IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2023 Vol.:27, Issue:1

Survey of Microbial Contamination in the Washbasin Floor of a University Toilet

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Jun Kobayashi*[1], Ami Yano[1], Keiichi Ikeda[2]

[1]Faculty of Nutrition, University of Kochi, 2751-1 Ike, Kochi, Kochi 781-8515, Japan;

[2]Faculty of Pharmaceutical Sciences, Hokuriku University, Ho 3, Kanagawa-machi, Kanazawa, Ishikawa 920-1181, Japan.

Submitted: 21 March 2023
Accepted: 27 March 2023
Published: 30 April 2023

Keywords: Microbial contamination survey, Toilet washbasin floor, General live bacteria, *Escherichia coli*, Fungus

ABSTRACT

Infectious diseases are caused by pathogens, such as viruses and bacteria that invade and proliferate in humans, exerting various adverse effects on the body. To prevent such diseases, it is important to sterilize and remove causative microorganisms, and prevent the introduction and transmission of microorganisms from the outside. We have been investigating the microbial contamination status of indoor footwears and floors in the university. Our previous results confirmed that the degree of microbial contamination differs depending on the location. The aim of this study was to understand the microbial contamination status of the floor surface near eight toilet wash basins from the viewpoint of public health, considering the number of users. We investigated five bacterial groups (general live bacteria, fungi, *Staphylococcus aureus*, *Escherichia coli*, and coliform bacteria).

1. INTRODUCTION

Microorganisms are used to process food, manufacture pharmaceuticals, and clean the environment, benefitting people's lives[1)-3)]. However, some are known to cause infectious diseases and food poisoning, particularly opportunistic nosocomial infections caused by drug-resistant bacteria and food poisoning in food service facilities and at home. To prevent the adverse effects brought about by the infections caused by these pathogenic microorganisms, thorough disinfection of hospital equipment and proper hand hygiene are being implemented, while quality standards of tap water and cooking with heat in large quantity cooking facilities are stipulated[4),5)].

For microorganisms to grow, the required conditions that facilitate growth, such as nutrients, temperature, and moisture, are necessary. Toilets are likely to introduce organic matter in the environment, serving as a nutrient source for microorganisms. Because a large amount of water is used in the toilet bowl and wash basin, the necessary conditions microbial growth are met. Therefore, toilets are among the places where microbial contamination occurs[6)-8)]. In a previous study, several bacteria were detected at the bottom of hand-washing sinks and on drain surfaces in medical wards[9)]. In addition, resident skin and intestinal bacteria were detected in the pooled water from a hand warm air dryer in the toilet[10)]. These results suggest that the water used for washing hands is likely to be splashed; thus, it is particularly susceptible to microbial contamination. Contamination level around the wash basin is considered to be high.

Previously, we conducted a microbial contamination survey on the floor surface of the toilet entrance in a university building and found that the number of microorganisms tended to be high in toilets that are frequently used[11)]. We also conducted a survey on the microbial contamination of shared indoor footwear in the university and found that the floor surface was the source of contamination of the footwear[12)]. Therefore, a survey on the microbial contamination status on the floor surfaces of university toilets is of high importance. In this study, we investigated the microbial contamination status of eight locations on the floor under washstands, which are considered to be highly contaminated, in three toilets frequently used at K University. Furthermore, the results were compared within and between the toilets.

2. METHOD

2-1 Outline of survey target

In this experiment, four wiping surveys were conducted on June 27, August 9, September 7, and October 10, 2021, targeting the women's toilet floor on the third floor of K University. Women's toilets are installed on all floors of the building; however, those in the third floor were targeted, since these are used frequently owing to the several classrooms installed in the same floor. As shown in Table 1, the three toilets on the third floor were labeled as **A**, **B**, and **C**. Toilets A and B are located on the southwest side of the building, and toilet C is located on the northeast side. All floors are tiled, and people enter the toilets wearing outside footwears. All toilets have ventilation fans and windows but have no doors. The ventilation fan runs for 24 h a day; however, whether the windows are open is unknown. For the eight floors used for sampling, we selected the places where people stand on the front side of the edge of the wash basin, where splashing of water droplets is likely to occur. An outer frame made from a cardboard box was placed on the floor only when samples were collected, and the inside of the guide was wiped so that a floor area of 50 cm ×50 cm was used as the measurement site at any point.

