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Evaluation of bacterial contamination on three different surfaces within domestic refrigerators

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Abstract

The objectives of this study were to analyze the number of microorganisms, fungal composition and the correlation between bacterial enrichment and air quality on three internal surfaces (the inner wall, shelf, and basket) of domestic refrigerators. The results showed that the inner wall had a significantly lower number of coliforms ($P < 0.05$), and the range was 0.2–2.5 log MPN cm^{-2}. The total bacterial counts and psychrophilic bacterial counts on three internal surfaces in the same refrigerator tended to be consistent. Moreover, the inner wall owned a simpler bacterial community structure. At the genus level of fungi, the dominant flora of both the inner wall and shelf were Saccharomyces spp. and Candida spp., while Saccharomyces spp., Candida spp. and Fistulina spp. took superiority in the basket. Specifically, Shannon index and Simpson index, which represent the bacterial community diversity, were the lowest on the wall, and six bacterial species on the inner wall had relative abundance higher than 0.5% of the total operational taxonomic units (OTUs), while for the shelf and basket, there were 12 and 11 bacterial species respectively. Also, there was a significant negative correlation in the basket between the chao1 index and $PM_{2.5}$. This study could provide guidance for the sanitation and recommend adequate packaging of foods stored in refrigerators.

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INTRODUCTION

Domestic refrigerators are one of the essential electrical appliances and play an important role in keeping food fresh and preventing food spoilage[1−3], but domestic refrigerators have the potential to cause food contamination if they are not managed well[4,5]. Bacterial contamination from unwashed raw foods, leaking packages, hands etc. introduced to domestic refrigerators may directly contaminate other stored foods, or attach to and persist on the internal surface of the refrigerator posing risks of indirect contamination during food preparation activities[6]. Moreover, the improper use of domestic refrigerators will greatly pose a risk of bacterial contamination of the food[1,7,8]. Therefore, the determination of bacterial contamination inside domestic refrigerators has practical and important significance for food storage[7].

The surfaces within domestic refrigerators are a major source of bacterial contamination due to the biofilms that bacteria may form on them[9]. The internal surface of domestic refrigerators usually created an unfavorable environment for many harmful bacteria but most of them are still capable of growing at low temperatures[5,10,11]. It is generally observed that different internal surfaces of domestic refrigerators have different features such as the frequency of contact with food and types of food stored. Some studies had considered that bacterial contamination on different surfaces within domestic refrigerators may be different. Currently, some research regarding contamination of different surfaces within domestic refrigerators have been published[4,10,12], but the detection methods were traditional culture-dependent technology, which can only provide the incidence of some target bacteria. For example, Otu-Bassey reported the frequency of bacterial isolates (such as *Staphylococcus aureus, Salmonella typhi,* etc.) on different surfaces within refrigerators using the culture-isolation-identification method[4]. However, comprehensively understanding bacterial communities in terms of their diversity and composition on different internal surfaces is of great significance to direct the management of domestic refrigerators and to prevent microbial contamination of refrigerators. Bioinformatics analysis based on metagenomics sequencing has been used by many researchers to investigate microbial communities in various fields. Therefore, further effort is required to study the bacterial contamination including the contamination levels and its composition on different internal surfaces within domestic refrigerators.

This study provided a general overview of the sanitary status of three different internal surfaces of domestic refrigerators and analyzed its bacterial community including the composition and diversity with high-throughput sequencing techniques. The results may contribute to designing refrigerator management to mitigate the risk of bacterial contamination and promote food safety.

MATERIALS AND METHODS

Sampling

Swab samples were obtained from surfaces of eight household refrigerators. These home refrigerators were selected from 27 different homes, including three different refrigerator models and two different purchase years (2016 and 2017). For each refrigerator, the inner wall, shelf, and basket were wiped over with sterile cotton swabs moistened with Buffered Peptone Water (BPW, Luqiao Co., Ltd., Beijing, China) respectively. The sampled cotton swabs were placed in a sterile homogenous bag and transported back to the laboratory under chilled conditions (4 ± 1 °C).

Pre-treatment

At the laboratory, sterile saline was added to the homogenous bags containing cotton swabs to achieve 10^{-1} diluent. The bag containing the diluted samples was agitated for 2 min in a stomacher (BagMixer, Interscience). Serial dilution was then performed on the diluted samples.

Microbiological counting

To analyze the contamination levels of different bacteria, the diluted solution from the three internal surfaces was inoculated onto the following agars: 1) plate count agar (PCA, Luqiao Co., Ltd., Beijing, China) and incubated at 37 °C for 48 h for the total bacterial counts; 2) plate count agar (PCA, Luqiao Co., Ltd., Beijing, China) and incubated at 4 °C for 10 d for the count of psychrophilic bacteria; 3) double lactose bile salt fermentation medium (Luqiao Co., Ltd., Beijing, China) containing an inverted tube incubated at 37 °C for 24 h. The fermentation liquid that turned yellow and produced gas was streaked onto the eosin-methylene blue agar plates (EMB, Luqiao Co., Ltd., Beijing, China) to observe the colony morphology. The tubes that observed typical colony characteristics of coliforms (green and shiny or with dark or purple centers) were eventually counted. Internal Transcribed Spacer (ITS) sequencing was used to analyze the fungal community.

