 21, September 18, and October 16, 2023, the tops of the target disinfectant bottles were wiped and numbers of different microorganisms, including general bacteria and fungi, *S. aureus*, *E. coli*, and coliform bacteria, were measured. A sterile cotton swab was moistened with sterile water in a centrifuge tube, and one measurement location was continuously wiped in three directions (horizontal, vertical, and diagonal) for 3 min. To prevent any bias in the results due to the order of measurements, the measurements were taken in the order of **A1** → **B8** on the first, third, and fifth measurement days and **B8** → **A1** on the second and fourth measurement days (Table 1). The nearby floor surfaces were wiped with a sterile frame at the same time as the disinfectant bottles at **A1**, **A3**, **B6**, and **B7** were wiped during the fifth measurement. Then, taking care not to touch the cotton swab with bare hands, it was returned to the centrifuge tube containing the remaining sterile water (approximately 6 mL).

After wiping, centrifuge tubes with the sterile cotton swabs were sonicated for 5 min and stirred on a clean bench. Then, 1 mL of the microbial suspension was plated onto a simple culture medium, after which general bacteria were cultured at 35 °C for two days, fungi at 25 °C for three days, and *S. aureus* and *E. coli*/coliform bacteria at 35 °C for four days. *S. aureus*, *E. coli*/coliform bacteria, fungi, and general bacteria were seeded in this order to minimize the contamination of components among media. Then, regardless of the color for fungi, numbers of colonies red for general bacteria, light blue-to-blue for *S. aureus*, blue-to-bluish-purple for *E. coli*, and pink-to-reddish-purple for coliform bacteria were counted. To increase the measurement accuracy, one person observed the front and another person observed the back of each culture medium, and average of the two measurements was converted per 100 cm^2 to determine the total number of microorganisms.

Floor was wiped in an additional experiment on October 16. During wiping, temperature and humidity were measured on the floor at the disinfectant site (data not shown).

3. RESULTS AND DISCUSSION

3-1 General bacteria

Figure 1 shows the number of bacteria at each location. Several general bacteria were detected at **B6** throughout the survey period. Number of people using the cafeteria and use of hand-operated disinfectants possibly affected the number of microorganisms detected at each location. The number of detections started to decrease from the third measurement day, with no detections made on the fourth day. However, number of detections increased again on the fifth day. This was possibly because the cafeteria was closed during summer vacation, resulting in no customers; however, upon reopening, the number of people using the cafeteria increased. Installation of disinfectants on the walkways people passed through before meals possibly increased their disinfection awareness. In the fifth experiment, significant detections were made at **B5**. General bacteria at **A2** and **A3** were detected in all five experiments. However, detections at **A1** were only made in the first and fifth experiments, at **A4** in the first experiment, and at **B8** in the fifth experiment, indicating that these places were not always contaminated.

Numbers of microorganisms detected at all five sampling sites decreased from the first to the second sampling, with no change from the second to the third sampling in many locations. Our laboratory previously reported that microbial counts increased and decreased with increasing and decreasing temperature, respectively[8]. Therefore, we considered temperature and humidity in this study; however, no similar trends were observed. The number of microorganisms decreased or remained unchanged from the third to fourth measurements but increased from the fourth to fifth measurements in six out of eight locations. The third and fourth measurements were during summer vacation, and fifth measurement was during the second semester.

More bacteria were detected in building **B** than in building **A**. Compared to those in building **A**, sampling locations in building **B** included places where people tended to gather, such as the co-op and cafeteria, and a library open to non-university personnel. Use of disinfectants was possibly higher in building **B** than in building **A**, even during university holidays, resulting in the higher detection of general bacteria in building **B**.

3-2 Fungi

Figure 2 shows the number of fungi detected at each location. Fungi were detected at **A3** but never detected at **A1** in all five experiments. During graduation research in our laboratory, large numbers of fungi were detected in unused sandals, but not in the frequently worn sandals[9]. Similarly, a previous study also reported places not used for long periods as optimal environments for fungal growth[10]. In this study, **A3** had a lot of dust during sample collection; therefore, fungal count was possibly influenced by the microorganisms contained in the dust. However, the amount of dust was only visually evaluated in this study, warranting further investigation.