Determining the number of toilet users is difficult. In addition to classrooms, the third floor has several faculty rooms, graduate student seminar rooms, and locker rooms. The primary users were assumed to be the class participants. As shown in Table 2, only the number of students from classrooms on the third floor, which were confirmed to be used for classes by the K University secretariat, were included in this study. An unspecified number of students used the classrooms where no classes were held for seminars or self-study, but the number of users was not determined.

We considered that the number of users of A304/A305, A306, and A316–A321 contributed to the usage frequency of toilets **A**/**B**, **B**, and **C**, respectively. Among these three (**A**-**C**), the number of users in the classroom near **B** was the largest. **A** was presumed to be farther away from the classrooms used in classes compared to **B**, and that the frequency of use was lower than that of **B**. Classrooms near **C** had fewer users than those near **A** and **B**. Many users of classrooms on the east side (A318–321) were assumed to be using **C**. From these facts, **C** may be used more frequently than **A**, and the expected frequency of use of the three toilet rooms were in the order of **B**>**C**>**A**. Eating and drinking are permitted in the classroom on

the east side; therefore, toilet **C** may be affected. Classes were not conducted from 10 August to 12 September 2021 due to the summer vacation period. From September 13 to September 30, classes such as intensive lectures were conducted; thus, the frequency of classroom use was low. Therefore, the frequency of toilet use was higher in the 4th time than in the 3rd experiments.

2-2 Equipment and devices

The pure water production equipment Auto Still WS200 (Yamato Scientific, Tokyo, Japan), ultrapure water filtration system RFU414BA (Advantech Toyo, Tokyo, Japan), and the automatic lab autoclave MLS-3020-PJ (Sanyo Electric Bio Medica, Tokyo, Japan) using a high-pressure steam sterilizer were used to prepare the sterilized water. A sterile cotton swab (code 06526) (Nissui Pharmaceutical, Tokyo, Japan) was used to collect microorganisms. The ultrasonic cleaner DG-1 (Iuchi, Osaka, Japan) with an oscillation frequency of 43 kHz was used for ultrasonication. The incubator MIR-154 (Sanyo Electric, Tokyo, Japan) and the clean bench ADS161SHUG (Yamato Scientific, Tokyo, Japan) were used for culturing and aseptic operations. The vortex mixer VORTEX-GENIE 2 (Scientific Industries, New York, NY, USA) was used to mix microbial suspensions. An air velocity meter (Temp/RH TM-413, Tenmars, Taipei, Taiwan) was used to measure temperature and humidity.

2-3 Medium and water

The compact dry Nissui medium kit (Nissui Pharmaceutical) was used to measure the population of general live bacteria, *E. coli* coliforms, *S. aureus*, and yeast/mold (rapid type). In this study, a yeast/mold assay kit was used for enumeration of fungi, following the manufacturer's instructions. The culture conditions were performed according to the manufacturer's instructions. General viable bacteria were cultured at 35°C for 2 days, fungi at 25°C for 3 days, and *S. aureus* and *E. coli*/coliforms at 35°C for 4 days. The microbial population were visually measured based on colonies immediately after incubation. Red colonies were considered as general live bacteria, light blue to blue colonies as *S. aureus*, blue to blue violet colonies as *E. coli*, pink to red purple colonies as coliforms, and all colors for fungi.

Ultrapure sterile water was obtained by the purification of water using pure water production equipment, followed by ultra-purification at a specific resistance value of > 18 MΩ · cm using an ultrapure water filtration system, and sterilization at 121°C for 15 min using a high-

pressure steam sterilizer. Aliquots (6 ml) were dispensed into sterile 15 ml centrifuge tubes using a micropipette in a clean bench for subsequent measurements.

2-4 Floor sampling method

The toilet floor was wiped at approximately 8:00–9:00 in the morning on all four survey days. General live bacteria, fungi, *S. aureus*, *E. coli*, and coliforms were measured. Since sampling was conducted before the start of classes, only a few people were using the toilet and were less likely to be a hindrance. At the time of sampling, we measured the temperature and humidity inside and outside the toilet (Table 3).

