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Survey of Microbial Contamination on the Floor of University Building

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ABSTRACT

In order to prevent infectious diseases and food poisoning, it is important not only to eradicate the viruses and bacteria that them but also to prevent the transmission of microorganisms from one person to another. In our previous study, we surveyed the microbial contamination of shared indoor footwear (slippers and sandals) in the university and found that the degree of microbial contamination varied depending on the place of use. We speculated that the floor of the room might be a potential source of microbial contamination of indoor footwear. Therefore, the purpose of this study was to investigate contamination on the floors of information practice room and toilets in the same university. Our results demonstrated that the number of locations where bacteria were detected and the number of bacteria were higher in the information practice as compared to the toilets. This can be attributed to the presence of dust, presence of carpet, high frequency of use, presence of conditioning, and contact of socks with the floor of the information practice room after removal of outdoor shoes. The number of fungi was remarkably high in the toilets as compared to the information practice room, probably due to the high humidity in toilets.

INTRODUCTION

In recent years, nosocomial infections and food poisoning have been recognized by not only medical professionals and specialists but also the general public. These infections are caused by microorganisms including viruses and bacteria, which multiply in the body and cause various adverse effects. Despite the various laws enacted and the guidelines created and disseminated by the national government, outbreaks still occur at restaurants, hospitals, schools, *etc*., every year[1)].

Previous studies have reported contamination in operating rooms and waiting rooms and on objects such as sheets, doorknobs, and toys for pediatric patients in hospitals; contact with such contaminated objects may cause nosocomial infections in patients. In addition, contact transmission may occur in waiting rooms because it is a place where many people gather[2-4)]. Handwashing is considered an effective strategy to prevent the spread of infections. Inadequate handwashing can lead to spread of infections through contact with an unspecified number of people. Surveys that measure the bacterial load in the toilet environment in hospitals and passenger planes have reported that bacteria are present on doorknobs, electric light switches, hand dryers, etc[5-7)]. Other studies have reported the presence of Trichophyton on hospital outpatient slippers[8)], and the presence of bacteria in household dust[9)]. It has also been reported that using a public bathroom causes dermatophytes to adhere to the soles of healthy individuals, which subsequently causes infections[10)].

In our previous study, we surveyed the microbial contamination of shared indoor footwear's in a university and found that the degree of bacterial contamination varied depending on the place of use; we speculated that the floor surface could be a potential source of bacterial contamination of indoor footwears[11)]. Therefore, the purpose of this study was to investigate microbial contamination on the floor of the information practice room and toilets in the same university as our previous report. The information practice room is one of the classrooms that was covered in the previous report and is used by many people on a daily basis. Though toilets are most likely to be contaminated, there was almost no survey of university toilets in the literature and the contamination characteristics are not known. Therefore, we surveyed the microbial contamination in university toilets in this study. We determined the load of total live bacteria, *Escherichia coli*, coliforms, *Staphylococcus aureus*, and fungi at these two locations.

MATERIALS AND METHODS

Equipment and devices

Compact dry "Nissui" medium kits (Nissui Pharmaceutical, Tokyo, Japan) were used for measuring a load of total live bacteria, *E. coli* coliforms, *S. aureus*, and yeast/mold (rapid type). In this study, the yeast/mold assay kit was used for enumeration of fungi, after confirming with the kit manufacturer. A sterile cotton swab (code 06526; Nissui Pharmaceutical) was used to collect the microorganisms.

For ultrasonic treatment, DG-1 ultrasonic cleaner (Iuchi, Osaka, Japan) with an oscillation frequency of 43 kHz was used. MIR-154 incubator (Sanyo Electric, Tokyo, Japan) and ADS161SHUG clean bench (Yamato Scientific, Tokyo, Japan) were used for culturing and aseptic operation.