Almost no change in the number of microorganisms was observed on all five sampling experiments. In many places, it increased from the fourth to fifth experiment. This was possibly influenced by humidity, as fungi are frequently found in humid and condensation-prone places[11]. However, no change in the number of microorganisms was observed with increasing or decreasing humidity. Fungal count increased in the fifth experiment. As humidity was the lowest on the day of and immediately before the experiment, it possibly did not affect the fungal count.

Many microorganisms were detected in places susceptible to external influences. In our previous study investigating the number of microorganisms on the floor of a computer room, fungi were abundant on the floor facing the corridor and around the windows, suggesting their transmission from the outside environment by the flow and movement of people[12]. The number of microorganisms detected in this study was also possibly influenced by microbial movement from the outside environment.

Fungi were more abundant in building **A** than in building **B**. Compared to building **A**, building **B** was a place where students from various faculties tended to gather and outsiders could freely enter/exit. This suggests that the use of disinfectants was lower in building **A** than in building **B**. Moreover, as fungi are often detected in long-term unused places, more fungi were detected in building **A** than in building **B**.

3-3 *S. aureus*

Figure 3 shows the *S. aureus* count at each location. *S. aureus* was frequently detected at **B6**. *S. aureus* was detected more in the

hand-operated dispensers than in the foot-operated dispensers in all five experiments. *S. aureus* is a normal skin bacterium distributed in various places, including the nasal cavity and intestinal tract[10]. Our findings suggest that these bacteria are transmitted via direct contact with hand-operated dispensers at the sampling location. Several people used hand-operated dispensers installed near the toilet, which caused skin damage after hand wash, resulting in considerably high contamination at **B6**[13].

Comparison of the changes in *S. aureus* counts over five experiments revealed decreased counts from the first to second experiment, with no changes from the second to third or third to fourth experiment in many locations. However, *S. aureus* counts increased from the fourth to fifth experiment. The fourth experiment was conducted during summer vacation, whereas the fifth was conducted during the second-semester classes. Number of users decreased during summer vacation and increased with the initiation of second-semester classes, thereby affecting the microbial count.

3-4 *E. coli* and coliform bacteria

Notably, no *E. coli* and coliform bacteria colonies were detected at any location on any sampling date. Figure 4 shows the average results of the additional experiment, along with the average number of microorganisms on the hallway floor for comparison[12]. Comparing the counts of general bacteria, fungi, and *S. aureus*, no major differences were noted between the previous and current floor surface measurements. However, no *E. coli* and coliform bacteria were detected on the floor. This was possibly because the tested locations were not optimal habitats for *E. coli* and coliform bacteria, with no contamination from toilets. Several microorganisms were detected on the top of disinfectant bottles, indicating that the top of disinfectant bottles is not always clean.

4. CONCLUSION

This study conducted a wiping survey of disinfectant bottles at various locations to examine and compare microbial contamination considering factors, such as the operation mode (hand- or foot-operated).

Many bacteria were detected in the disinfectant bottles in the cafeteria throughout the study period, possibly due to the large number of people using the cafeteria and hand-operated type of bottle. No association was observed between the number of general bacteria and disinfection location or time. Several fungi were detected in the bottles in entrance halls or by windows, suggesting the entry of microorganisms, along with soil and dust, from outdoor environments. *S. aureus* was detected in large numbers in the cafeteria and locations with hand-operated dispensers. *S. aureus* is a normal skin bacterium that was possibly transmitted via direct contact at the sampling location. As the number of students increased in the fifth measurement with ongoing classes compared to that in the fourth measurement during summer vacation, number of cafetoria users possibly influenced the microbial contamination in these places. *E. coli*/coliform bacteria were detected in all five experiments. However, no *E. coli* and coliform bacteria were detected on the surrounding floor, suggesting the disinfectant bottle and its surroundings as environments with low levels of *E. coli* and coliform bacteria.

The tested conditions were often inconsistent in this study. Therefore, future studies should set common conditions and measurement locations, such as only entrances or places using disinfectants with the same frequency, to reveal more specific microbial contamination trends. As the area wiped with the disinfectant bottle was small and area correction possibly resulted in larger-than-actual values, relatively large areas should be surveyed in future studies.

5. ACKNOWLEGDMENT

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Table 1. Survey location characteristics.