A sterilized cotton swab was moistened with 6 ml of sterilized water, and each measurement site was wiped with a swab in three directions: horizontal, vertical, and oblique. To prevent the results from being biased by the order of sample collection, wiping was performed in the order of **a→h** (Table 1) on the first and third days and in the order of **h→a** on the second and fourth days. The swab was then placed in a centrifuge tube with sterilized water; the swab should not be touched with bare hands (assuming all 6 ml of sterile water remained). The centrifuge tube containing the cotton swab for 5 min was sonicated and mixed using a vortex mixer on a clean bench, and then 1 ml of the suspension was seeded into each medium kit and cultured. To minimize contamination of the media components, *S. aureus*, *E. coli*/coliforms, fungi, and general live bacteria were seeded in this order. To increase the measurement accuracy, two people counted the number of colonies per kit, and the average value was defined as the number of microorganisms.

2-5 Measurement of temperature and humidity

Temperature and humidity were measured on a 75 cm-high steel rack, avoiding direct sunlight, at five locations: the center of the three toilets and the landings of the exterior stairs located in the southeast and southwest. The results are presented in Table 3. To confirm the influence of changes in temperature and humidity due to measurement time and to prevent bias in the results depending on the order of measurement, measurements were taken in the order of the outside stairs (west)→**A**→**B**→**C**→outside the stairs (east) on the first and third measurement days, while the order was reversed for the second and fourth measurements. As shown in Table 3, the outside temperature tended to be lower, and the humidity was higher than that inside the toilet. There seemed to be fewer changes in the toilet. In **B** and **C**, humidity increased from the first to the second day of sampling, and decreased from the

second to the third, and from the third to the fourth. In A, humidity decreased from the first to the second day of sampling, and a difference was observed between the toilets. In this survey, the higher the temperature, the closer the temperature was to 35°C, which is the optimal temperature for growth of general live bacteria, *S. aureus*, and *E. coli*/coliform bacteria. The humidity was highest during the period from the second to the third day of sampling.

3. RESULTS AND DISCUSSION

3-1 Difference in pollution levels among toilets

We compared the number of microorganisms detected among toilets. Fig. 1 shows the number of microorganisms detected at each location of the live bacteria. The highest number of microorganisms detected was observed in **C**. *Trichophyton* was previously isolated from the household dust of patients[13], and the amount of dust found in the centrifuge tube after sample collection tended to be greater in **C** in the first measurement. This may be due to the presence of microorganisms in the dust (data not shown). In all four measurements, the amount of dust in the centrifuge tube was considered to be the same from the second sampling day onwards, and quantification was performed visually; therefore, there was no sufficient basis for microbial population in dust.

Fig. 2 shows the number of fungi at each location, in the order of **A**>**C**>**B**, especially in **A** and **C** were significantly high. In our previous study, many fungi were detected in unused sandals, and only a few were detected in sandals that had been worn for many days[14]. Furthermore, we previously observed that places that have not been used for a long period are an optimal environment factors for fungal growth[15]. As shown in the results in Table 2, we speculate that **A** and **C** were less frequently used than **B**.

Fig. 3 shows the number of *S. aureus* detected at each location. In particular, this microorganism tended to occur in **C**. *S. aureus* is a resident skin bacterium distributed in the nasal cavity, skin, and intestinal tract of the human body[16]. As shown in Table 2, many classrooms near **C** permit eating inside. A previous study detected bacteria from the skin in contact with dust mask after use[17]. From these results, bacteria are speculated to have spread from the mask to the fingers when the mask was removed when eating, and the contamination spread to the floor through the water splashed during hand washing and the water adhered to the fingers. However, classrooms that do not permit eating, as well as teachers' rooms, may

have been used for eating meals. Therefore, determining the impact of the use these rooms before and after meals, and comparing to other toilets, are not possible.

Figs. 4 and 5 show the number of *E. coli* and coliforms detected at each location. That of **B** was particularly high, followed by **C** and **A**. Because *E. coli* exists in the human and animal intestines, it is regarded as an indicator of fecal-derived contamination. In addition to *E. coli*, coliforms include bacteria that exist in the environment, such as plants, soil, and water[16),18)]. From Table 2, **B** had the highest frequency of use, followed by **C** and **A**. These results indicate that the higher the frequency of use, the higher the chance of fecal-derived contamination, as well as environment-derived, such as soil and moisture, through footwear.