Ultrapure sterile water was obtained by purification of water using Auto still WS200 pure water production equipment (Yamato Scientific), followed by ultra purification at a specific resistance value of 18 MΩ·cm or more using KRFU414BA ultrapure water production equipment (Advantech Toyo, Tokyo, Japan), and finally sterilization at 121 °C for 15 min using MLS-3020 high-pressure steam sterilizer lab autoclave (Sanyo Electric Biomedica, Tokyo, Japan). Aliquots of 6 mL of sterile water were dispensed into sterile 15 mL centrifuge tubes using a micropipette in a clean bench for subsequent measurements.

Survey target

The survey targets were the floor of the information practice room of K University and the floor of the women's toilet on the 1st to 4th floors. The floor of the information practice room was covered with a carpet and was used for switching from outdoor footwear to shared indoor footwear (slippers). However, many users did not wear indoor slippers as it was not mandatory. Based on the timetable defined by the university, the room was used for about 17 h per week. However, depending on the content of the class, the time period could vary, and the usage status could be changed exceptionally. As this room was open outside of class hours and about 50 personal computers were installed, there were times when nearly 40 people used it regardless of the class; however, it should be noted that the number of people was not fixed by date or time. In the information practice room, we selected 10 locations (***a–j***) that were considered to contact different people's feet. The floor near the doorway was labeled ***a***, and

the floor near the teaching table was labeled ***b***. Locations ***c*** to ***h*** represented floors near the desks where student computers were installed, and locations ***i*** and ***j*** represented the floor of the passages.

The floor of the toilet was tiled to allow usage while wearing outdoor footwear. Ventilation fans were installed in all toilets and they operated for 24 h. The toilets on the 2nd to 4th floors had windows, but we were unaware of their opening and closing conditions. For measurement of microbial load on the floors of toilets, we selected nine locations (***A-I***), which included the entrances of the toilets where people were supposed to pass. The locations on the 1st floor were labeled ***A*** and ***B***, and that on the 2nd floor were labeled ***C*** and ***D***. ***E***, ***F***, and ***G*** represented the locations on the 3rd floor, and ***H*** and ***I*** represented the locations on the 4th floor. ***A***, ***C***, ***E***, ***F***, and ***H*** were west side toilets, and ***B***, ***D***, ***G,*** and ***I*** were east side toilets. An area of 50 × 50 cm^2 was surveyed on all floors. Few meeting rooms were located on the east side of the 2nd floor of the same building, and many classrooms were located on the 3rd floor.

Microbial sampling and measurement

On the morning of June 3, July 1, August 2, August 30, and September 30, 2019, the information practice room and women's toilet were sampled for microorganisms using sterile cotton swabs before the start of class. Table 1 shows the average temperature and average humidity measured one week before the sampling date. Owing to the summer vacation between August 2, 2019, and September 30, 2019, we assumed that there would be fewer users than usual during this period.

A sterile cotton swab was moistened with sterile water contained in a tube, and the location to be sampled was wiped three times using the moistened cotton swab. Next, the swab was carefully placed in the same tube with sterile water (assuming all 6 mL of sterile water remained), taking care not to touch it with hands. The centrifuge tube containing the cotton swab was sonicated for 5 min, the suspension was stirred using a touch mixer in a clean bench, and 1 mL of the microbial suspension was seeded on a simple medium kit. The culture conditions were according to the instruction manual. For quantification of total live bacteria, the culture conditions were 35 °C for 2 days; for quantification of *E. coli*/coliforms and *S. aureus*, the culture conditions were 35 °C for 4 days; and for quantification of fungi, the culture conditions were 25 °C for 3 days. The number of microorganisms was visually

counted as the number of colonies. To increase the accuracy of the results, two people independently counted one petri dish, and the result was expressed as the average value. Based on the instruction manual, red colonies represented total live bacteria, blue to blue-purple colonies represented *E. coli*, pink to red-purple colonies represented coliforms, and light blue to blue colonies represented *S. aureus*. For quantification of fungi, all colors of fungal colonies were counted.

RESULTS AND DISCUSSION

Comparison among locations

Fig. 1 shows the number of locations in which *E. coli*, coliforms, and fungi were detected. *S. aureus* was detected in all the sampled locations. The number of *E. coli* was more in the information practice room than in the toilets, and the number of coliforms varied according to the sampling date. Fungi were detected at all the sampled locations in the toilet. Figs. 2-6 show the number of microorganisms detected at each location.