Sample No.	Disinfectant bottle installation location			Bottle type	Wiping order		Susceptibility to outdoor influences	Amount of foot traffic	Disinfectant refill amount
					① June 26 ③ August 21 ⑤ October 16	②July 24 ④September 18			
A1	**A** building	First floor	In front of the office	Hand-operated	1	8	Less susceptible	Low	Unknown
A2		First	In front of the security room	Foot-operated	2	7	Susceptible	High	High (1200 mL)
A3		First	In front of the bicycle parking lot	Foot-operated	3	6	Susceptible	High	High (200 mL)
A4		Second	Computer room	Hand-operated	4	5	Less susceptible	Low	Low (0 mL)
B5	**B** building	First	In front of the Co-op store	Foot-operated	5	4	Less susceptible	High	High (1200 mL)
B6		First basement	Inside the cafeteria	Hand-operated	6	3	Less susceptible	High	Unknown
B7		Second	In front of the counseling room	Foot-operated	7	2	Less susceptible	Low	Low (0 mL)
B8		Second	Computer room	Hand-operated	8	1	Less susceptible	Low	Low (0 mL)

"Susceptibility to outdoor influences" was evaluated based on whether pollutants, such as dust, were easily dispersed from outdoor environments.

"Foot traffic" was measured by determining the number of people passing through the disinfectant installation sites based on the number of classes and characteristics of the surrounding facilities.

The replenished amount of disinfectants for which no replenishment was recorded during the entire survey period was indicated as "unknown."

Table 2. Average temperature and humidity in Kochi city.

Period	Average temperature (℃)	Average humidity (%)
May 30 to June 25	22.2	82
①	25.2	84
June 27 to July 23	27.1	83
②	26.7	90
July 25 to August 20	28.6	79
③	29.6	78
August 22 to September 17	27.5	80
④	27.6	81
September 19 to October 15	23.5	69
⑤	19.4	62

Wiping days are indicated as ①–⑤.

As surveys were conducted approximately every five weeks, average temperature and humidity before the first measurement were taken as the average values for the five weeks before the first measurement.

Table 3. Numbers of people weekly using the computer rooms in buildings A and B.

Period	Building **A** computer room (person/week)	Building **B** computer room (person/week)
First semester (April to August)	93	254
Second semester (October to February)	84	155

Numbers of people weekly using the building **A** and **B** computer rooms were determined based on the numbers of people attending classes in those buildings.

As many students used other rooms, these results may be inaccurate.

Disinfectants were installed next to the entrance of the computer room at **A4** in building **A** and **B8** in building **B**.

Table 4. Number of people using the university cafeteria.

Period	Number of users
May 30 to June 25	3935
June 26 to July 23	3558
July 24 to August 20	1869
August 21 to September 17	0
September 18 to October 15	1557

Number of daily cash register transactions was considered as the number of daily users and used to calculate the total number of people using the cafeteria for approximately five weeks before the survey.

Note: No users were recorded during the summer vacation period from August 8 to October 1.