3-2 Differences in toilets

We compared the number of each microorganism in toilets. As shown in Fig. 1, in **A** and **C**, general live bacteria tended to increase from the entrance side to the back side. However, in **B**, no significant difference was observed based on location. These results suggest that in **A** and **C**, where three wash basins are available, many people prefer to use the entrance side. Meanwhile, in **B**, both wash basins are frequently used because of the large number of users.

As shown in Fig. 2, fungi tended to be less abundant in the area under the washbasin located in the center of **A** and **C**. For example, a large number of fungi were detected in dust[19)]. Dust tends to accumulate in the corners of the room; therefore, the environment under the central washbasin is less likely to accumulate dust than that under the washstands at both ends, and the fungus contained in the dust is less affected than the areas at both ends. However, because the amount of dust was only visually observed (results are not shown), the tendency of less dust at both ends was not confirmed, and no sufficient evidence is available.

As shown in Fig. 3, *S. aureus* tended to be abundant on the entrance side in **A** and **C**. However, in **C**, it was also detected in large numbers on the back side. We speculate that this may be due to the difference in the state of the hands and fingers of toilet users and that of bacterial carriage. A large amount of *S. aureus* was detected in the hands of people who tended to have rough hands[20)], and individual differences in the tendency of nurses working in neurosurgery wards were observed, which in turn transmit MRSA in their nasal cavities[21)].

As shown in Figs. 4 and 5, *E. coli* and coliforms tended to be abundant in the inner parts of **A** and **B**. However, in **C**, they were detected in large amounts in the central part. We speculate

that the frequency of fecal- and environment-derived contamination through footwear was affected by the use of the toilet and the state of footwear of each user.

4. CONCLUSION

Different trends in microbial species were observed when the toilets were compared. Generally, the abundance of live bacteria and *S. aureus* were high in **C**, that of fungi in **A** and **C**, and that of *E. coli* and coliforms in **B**. When the insides of the toilet were compared, the number of general live bacteria tended to be higher at the entrance side, and the number of fungi was lower under the washstand located in the center. *S. aureus* and *E. coli*/coliforms did not show a clear trend in each toilet. Furthermore, when the sampling dates were compared, the abundance of general live bacteria is positively correlated with temperature. Additionally, the number of detected microorganisms increased as the frequency of use increased. The number of fungi was positively correlated with humidity.

We investigated the floor surface under the wash basin in three toilets and found similar and different contamination status between and inside the toilets. Thus, the investigation of the contamination status of the floor under the window and around the toilet bowl could not be done in the present study. Furthermore, of the distribution of contamination distribution should be examined in one toilet.

5. ACKNOWLEDGEMENT

We would like to express our gratitude to Assistant Professor Koyama, a faculty member of the Department of Nutrition, Faculty of Nutrition, University of Kochi, for reviewing this manuscript. We would also like to express our sincere gratitude to Mr. Fujita of the Financial Facilities Division, University of Kochi; and Ms. Okubo of the Educational Affairs Division, Student Support Division, University of Kochi, for their cooperation in understanding the usage status of ventilation systems and classrooms.

6. REFERENCES

1) Eiji Emoto. (2013) Flavors produced by lactic acid bacteria and those utilization. Japanese Journal of Lactic Acid Bacteria, 24, 71-78.
2) Satoshi Soda. (2019) Degradation and transformation of dioxins and dioxin-like compounds in bioremediation technology. Material Cycles and Waste Management Research, 30, 194-200.
3) Junji Sakamoto. (2008) Microbiology -Protecting the Earth and Health-. Shokabo, pp.137-144,149-157,172-177.

