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

Human Journals
Research Article
August 2019 Vol.:16, Issue:1

Survey of Microbial Contamination in Shared Indoor Footwear at University

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

Jun Kobayashi*[1], Yoshino Chikamori[1], Keiichi Ikeda[2], Hideo Sugiyama[3]

[1]Faculty of Nutrition, University of Kochi; 2751-1 Ike, Kochi, Kochi 781-8515, Japan; [2]Faculty of Pharmaceutical Sciences, Hokuriku University, 3 Ho, Kanagawa-machi, Kanazawa, Ishikawa 920-1181, Japan; [3]Department of Environmental Health, National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 251-0197, Japan.

Submission: 28 July 2019
Accepted: 2 August 2019
Published: 30 August 2019

Keywords: Microbial contamination survey, University classroom, General live bacteria, *Escherichia coli*, Fungus

ABSTRACT

To prevent infectious diseases and food poisoning, it is important to not only sterilize and eliminate causative viruses and bacteria but also to prevent the transfer of microorganisms attached to surfaces. We sought to identify the state of microbial contamination in shared footwear (slippers, sandals) used at university in Japan. We conducted a survey of contamination levels among common indoor footwear used in three classrooms of K University. The degree of contamination of indoor shared footwear was higher in rooms used more frequently and in footwear that was used more frequently. The high level of contamination in the cooking practice room was considered to be because almost all footwear was used during class, such that the frequency of footwear use was high. Usage times of the rooms, the presence or absence of dust, and structural differences in the types of footwear also influenced microbial contamination levels. The degree of contamination decreased with each subsequent survey. Thus, not only the frequency of room and footwear use but also conditions of temperature and humidity and conditions of the wearers' feet (perspiration level, type of socks, and so on) may affect the number of microorganisms present in shared footwear.

INTRODUCTION

In hospitals and nursing facilities where nosocomial infections are a major problem, many surveys of microbial contamination are conducted for various environments, places, and objects. There are many published reports of microbial contamination on beds and shelves, toys, doorknobs, nurse call buttons, and so on,[1,2] as well as investigations of free-floating microorganisms in the rooms of hospitalized patients.[3,4] To prevent the spread of infectious diseases caused by microorganisms, it is considered important to not only sterilize and eradicate the causative bacteria and viruses but also to prevent the spread of microorganisms and their introduction from the outside. There are also reports of methicillin-resistant *Staphylococcus aureus* (MRSA) found on slippers used in pediatric clinics,[1] and trichophytes have been isolated from slippers used by outpatients.[5] Footwear is an important factor that may mediate microorganisms inside and outside of health facilities. Previous studies have reported that trichophytes have been isolated from airborne substances and vacuum cleaner dust in the home,[6,7] skin filamentous fungi adhere to the foot soles of users of public pools and public baths,[8] and dermatophytes can adhere to the feet, depending on the type of socks used.[9] Microorganism possessed by an individual can easily spread and attach via the floor of public facilities or via shared footwear. As a result, there may be a risk of food poisoning in meal provision facilities or restaurants as well as a risk to the health of users of public facilities, such as hospitals, owing to contaminated footwear.

Prior research focusing on the investigation of indoor free-floating microorganisms has been conducted at educational institutions such as universities; however, those studies examining footwear contamination have only been conducted in hospitals. Therefore, we investigated indoor footwear (slippers, sandals) routinely used at universities, to determine the state of microbial contamination from a public health perspective. Shared footwear is used by students and teachers during classes at university, but this footwear is cleaned infrequently. In this study, we examined the contamination levels of *Escherichia coli*, coliform bacteria, general live bacteria, and fungi (including molds and yeasts) on shared footwear provided in university classrooms where changing from outdoor to indoor footwear is required.

MATERIALS AND METHODS

Equipment

To detect microorganisms, we used the Compactdry Nissui kit (Nissui Pharmaceutical, Tokyo, Japan). After confirming the conditions of use with the manufacturer, we also used a Compactdry YMR kit for yeast and mold measurement in this study. Sterile cotton swabs from Nissui Pharmaceutical were used for sampling microorganisms.