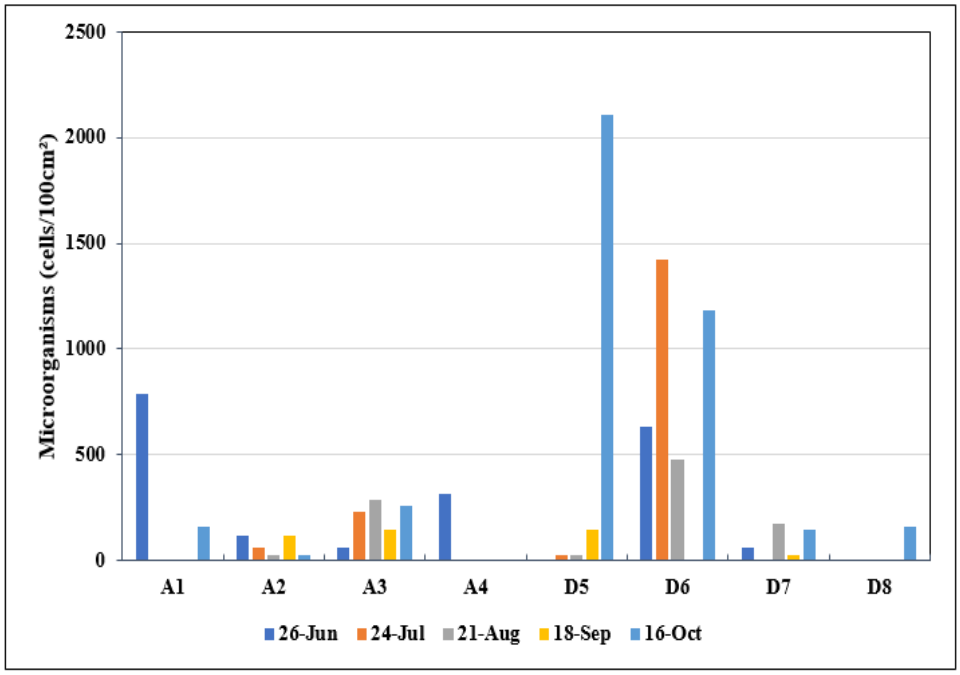

Figure 1. Numbers of general bacteria at all tested locations.

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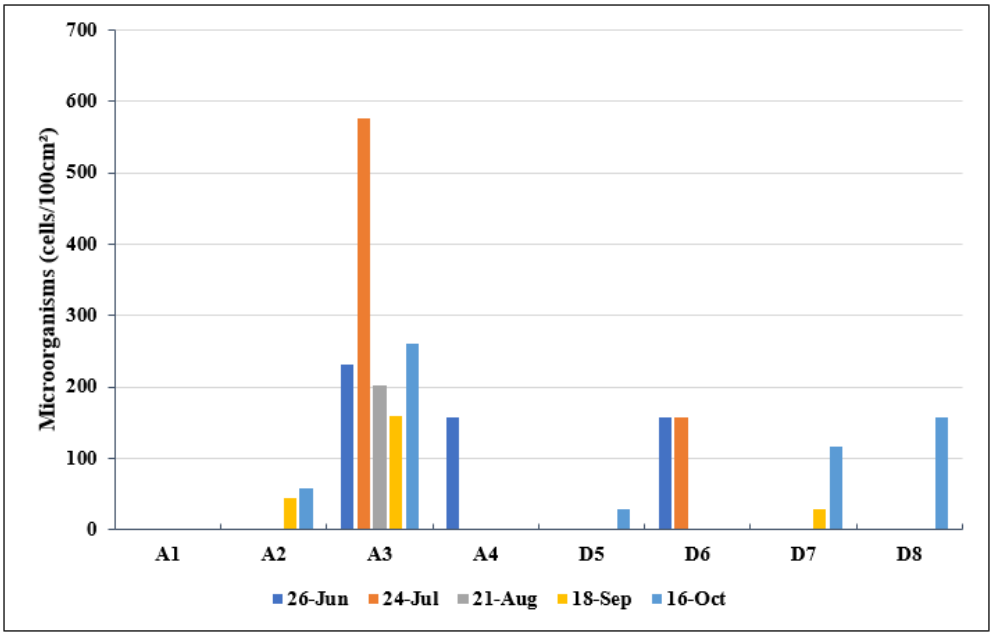