Live bacteria were detected at all the sampled spots in the information practice room and toilets, probably because of the large number of microbial groups existing in the environment. As shown in Fig. 2, the number of bacteria in the information practice room was higher than that in the toilets. This can be attributed to the fact that the information practice room was used more frequently (total number of people × usage time) as compared to the toilets. Though toilets were used on a daily basis, the usage time was short and the number of users may vary from floor to floor. In addition, as the information practice room was equipped with items such as desks and personal computers and the floor was covered with a carpet, the environment was prone to accumulation of dust. It has been reported that, though bacteria are separated from domestic dust[9], the presence of such dust is one of the factors for multiplication of bacteria.

We expected that the number of *E. coli* and coliforms would be more in the toilet than in the information practice room, but the opposite result was observed. This finding can be attributed to the presence of toilet on the opposite side of the information practice room, as the users of the toilet could subsequently carry microorganisms to the information practice room. The entrances and floors of passages had the highest number of bacteria probably because these places are used by many people. Therefore, it was speculated that the number of bacteria at a location is related to the frequency of use of that location. Most of the *E. coli*

and coliforms detected in toilets were considered to be derived from feces; however, in addition to feces-derived coliforms, coliforms are present in the environment such as water, soil, and air. For this reason, it was speculated that soil and moisture adhering to the soles of outdoor footwear were also potential carriers of coliforms. Therefore, it is considered that the number of coliforms in the toilet should be higher than that in the information practice room.

S. aureus was detected in all sampled locations of the information practice room and toilets, probably because it is a resident skin bacterium. As shown in Fig. 5, the number of bacteria in the information practice room was relatively high as compared to that in the toilets, although there were a few differences depending on the sampling date and location. This could be because, in the information practice room, it is possible that the hands of a person may come in contact with the floor while switching from outdoor footwear to indoor slippers or while placing luggage on the floor. In addition, it was observed that many people used the room without wearing slippers. Considering that bacteria reach the feet even while wearing socks[13], it is possible that bacteria from the fingers and soles adhere to the floor of the room. In the toilet, it was observed that water scattered on the floor during handwashing, and the water that adhered to the hands fell on the floor after handwashing. It has also been reported that *S. aureus* and intestinal indigenous bacteria were detected in the water receiver of a hand dryer[6]. At K University, there were handwashing facilities, but there are no hand dryers or paper towels. Therefore, it is considered that when water droplets attached to the hands fall onto the floor surface, the bacteria present in the water droplets after insufficient hand-washing adhere to the surface of the floor.

As shown in Fig. 6, the number of fungi detected in the toilet was remarkably high. Mold, a group of fungus, grows rapidly at a humidity of 92–95 % or more. It has been reported that molds easily grow in bathrooms, kitchens, and toilets owing to the high humidity at these locations[14]. Therefore, it is considered that mold growth is greatly influenced by humidity. Unlike the information practice room, the toilet had high humidity due to the presence of handwashing facilities, toilet flushing, and absence of air conditioning; and the humidity was inclusive of the rainy season during the sampling period. Therefore, we speculated that fungi could easily survive in toilets. On the other hand, the information practice room had an air-conditioner, and even during the period of this experiment, people were working from the room itself. Therefore, the information practice room was considered to be less affected by outside air and was in a dry state as compared to the toilets.

In general, the degree of pollution in the information practice room tended to be high. The information practice room was cleaned using a vacuum cleaner thrice a week; however, it was not easy to move the desks because personal computers and copy machines were installed on the desks. Therefore, we consider that adequate cleaning could not be performed. Toilets were cleaned daily. As the toilet is smaller than the information practice room and there are almost no items or machines, it is considered that dust is not easily accumulated in toilets and they were adequately cleaned. Though dust was observed in both information practice room and toilets when samples were collected, it was frequently observed in the information practice room. As it is known that microorganisms also exist in dust, the amount of dust influences the degree of pollution in the information practice room. From the viewpoint of preventing microbial contamination, it is also necessary to take into consideration the cleaning method and cleaning frequency.