Sterile water used in the experiment was prepared as follows. First, purified water was produced by distillation (Auto Still WS 200; Yamato Scientific, Tokyo, Japan). This water was then treated with an ultrapure water filtration system (RFU414BA; Advantec Toyo, Tokyo, Japan) to yield ultrapure water with a specific resistance value of 18 MΩ × cm or more. This ultrapure water was subjected to high-pressure steam sterilization using an automatic laboratory autoclave (MLS-3030-PJ; Sanyo Electric Bio Medica, Tokyo, Japan). An ultrasonic cleaner (DG-1; Iuchi, Osaka, Japan) with an oscillation frequency of 43 kHz was used for ultrasonication. Microorganisms were cultured in an incubator (MIR-154; Sanyo Electric, Tokyo, Japan); fungi were incubated at 25°C and the remainder at 35°C.

Survey target

We collected 12 samples each of indoor slippers or sandals from a shoebox in the information practice room (A), school lunch practice room (B), and cooking practice room (C) of K University (A, slippers made of vinyl chloride; B, open-toed sandals made of vinyl chloride; C, slippers made of synthetic rubber). An overview is shown in Table 1. There was no information regarding whether the collected footwear had been treated with antimicrobial products. From room A, we selected six "new" and six "old" samples (new, labeled number 1–6; old, 7–12). From the B room, we selected S (numbered 13–18) and M (19–24) sized footwear samples; samples were S and M sizes, which are the most frequently used. From room C, we selected 12 samples (labeled number 25–36). We selected the righth and slipper or sandal when right and left could be discriminated; footwear in which right and left were indistinguishable were randomly sampled. The numbered vinyl tape was affixed to the bottom of the footwear samples or on the surface contacting the back of the foot, to identify each sample. Because the tape attached to samples from room A had peeled off, these could not be identified in the second sampling round. A new sample from room A was then

randomly selected as the reference for each experiment.

Microbial sampling and measurement method

We took microorganism samples in the early morning, as follows: the first sampling was on September 3, 2018 (temperature: minimum 21.3°C, maximum 31.9°C; humidity: minimum 54%, average 77%), the second was on October 1 (temperature: 17.0–27.4°C, humidity: 26%; 54%), and the third sampling was October 28 (temperature: 9.6–23.2°C, humidity: 25%, average 61%). We considered that about 3 weeks had passed since the university summer vacation period on September 3 and that the sampled footwear had only been used during the first semester. October 1 was the start date of the second semester period, and we assumed that no footwear was used during the summer vacation period. About 3 weeks after the start of the second semester on October 28, we considered that the footwear was used again. In this study, we set the date of sampling to conduct experiments at equal intervals.

A sterile cotton swab was moistened with 5 mL of sterile water, which had been aliquoted into sterile 15 mL centrifuge tubes, and the entire inside of each footwear sample (at places where the foot would be in contact with the footwear) was wiped three times. The cotton swab was placed in a centrifuge tube filled with sterile water, taking care not to touch the swab with bare hands. At this time, it was assumed that all 5 mL of sterile water remained in the swab. Footwear samples were then wiped with a paper towel and returned to the original shoe box, where they were left until the next experiment.

Each centrifuge tube was subjected to ultrasonic treatment for 5 minutes, and immediately after vortexing inside a clean bench, 1 mL of each sample suspension was seeded onto each kit and cultured. The culture conditions were according to the manufacturer's instructions. *E. coli* and coliform bacteria were cultured at 35°C for 4 days; general live bacteria at 35°C for 2 days, and fungus at 25°C for 3 days. The number of microorganisms was assessed immediately after culture. To increase measurement accuracy, two people counted the number of colonies per kit, and the average value was defined as the number of microorganisms. In this study, the area of the footwear was not considered.