DNA extraction and PCR amplification

The diluted swab samples were inoculated on plate count agar (PCA, Luqiao Co., Ltd., Beijing, China) to enriched bacteria. The surface of the cultured agar medium was washed and suspended in 1 ml of the extraction buffer from a Fast DNA extraction kit (TIANamp DNA Kit, Beijing Tiangen) after 24 h. Bacterial DNA from the washed plates was then extracted according to the manufacturer's instructions. The extracted microbial DNA was amplified by PCR with a concentration greater than 50 ng ml^{-1} detected by a nucleic acid microspectrophotometer (NANODROP-2000, Thermo Scientific). For PCR, the primer and specific steps were carried out according to the parameters of Ye et al.[13].

High-throughput sequencing

16S rRNA sequencing was conducted to get the bacterial diversity and composition. The amplified fragment was modified and sequenced on the HISEQ platform with a paired-end sequencing method. The pairwise reads were spliced into long sequences and quality control was performed to get clean reads. Usearch software was used to cluster data and operational taxonomic units (OTUs) were obtained with 97% similarity. To correct sequencing errors, representative sequences in clusters of trimmed sequences were chosen and considered for taxonomy identification. The compositions and diversity of the bacterial community were obtained by assigning the representative sequences to taxonomic positions according to the database of known species (GreenGenes, http://greengenes.lbl.gov) with RDP methods.

The air quality in domestic refrigerators

The air quality was evaluated by $PM_{2.5}$ value, which was detected with instrument LB-S06 (Fengbao Co., Ltd., Nanjing, China). It was placed on the shelves for 30 s with the door of the refrigerators closed, and repeated three times.

Statistical analysis

The number of coliforms was calculated by referring to the most probable number of coliform bacteria identification table (MPN table) given in GB4789-2016 (China). The significant difference of coliforms among different surfaces within domestic refrigerators was calculated by Duncan·s multiple-range test using SAS. The relationship between the $PM_{2.5}$ values and the chao1 index was assessed by Pearson's correlation coefficients with sigmaplot. UniFrac utilizes evolutionary information to measure the differences of species between samples. Samples with large species differences tend to have a higher UniFrac value.

RESULTS AND DISCUSSION

The number of microorganisms on three surfaces within domestic refrigerators

Figure 1 showed the number of coliforms, total bacterial counts and psychrophilic bacteria on three internal surfaces of domestic refrigerators. The coliform contamination levels on the inner wall were significantly lower than the shelf or basket ($P < 0.05$). Considering the low temperature environment of the refrigerator, psychrophilic bacteria was also detected. It can be seen from Fig. 1, in the baskets, the number of coliforms, total bacterial counts and psychrophilic bacteria were ranged from 1.3–5.5 log MPN 100 cm^{-2}, 3.1–7.9 log CFU 100 cm^{-2}, and 0–9.0 log CFU 100 cm^{-2}, respectively. On the shelves, the number of coliforms, total bacterial counts and psychrophilic bacteria were ranged from 0.5–4.6 log MPN 100 cm^{-2}, 3.1–7.1 log CFU 100 cm^{-2}, and 1.8–8.3 log CFU 100 cm^{-2}, respectively. On the inner walls, the number of coliforms, total bacterial counts and psychrophilic bacteria ranged from 0.2–2.3 log MPN 100 cm^{-2}, 2.2–7.8 log CFU 100 cm^{-2}, and 2.6–8.5 log CFU 100 cm^{-2}, respectively. Results revealed that the total bacterial counts and psychrophilic bacterial counts on three internal surfaces in the same refrigerator tended to be consistent.

Coliform is one of the important indicators to evaluate the hygienic quality of food[11] and food is often stored in household refrigerators, therefore, coliform on three internal surfaces was detected. It was reported that higher coliform counts (higher than 3 log CFU 100 cm^{-2}) in household cloths and refrigerator drawers were related to *S. aureus*[8]. In this study, the shelf and basket had samples exceeding this level, but all samples on the inner wall were below this number. This reveals that the hygienic conditions of the wall within domestic refrigerators is better than the shelf or basket, which coincides with a previous study[12]. This may be caused