Comparison by sampling date

For each microorganism, the changes in the number of microorganisms during all four sampling days were compared. Fig. 2 shows the trend in the number of total live bacteria. At many locations, it was observed that the number of bacteria increased in the third sampling as compared to the second and decreased in the fourth sampling as compared to the third. This trend is possibly related to changes in temperature and humidity. As shown in Table 1, the temperature was also higher on the third sampling day as compared to the second and lower on the fourth sampling day as compared to the third. Moreover, a similar tendency was observed for average temperature of the week before the sampling day. Even though the temperature of the information practice room was adjusted by air conditioning, it was affected by outside air. Therefore, it is considered that temperature is one of the factors that influence the increase or decrease in the number of bacteria.

Changes in the numbers of *E. coli* and coliforms are shown in Figs. 3 and 4, respectively. It was observed that the number of *E. coli* and coliforms decreased in the fourth sampling as compared to the third, and few areas showed absence of these organisms in the fourth sampling. One reason for this could be the summer vacation between the third and fourth sampling, which resulted in a reduction in the number of users than usual. As the temperature was high between the second and fourth sampling, we expected that the number of bacteria would increase owing to the favorable environment, but this tendency was observed only partially. In addition, the tendency of *E. coli* and coliforms to increase or decrease was

different at each location in the second sampling as compared to the first. These results indicate that the effect of frequency of use is greater than the effect of temperature or humidity on the number of bacteria.

Fig. 5 shows the changes in the number of *S. aureus* according to sampling day and location. In both information practice room and toilets, it was observed that the number was higher in the third sampling as compared to the second and lower in the fourth sampling as compared to the third. A similar trend was observed for temperature, which suggests that temperature is one of the factors affecting the number of *S. aureus*. Another factor that affected the number of *S. aureus* was the number of users, which was low during the third and fourth sampling owing to the summer vacation at the university. However, from the first sampling to the second, there was a decreasing or an increasing trend in the number of *S. aureus* at different locations, which was not observed for temperature. Considering that *S. aureus* is a skin-resident bacterium, we speculate that in addition to temperature, humidity, and frequency of use, another factor that influenced the number of *S. aureus* was the state of handwashing.

Fig. 6 shows the changes in the number of fungi according to sampling day and location. We observed that humidity was highest on the second sampling day. In addition, the rainy season increased the humidity on the second and third sampling days. As the fungus easily survives in a humid environment, it was expected that the number of fungi will be high in the second and third sampling, and it will decrease in the fourth sampling when the rainy season is over and the temperature decreases. However, this trend was mostly observed in the toilets and hardly observed in the information practice room. As the temperature of the information practice room was adjusted by air conditioning, the humidity is less likely to be higher than that in the toilet; therefore, the information practice room was in a dry condition. A study on Trichophyton revealed that Trichophyton dies in about one month on a normal dry floor, while they survive for more than half a year under conditions of high humidity[15]. Therefore, it is highly possible that temperature and humidity influence the increase or decrease in the number of fungi.

Comparison by location

We observed that the number of microorganisms varied depending on the location even in the same room/toilet; therefore, we compared the number of microorganisms among different

locations of the same room. In the information practice room, the number of microorganisms was the highest at location ***a***, which is the floor near the doorway and shoeboxes were installed beside it. It is considered that the contamination level was high at location ***a*** because most of the users passed through this point during entry and exit. Locations ***i*** and ***j*** had the second highest number of microorganisms because they were the floors of the passage. At location ***b***, personal computers were installed for teachers, but it was considered that the degree of pollution was low at this location because it was not used on a daily basis and its usage depended on whether class was conducted or not. Personal computers for students were installed near locations ***c*** to ***h***, but its usage was not known clearly. However, among them, locations ***f***–***i*** were close to the entrance and tended to have a larger number of microorganisms as compared to locations ***c***–***e***; therefore, it can be assumed that there were many users.