Classrooms

An outline of the three classrooms is given in Table 1. The use time of each classroom was calculated based on the regulations of the university; depending on the class content, the

times could vary.

The A room was used 15 hours a week for late lessons. However, except for class time, it remained open for self-study and at times, nearly 40 people used the room; We could not determine the number of users for each date and time. The footwear in room A was the same as slippers used in hospitals. Many students do not use the footwear in this room because is it carpeted and changing footwear is not required. The footwear in room A was never cleaned and was only replaced every few years. As of 2019, the experimental footwear had been discarded and replaced.

The B room is used for 1.5 hours a week. The footwear was the same as the sandals often used in toilets. Users change into sandals in the corridor outside the classroom and enter the room. This footwear is to be worn when eating and is replaced with another set of footwear at the location where cooking takes place. In addition to class time, 6 to 8 people use this footwear daily; in exceptional cases, the footwear may be used in group and other activities. The footwear is cleaned twice a year when only the bottom (the surface that touches the ground) is wiped with disinfecting alcohol. The same sandals have been used for as long as the authors are aware (at least a few years).

The C room is used for 7 hours a week. The slippers were similar to those used in the A room, but they had a lot of surface irregularities and were slightly harder. Almost all slippers are used during class meeting times. This room may be used for group activities, among other things, but unlike rooms A and B, footwear is not always used because the room entrance is locked. Cleaning is conducted twice a year by students during class. After applying a mild detergent with a shoe brush and scrubbing the whole slipper, it is washed with tap water and air-dried. However, these slippers have also been used for several years (or as long as the author can remember). From 2019, the policy has changed to permit individuals to wear their footwear.

RESULTS AND DISCUSSION

Comparison between classrooms

Figure 1 shows the number of footwear samples in which microorganisms were detected. *E. coli* and coliform bacteria tended to be less frequent in room A and more frequent in room C. General live bacteria were detected in all footwear, and no difference was found among the

classrooms concerning fungi. Figures 2 to 5 show the number of microorganisms detected for each footwear sample. The number of microorganisms in room C was the largest for all species. Footwear from room B tended to be more frequently contaminated, but the contamination in footwear from all three rooms varied and was sometimes the same, depending on the sampling date and the microorganism species.

A large number of microorganisms in samples from room C was considered to be because the chance of contact with water and food materials used during cooking was higher than in rooms A and B. A is a room predominantly for computer use and B is a front room used for cooking. Before the experiment, we assumed that the number of microorganisms in room A was large, but the observed difference as compared with rooms B and C was larger than expected. Despite having the lowest frequency of use, the factor that increased the degree of contamination in C was that shared footwear was used more frequently in C than in other rooms. One reason for this is that most of the provided slippers are used during each class. Also, dust contamination was observed more frequently for room C than for the other classrooms (data not shown). Previous studies have reported skin mold fungus isolated from household vacuum dust,[8] so the presence or absence of this dust may influence the degree of contamination. Furthermore, the shape of footwear, with fine irregularities on the surface, is also a consideration.[5] In-room C, footwear was often worn continuously for about 2 hours, so it is possible that moisture could easily build up inside the footwear, where microorganisms can easily survive.

The low degree of contamination of footwear from room A is considered to mostly be related to the low frequency of use of the slippers. As mentioned, many people do not wear these slippers because there is no requirement to do so; small classes are also held in this room. Also, it is conceivable that some people remove their footwear while using a computer, even though they initially changed into the shared slippers. It has been shown in previous research that trichophyte spores spread and adhere to tatami mats, concrete floors, and cushions.[10] In-room A, with carpeted floors, a survey of microbial contamination should be conducted not only of shared footwear but also of floor contamination.

In sandals from room B, only a small amount of dust contamination at the time of sampling was observed. Not all footwear is used in each classroom, and good breathability is related to low contamination levels.