4) Ministry of Health, Labour and Welfare. Ministerial Ordinance on Water Quality Standards (Ministry of Health, Labor and Welfare Ordinance No. 101, May 30, 2003), Ministry of Health, Labor and Welfare website, https://www.mhlw.go.jp/web/t_doc?dataId=79aa4934&dataType=0&pageNo=1 (browsed November 2021).
5) Ministry of Health, Labour and Welfare. Revision of the "Manual for Hygiene Management of Large Quantity Cooking Facilities". Ministry of Health, Labor and Welfare website, https://www.mhlw.go.jp/file/06-Seisakujouhou-11130500-Shokuhinanzenbu/0000168026.pdf (browsed October 2021).
6) Shigehiko Ito, Yuko Nakagawa, Hiroko Minami, Osamu Hashimoto, Yasuko Horie, Miki Hiwatashi, Yukiko Moronaga, Kazuyo Motoishi, Hatsumi Taniguchi, Tetsuro Matsumoto. (2016) Relationship of frequency of wiping with disinfectant and decrease in bacteria on environmental surfaces in general wards. Japanese Journal of Infection Prevention and Control, 31, 165-172.
7) Yoshiki Ishida, Rahul Vyas, Garima Chauhan, Liliana Delgado, Hiroki Kourai. (2021) Bacterial contamination analysis of residential toilet room environment and delivery of residual bacterial growth prevention efficacy on residential toilet permeable surfaces using toilet air freshener product. Biocontrol Science, 26, 85-93.
8) Gilberto E. Flores, Scott T. Bates, Dan Knights, Christian L. Lauber, Jesse Stombaugh, Rob Knight, Noah Fierer. (2011) Microbial biogeography of public restroom surfaces. PLoS ONE, 6, e28132.
9) Miho Uchida. (2019) Effects of drying on the amount of contaminated bacteria of the hand-washing sink. Journal of Healthcare-associated Infections, 12, 15-24.
10) Satoshi Kimura, Hisako Aizawa, Tomoko Masuyama, Emiko Nakama. (2009) Bacterial contamination of electric air hand dryer -A study in a Japanese university hospital. Japanese Journal of Infection Prevention and Control, 24, 21-26.
11) Jun Kobayashi, Mayuko Takarabe, Keiichi Ikeda. (2020) Survey of microbial contamination on the floor of university building. International Journal of Pharmacy & Pharmaceutical Research, 18, 295-309.
12) Jun Kobayashi, Yoshino Chikamori, Keiichi Ikeda, Hideo Sugiyama. (2019) Survey of microbial contamination in shared indoor footwear at university. International Journal of Pharmacy & Pharmaceutical Research, 16, 303-315.
13) Rie Sugimoto, Takuroh Katoh, Kiyoshi Nishioka. (1995) Dermatophyte infection of plural family members detected by isolation of dermatophytes from house dust. Nippon Ishinkin Gakkai Zasshi, 36, 291-295.
14) Jun Kobayashi, Aika Tanaka, Keiichi Ikeda. (2021) Study on the microbial contamination of sandals used on campus. Bulletin of University of Kochi, Series of Faculty of Nutrition, 70, 1-7.
15) Kosuke Takatori. (2005) Ecological and mycological review on the fungi in dwelling environments. Japanese Journal of Allergology, 54, 531-535.
16) Shoji Yamazaki. (2002) Color Atlas Environmental microbes. Ohm, pp.12-13,30-31.
17) Mitsuo Hinoue, Yuka Simada, Sumiyo Ishimatsu, Toru Ishidao, Yukiko Fueda, Hajime Hori. (2014) Bacterial contamination after use and assessment of countermeasures a dust mask facepiece. Sangyo Eiseigaku Zasshi, 56, 237-244.
18) Food Processing Research Institute, Hilly and Mountainous Agriculture and Livestock Division, Fukui Prefecture. Food processing Q&A. Fukui Prefecture Homepage, https://www.pref.fukui.lg.jp/doc/021115/qa12.html (browsed November 2021).
19) Ayako Ishiguro, Matsuko Doi. (1994) Improvement of indoor environment for children with asthma -From the survey of fungal flora in the air and dust. Journal of Japanese Society of Pediatric Nursing, 3, 108-113.
20) Hideo Miura, Kazuo Ishida. (1994) Distribution and antibiotic resistance of Staphylococcus aureus isolated from healthy human bodies. Journal of Nagoya Bunri College, 19, 53-57.
21) Tomoko Matsuo, Keiko Oshima, Eiko Shigetomi, Michiyo Nojima, Noriyuki Murakami, Kenji Kono. (1994) Detection of methicillin-resistant Staphylococcus aureus from patients and personnel in a neurosurgery ward. Japanese Journal of Infection Prevention and Control, 9, 1-5.