In case of women's toilet, the number of microorganisms tended to be high at locations ***E*** and ***G***, suggesting that the degree of contamination was high at these locations. ***E*** and ***G*** were located on the 3rd floor, which is the floor where classes are held. Therefore, it is considered that the number of microorganisms was high due to the high frequency of use by teachers and students. Though ***F*** was located on the same 33rd floor, it had fewer users compared to ***E*** and ***G*** as it was far from the classroom; therefore, the number of microorganisms at location ***F*** was also small. There were practice rooms and laboratories on the 1st, 2nd, and 4th floors, but their frequency of use was lower than that of the classrooms on the 3rd floor. Therefore, it is considered that the number of microorganisms was smaller and the degree of contamination was lower at all other locations as compared to ***E*** and ***G***.

CONCLUSION

In this study, we investigated the number of microorganisms on the floor of information practice room and women's toilet in the university. We observed that the number of locations where bacteria were detected and the number of bacteria were high in the information practice room than in the toilets. However, the number of fungi was significantly higher in the toilets as compared to the information practice room. The reasons for high level of contamination in the information practice room were presence of dust, presence of carpet, high frequency of use (number of users × time), presence of air conditioning, and use of outdoor footwear. Fungi commonly grow under conditions of high humidity. Therefore, the number of fungi was large in the toilets as the humidity in toilets was higher than that in the

information practice room.

Comparison according to sampling data revealed that the number of bacteria increased in the third sampling day as compared to the second and decreased in the fourth sampling as compared to the third. This phenomenon was considered to be the effect of temperature. In addition, as the fourth survey was conducted after the summer vacation, we speculated that the frequency of use was also an important factor influencing the number of microorganisms.

Comparison of number of microorganisms at each location of the information practice room showed that that the floor of entrances and passages, which is considered to be used by many people, had the highest degree of contamination. Therefore, it is considered that contamination was introduced by microorganisms attached to the soles of people's feet, and it progressed further depending on the frequency of use, temperature, and humidity of the place.

In this study, we did not measure the actual temperature and humidity at the target location and referred to only the outside air data from public institutions. However, in future, it is necessary to measure the temperature and humidity simultaneously at the target location to better understand the more accurate relationship.

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Table No. 1: Average temperature and humidity on the sampling date and the week before the sampling date

Number of measurements	Date	Average temperature (℃)	Average humidity (%)
1	May 27–June 2	21.6	70.0
	June 3	22.7	76.0
2	June 24–30	24.4	87.6
	July 1	23.7	94.0
3	July 26–August 1	29.0	76.1
	August 2	29.8	72.0
4	September 23–29	24.9	76.0
	September 30	26.6	83.0

Based on the data in reference 12).