Comparison by sampling date

It has been reported that when culturing trichophytes from vacuum cleaner dust kept indoors at room temperature, most die in about 1 month.[8)] It has been thought that the usage and degree of contamination by microorganisms are related. Therefore, we predicted a tendency for the number of microorganisms to generally decrease by the second sampling period, as we expected a lower frequency of room use than during the first and third sampling times and a longer period of non-use. However, for footwear samples in which we detected microorganisms, only *E. coli* in footwear from rooms A and B, coliforms in samples from all locations, and fungi from footwear samples in room C decreased between the first and second sampling times. Coliforms in footwear from rooms B and C and *E. coli* and fungi from all classrooms decreased between the second and third sampling times.

When comparing the number of microorganisms in individual footwear among all three surveys, we could not confirm the change owing to the date of sampling for each sample as the identifying numbers taped to the samples had peeled off in footwear from room A. Therefore, in this experiment, changes were observed only for footwear that could be identified. General live bacteria showed a tendency to decrease by the second sampling and were significantly increased at the third sampling time. In the other footwear samples, the changes were different, with no observable tendencies between each day of sampling.

As shown in Figs. 2–5, similar to *E. coli*, there was a tendency to detect a large number of microorganisms in the first sampling for coliform bacteria from room B; we observed a slight similar tendency for fungus, which then decreased. For general live bacteria, more samples showed decreased numbers of microorganisms at the second sampling than the first and third sampling times.

In the case of *E. coli* and coliform bacteria from room C, microbial contamination at the second sampling time was increased by half for all samples. No particular trend was observed for general live bacteria. Although the change varied, as in general live bacteria, the overall number of microorganisms tended to decrease between the first and the third sampling times.

The influence of temperature and humidity can be considered factors that resulted in an overall downward trend from the first to the third sampling times. Because shared footwear is stored indoors, the daily fluctuation of temperature and humidity is smaller than outdoor

temperature and humidity, but the effects remain considerable. The temperature declined over the survey period. Average humidity on the first day of the survey was higher than in the second or third days, but both the average and the minimum humidity decreased if the average was taken 3 days before and 3 days after the survey day (total 7 days; data not shown). Therefore, it is possible that our results reflected the gradual change to environmental conditions in which it is difficult for microorganisms to survive.

The condition of the wearer's feet when wearing indoor footwear is another influencing factor. It is hot and steamy in early September, and feet perspire easily. It can be expected that many people use lightweight socks in the summer. It has been reported that thick, heavy socks and *tabi* (Japanese socks with a split toe) are useful for preventing the adhesion of microorganisms from the foot, whereas nylon stockings and cotton socks are not effective.[9] From these findings, microorganisms, as well as nutrient substances from the foot, can easily become attached to footwear.

The absence of a common trend among all footwear samples from the first to third sampling times may be attributed to effects owing to the condition of the wearers' feet and the interval from the last wear date to the sampling date.

CONCLUSION

Our results indicated that the degree of contamination in shared footwear used in university classrooms was higher in rooms C > B > A. The high contamination level in room C reflected the higher rates of shared footwear use than in other classrooms because almost all slippers are used during class. Room use times and structural differences in footwear also affected microbial contamination levels.

Across sampling days, more footwear samples showed decreased contamination levels. Because the temperature and humidity gradually had decreased with each subsequent sampling day, the change in microorganism counts may be related to the change in conditions of temperature and humidity as well as conditions of the wearers' feet (perspiration, type of socks, and so on). Further investigation of the contamination level of shared footwear should be conducted, including an examination of unused footwear and floor cleaning procedures.

ACKNOWLEDGMENTS

In this research, we would like to express our deep appreciation to three participants in their fourth year of Heisei fiscal year, Health Ecology Laboratory, Faculty of Nutrition, University of Kochi, who cooperated with the survey.

REFERENCES

1) Akiko Obayashi. Microbial contamination on environmental surfaces and infant toys in pediatric outpatient facilities and preventive measures. J. Environ. Infection 2013: 28: 142-146.