Table 1 Details of survey points in each toilet

Survey floor	Toilet type and location	Position in toilet	Presence of handrails
a	**A** (Southwest side)	Entrance side	+
b		Center	-
c		Back	-
d	**B** (Southwest)	Entrance	+
e		Back	-
f	**C** (Northeast)	Entrance	+
g		Center	-
h		Back	-

Two women's toilets (**A** and **B**) are installed on the southwest side, and **A** had only women's toilets.

Table 2 Number of students who used each classroom on the 3rd floor for classes

Classroom used for classes		A304/A305	A306	A316	A318	A319	A320	A321
Direction of classroom		Southwest		North	Northeast			
Usage permit for meals		-			+			
Toilets believed to have been used by classroom users		**A**, **B**	**B**	**C**				
Number of users	23 May–26 June	1875	3735	73	20	0	0	535
	28 June–8 August	1709	3384	73	20	0	0	526
	10 August–6 September	0	0	0	0	0	0	0
	8 September–9 October	707	879	0	154	120	21	21

Absences from classes and users outside of classes are not reflected.

A304 and A305 were observed together because the two rooms are connected and only a partition divides them.

Since the measurements were taken at intervals of approximately four to five weeks, the number of students using the classrooms before the first measurement was taken as the number of users during the five weeks before the measurement.

Table 3 Temperature and humidity on the day of sampling

Measuring date	Weather between 8-9 am	Measurement location	Order of measurement	Temperature (℃)	Humidity (%)
27 June	Rain	**A**	2	24.0	74.0
		B	3	25.1	70.2
		C	4	24.6	71.9
		Outside stairs (west)	1	21.6	82.5
		Outside stairs (east)	5	21.5	84.1
9 August	Cloudy	**A**	4	31.3	67.6
		B	3	29.5	73.9
		C	2	29.1	74.6
		Outside stairs (west)	5	29.5	67.8
		Outside stairs (east)	1	27.6	75.3
7 September	Sunny	**A**	2	31.8	54.6
		B	3	28.8	62.3
		C	4	28.2	64.3
		Outside stairs (west)	1	27.4	64.5
		Outside stairs (east)	5	27.9	62.9
10 October	Sunny	**A**	4	28.6	58.1
		B	3	27.3	61.4
		C	2	27.1	61.5
		Outside stairs (west)	5	29.6	52.4
		Outside stairs (east)	1	24.174.0	69.5