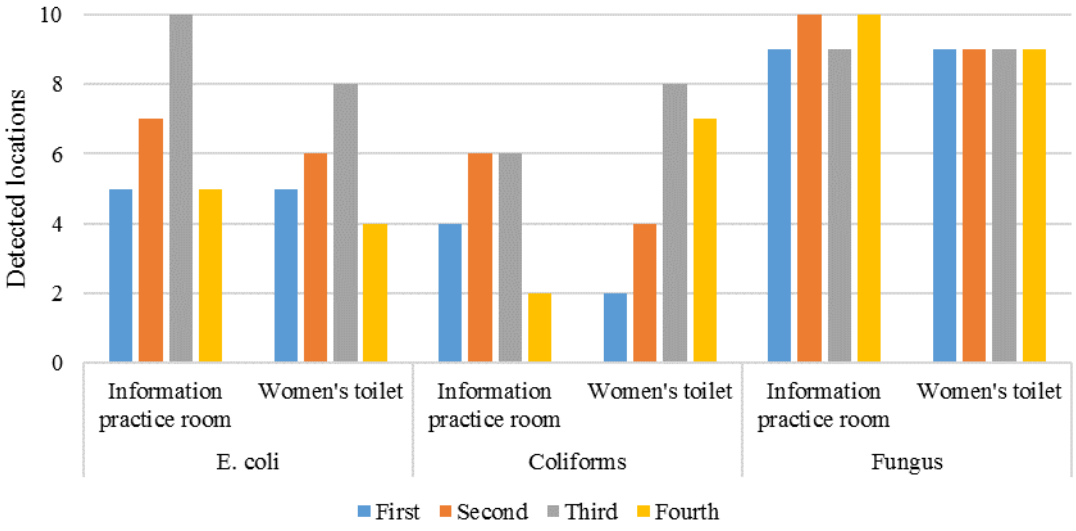

Figure No. 1: Number of locations where microorganisms were detected.

The number of microorganisms was measured at 10 locations in the information practice room and at 9 locations in the women's toilet. Different colors represent different sampling days.

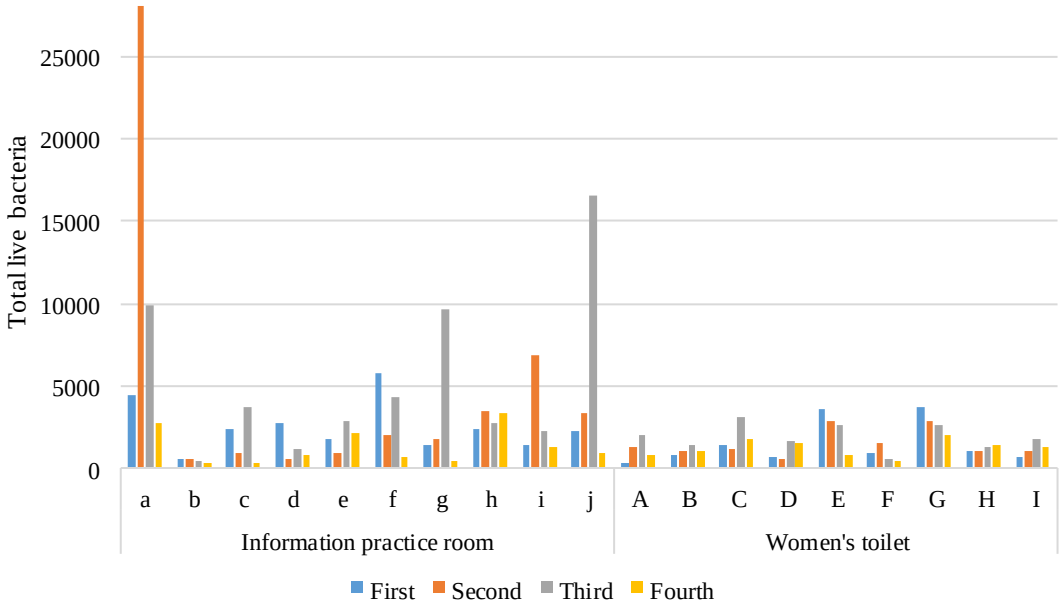

Figure No. 2: Number of total live bacteria detected at each location in the information practice room and women's toilet