2) Masayuki Ogata, Miki Iijima, Miho Matsubara, Hitomi Tsutsumi, Satoshi Hori, Shin-ichi Tanabe. Environmental surface contamination examined by ATP assay before and after terminal room cleaning inpatient rooms. J. Environ. Eng. AIJ 2016: 81: 723-729.

3) U Yanagi, Koichi Ikeda, Naoki Kagi, Naoya Nishimura, Hiroshi Yoshino. Field measurements on indoor airborne bacteria and fungi in nine hospital waiting rooms. Proceedings of the Conference on Heating, Air-conditioning Sanitary Engineers 2007: 1371-1374.

4) U Yanagi. Microbe environment and healthy influence of elderly-people dwellings. J. Natl. Inst. Public Health 2017: 66: 136-140.

5) Machiko Fujihiro, Kanao Noda, Hiroko Takahashi, Shouichi Mori. Isolation of dermatophytes forms slippers for outpatients. Jpn J. Infect. Prev. Cont. 1989: 4: 9-13.

6) Ryuji Maruyama, Kunitaro Fukuyama, Takuro Katoh, Rie Sugimoto, Hiroko Taniguchi, Kyoko Watanabe, Kiyoshi Nishioka. Prevention of dermatophytoses. Jpn J. Med. Mycol. 2003: 44: 265-268.

7) Yuji Kawakami, Kazuhiro Hashimoto, Yuma Fukutomi, Masami Taniguchi, Akemi Saito, Kazuo Akiyama. A survey on the distribution of booklice, other allergenic arthropods, and fungi in houses in Tokyo. Urban Pest Manag. 2014: 4: 65-77.

8) Takuro Katoh, Kyoko Kimura, Hiroko Taniguchi, Rhuji Maruyama, Kiyoshi Nishioka. Isolation of dermatophytes by the foot-press method from several, male and female volunteers after bathing in four public baths. Jpn J. Med. Mycol. 1996: 37: 223-227.

9) Kyoko Watanabe, Hiroko Taniguchi, Kiyoshi Nishioka, Ryuji Maruyama, Takuro Katoh. Preventive effects of various socks against adhesion of dermatophytes to healthy feet. Jpn J. Med. Mycol. 2000: 41: 183-186.

10) Takuro Katoh. Dermatomycosis and environment. Jpn J. Med. Mycol., 2006: 47: 63-67.

Table No. 1 Class usage time for each room and features of footwear

Classroom / Item		Information practice room (A)	School lunch practice room (B)	Cooking practice room (C)
Class time	Previous period	17:15	4:30	2:15
	Late	15:00	1:30	6:45
Footwear shape		Covers toe No irregularities	Only covers instep Large irregularities on sampling surface	Covers toe Small irregularities on sampling surface
Footwear material		Vinyl chloride	Vinyl chloride	Synthetic rubber
Footwear No.		New, 1–6; Old, 7–12	S, 13-–8; M, 19–24	25–36
Cleaning		None	Alcohol disinfection of the surface touching the ground, twice a year (by students)	Twice a year (by students)
Floor		Carpet	Linoleum	Linoleum
Storage (shoebox)		Near room entrance	Hallway in front of the classroom	Near room entrance