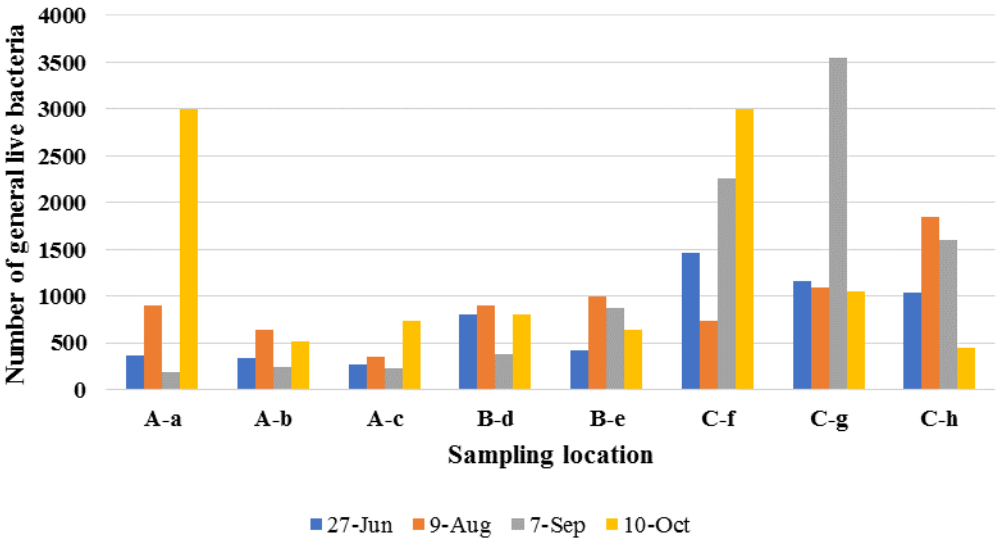

Fig. 1 Number of general live bacteria detected at each location

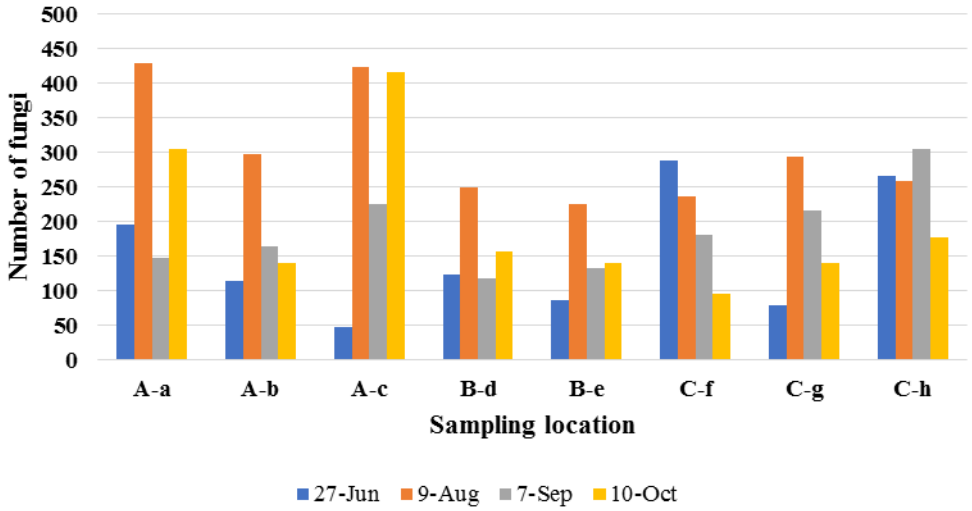

Fig. 2 Number of fungi detected at each location

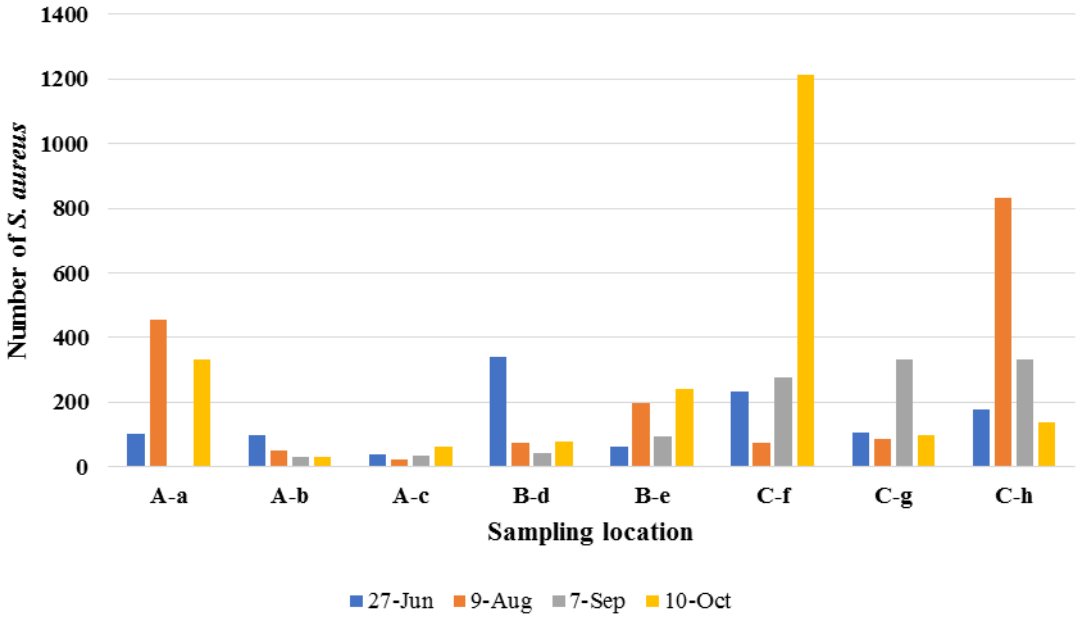