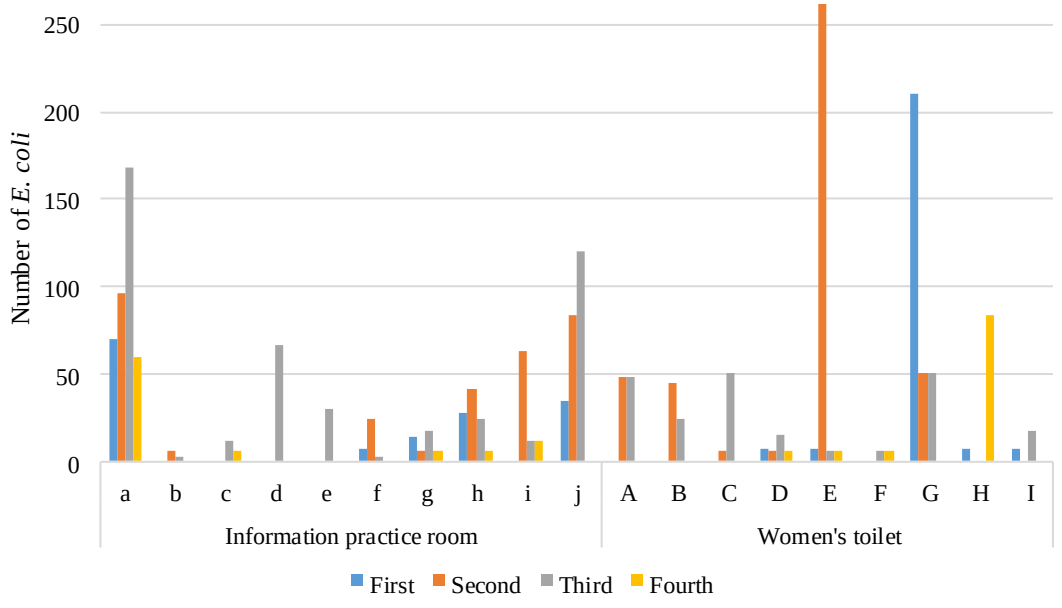

Figure No. 3: Number of *Escherichia coli* detected at each location in the information practice room and women's toilet

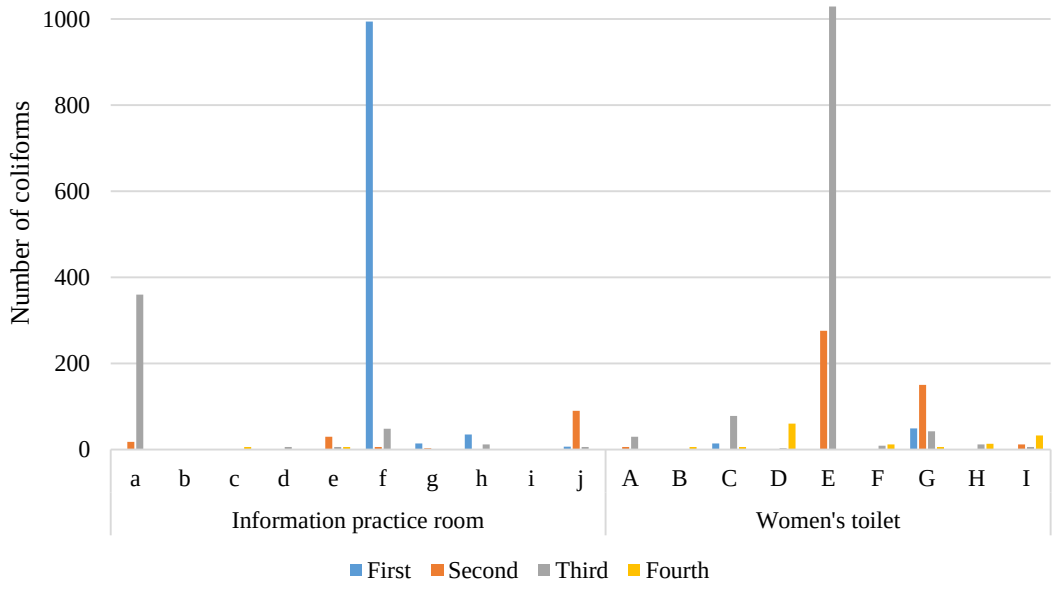

Figure No. 4: Number of coliforms detected at each location in the information practice room and women's toilet