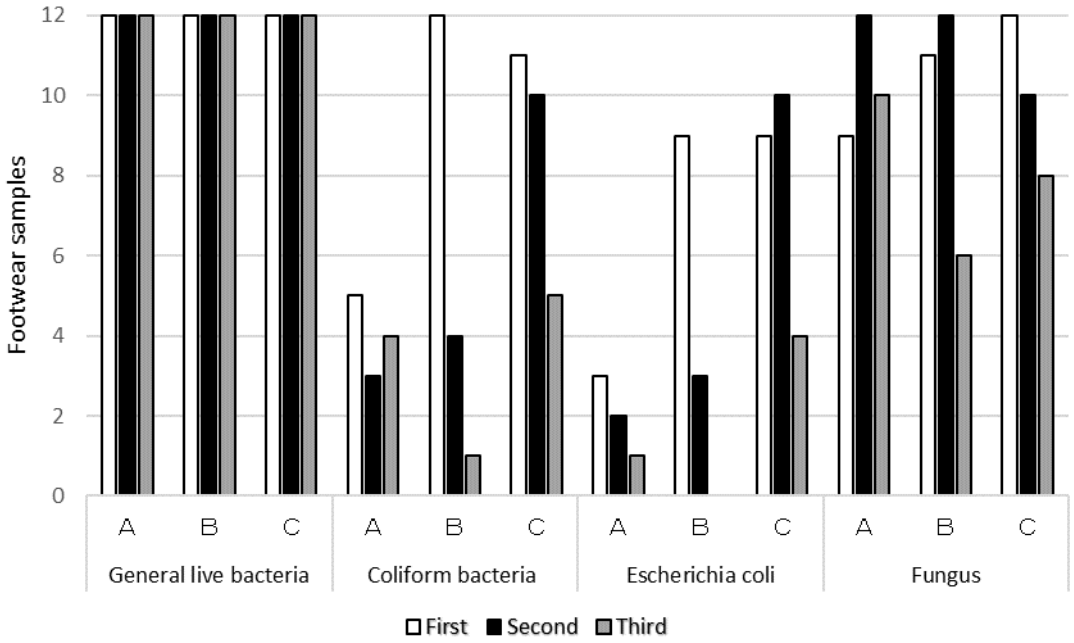

Fig. No. 1 Number of footwear samples in which microorganisms were detected. Twelve samples were collected from each room. First, Second and Third refer to the sampling times. An Information practice room; B, School lunch practice room; C, Cooking practice room.

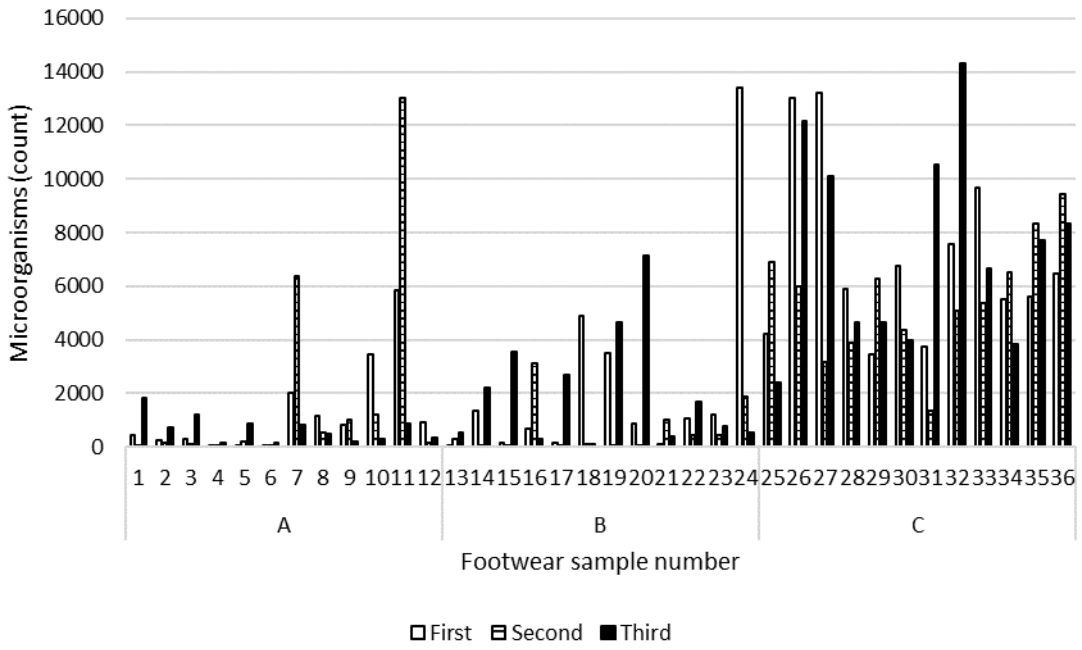

Fig. No. 2 General live bacteria counts in each footwear sample. First, Second and Third refer to the sampling times. An Information practice room; B, School lunch practice room; C, Cooking practice room.