Figure 2. Numbers of fungi at all tested location

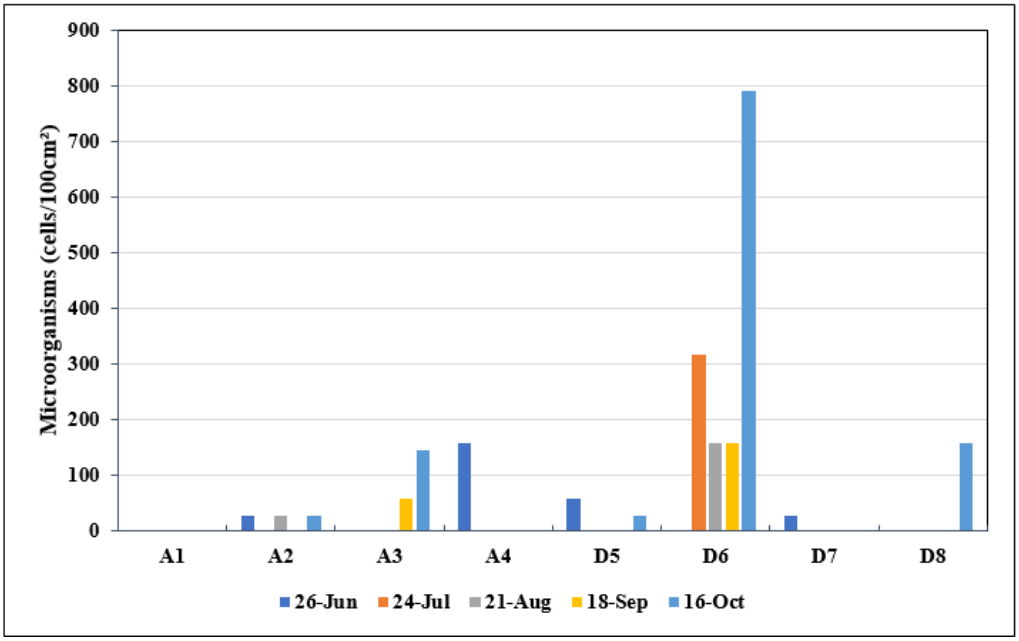

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Figure 3. Numbers of *Staphylococcus aureus* at all tested locations.

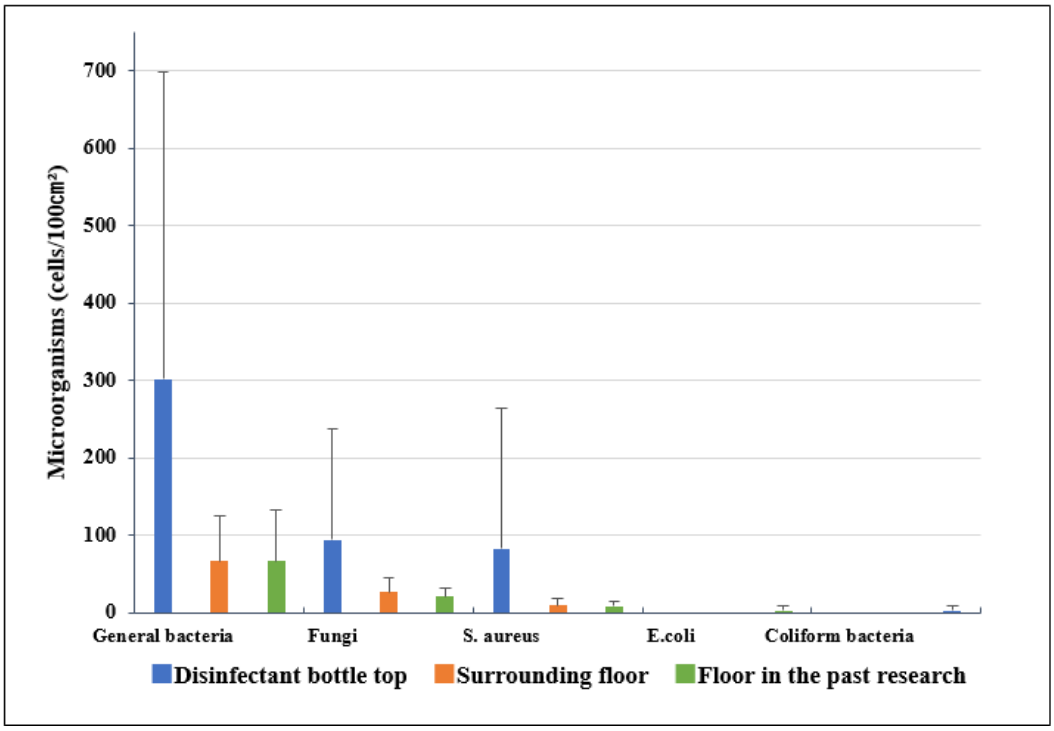

Figure 4. Numbers of different microorganisms detected on the floor.

In an additional experiment, different microbial counts on the floor near the disinfectant installation locations **A1**, **A3**, **D6**, and **D7** were averaged and are shown together with the average microbial counts on the top of the disinfectant bottles and floor of the hallway.

Number of microorganisms detected on the computer room entrance floor in the same university, previously investigated by our laboratory[12], was used for comparison.

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Conflict of Interest Statement: All authors have nothing else to disclose.