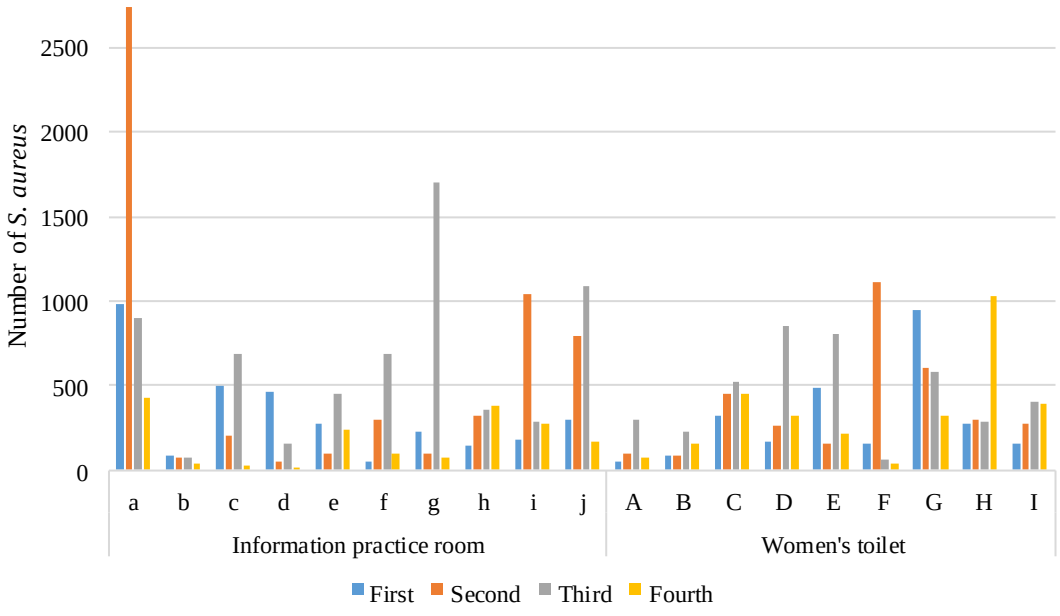

Figure No. 5: Number of *Staphylococcus aureus* detected at each location in the information practice room and women's toilet

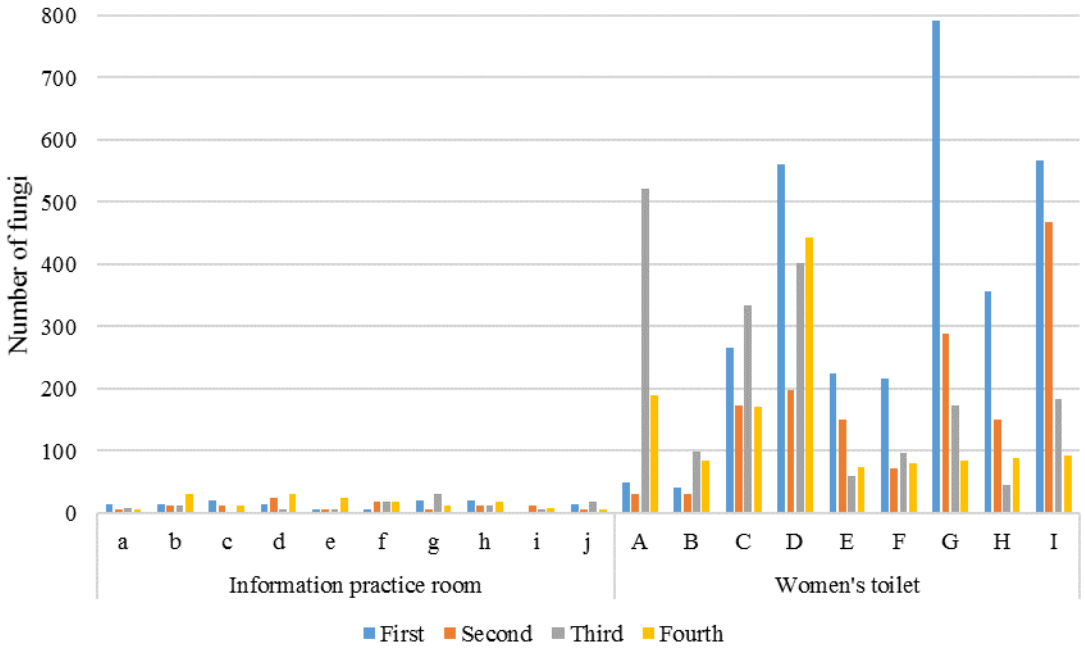

Figure No. 6: Number of fungi detected at each location in the information practice room and women's toilet