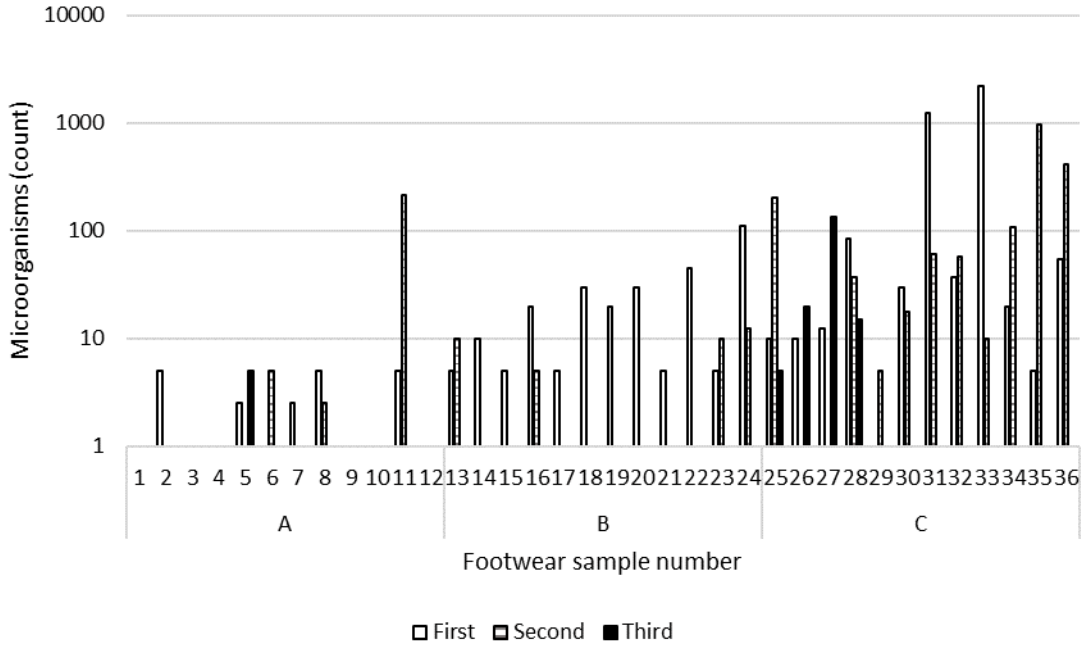

Fig. No. 3 Several coliforms detected in each footwear sample. First, Second and Third refer to the sampling times. An Information practice room; B, School lunch practice room; C, Cooking practice room.