Fig. 3 Number of *Staphylococcus aureus* detected at each location

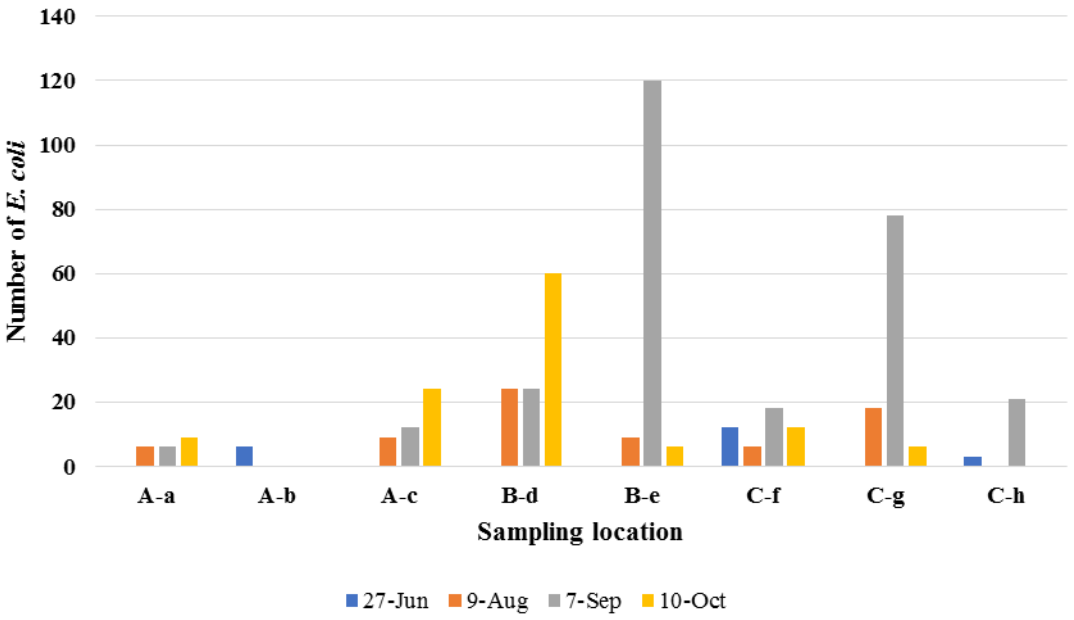

Fig. 4 Number of *Escherichia coli* detected at each location

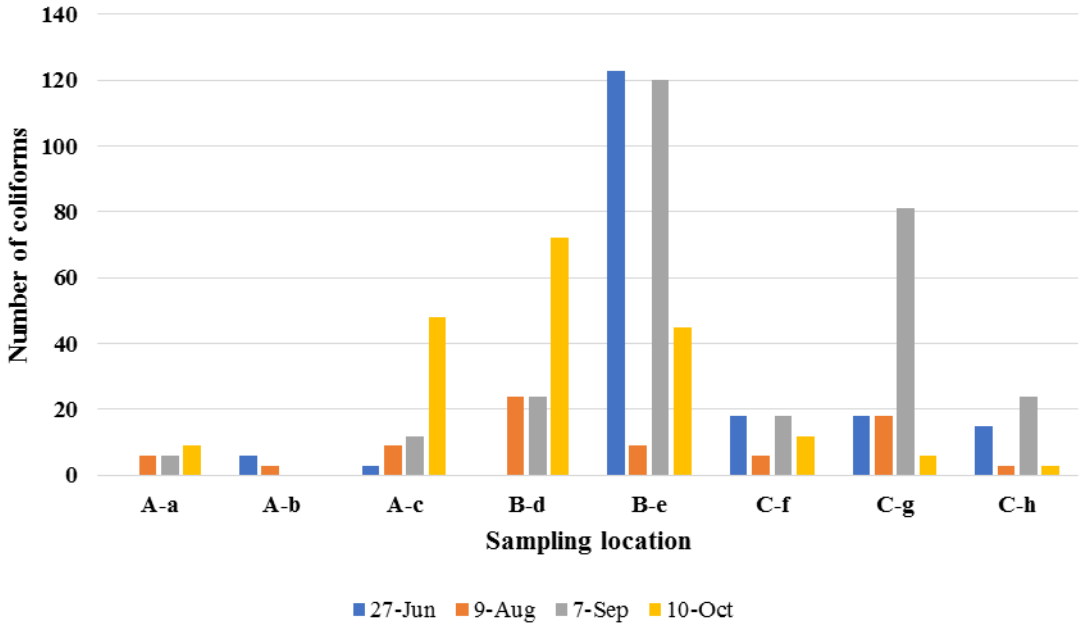

Fig. 5 Number of coliforms detected at each location