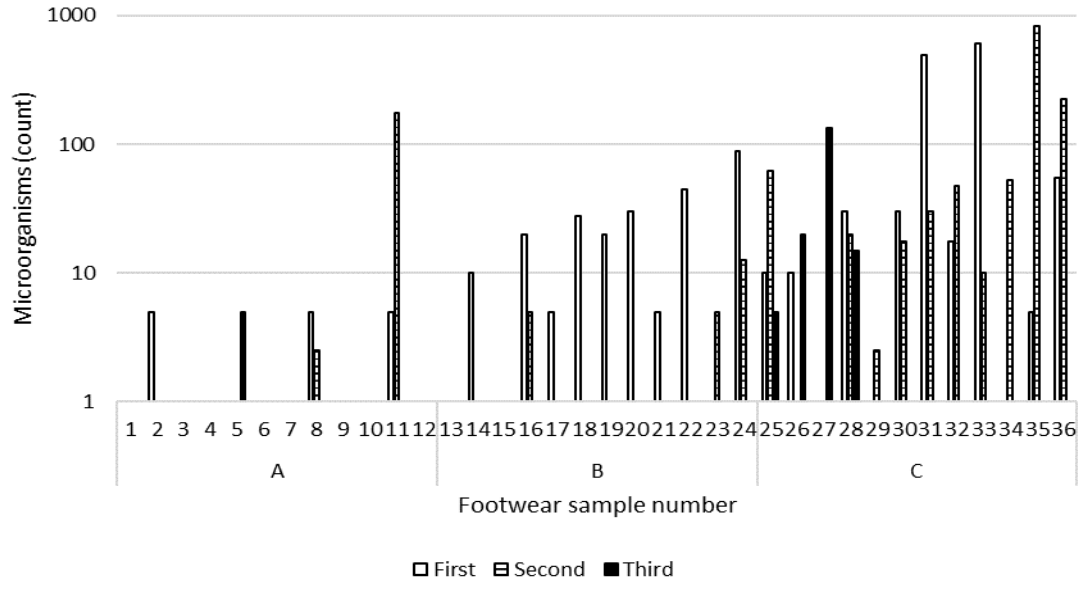

Fig. No. 4 *Escherichia coli* detected in each footwear sample. First, Second and Third refer to the sampling times. An Information practice room; B, School lunch practice room; C, Cooking practice room.

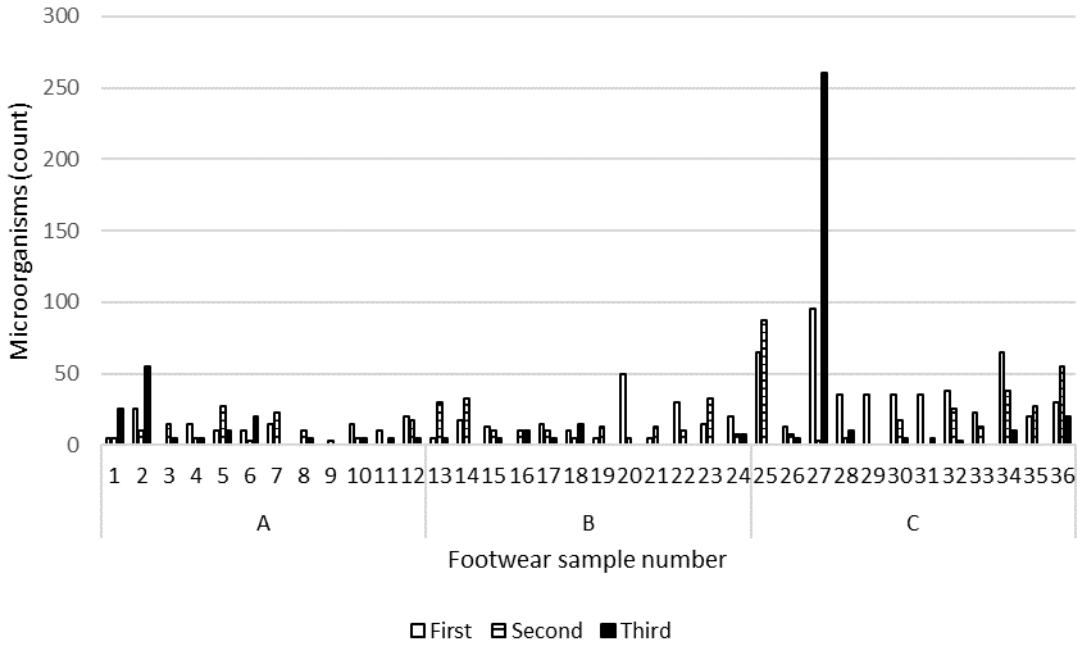

Fig. No. 5 Fungus detected in each footwear sample. First, Second and Third refer to the sampling times. An Information practice room; B, School lunch practice room; C, Cooking practice room.