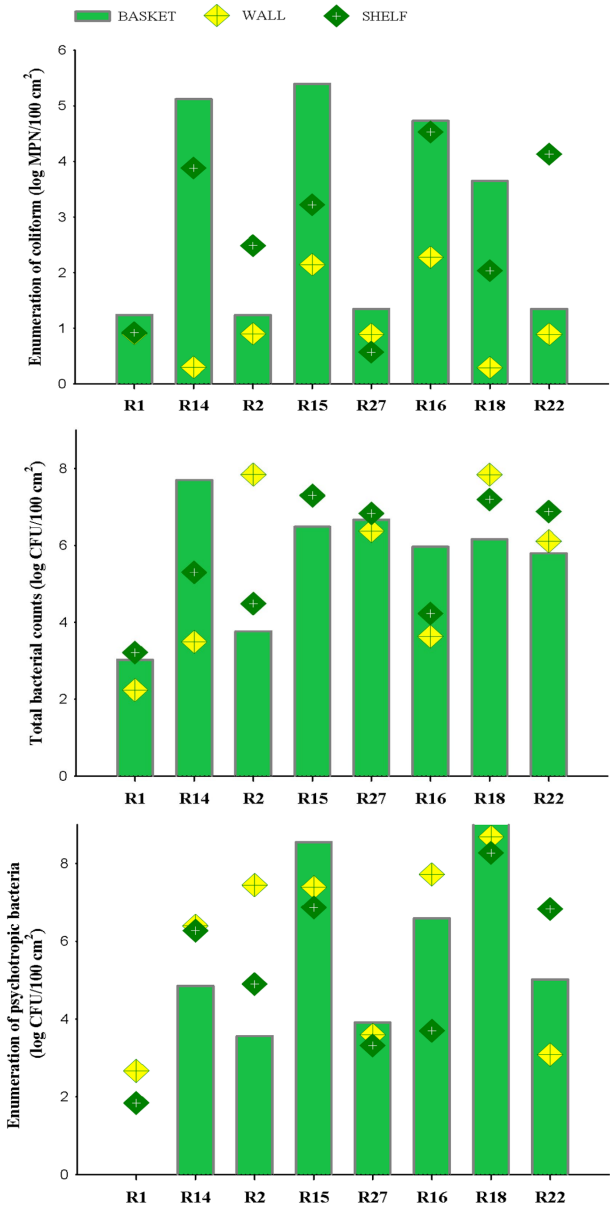

Fig. 1 The number of microorganisms on three surfaces within domestic refrigerators. (R1–R27: Different refrigerator numbers , BASKET WALL SHELF).

by the fact that the shelf and basket mainly played the role of supporting food in daily use, thus, they had more direct contact with food. Ye et al. found that it was the food stored in the refrigerator, rather than the bactericidal module, user age and use time of domestic refrigerators, that has a greater impact on microbial contamination of refrigerators[13]. Bassey et al. found that the microbial contamination level of the refrigerators was likely to be influenced by a range of factors including the levels of initial contamination introduced on contaminated foods, the efficiency and frequency of refrigerator maintenance and cleaning[4]. Similarly, due to individuals using domestic refrigerators, the total bacterial counts and the psychrophilic bacterial counts of three surfaces did not show differences in this study but showed a consistent trend in the same refrigerator.

Composition and diversity of fungal community on three different surfaces within domestic refrigerators

Figure 2 showed that at the genus level of fungi, the dominant flora of both the inner wall and shelf were *Saccharomyces* spp. and *Candida* spp., while *Saccharomyces* spp., *Candida* spp. and *Fistulina* spp. took superiority in the basket. The UniFrac values of fungal composition on three different internal surfaces of domestic refrigerators at the genus level showed that the diversity of fungal species was rather polarized, and the influence of different internal surfaces on the diversity of fungal flora is not clear. The composition of the fungal species on every surface may be completely independent or identical.

Fungi also play an important role in food safety for consumers in the process of food storage, in this study, due to the use of high-throughput sequencing, the detection rate and relative abundance of the dominant fungi on three internal surfaces were obtained. In addition to that, among the 24 samples, the relative abundance of 'other' exceeded 80% in 19 samples, which contained a lot of different fungi flora with lower relative abundances (usually lower than 0.5%). This may be due to the fact that the internal surfaces within domestic refrigerators were likely to be influenced by a range of factors, including the nature and levels of the initial contamination introduced on different foods, the hygiene of those preparing and placing foods into the refrigerator, and the efficiency and frequency of refrigerator maintenance and cleaning. The results show that at the genus level of fungi, the dominant flora of both the inner wall and shelf were with *Saccharomyces* spp. and *Candida* spp., and those of basket were with *Saccharomyces* spp., *Candida* spp. and *Fistulina* spp. from the results, the diversity of fungal species was rather polarized, the species of fungi on different surfaces were not consistent, this illustrated there were various factors having an influence on the fungal composition of the three internal surfaces.

The bacterial community diversity on three surfaces within domestic refrigerators

Figure 3 showed the Shannon index and Simpson index of three surfaces within eight domestic refrigerators. The Shannon index emphasizes the contribution of rare species and the Simpson index focus on the evenness. It can be seen that the indexes can properly reflect the frequency of contact between the surface and external environment. The shelf had the highest bacterial community diversity index and the wall has the lowest bacterial community diversity index.

Shannon and Simpson indices represent the species richness and species evenness of the community[14], which constitutes two aspects of bacterial community diversity. The results that the inner wall owned the lowest bacterial diversity may due to its position in the refrigerator, the inner wall rarely had direct contact with foods, thus, it had less access to different types of bacteria. Based on the results, reducing the direct contact between external foods and the surface within domestic refrigerators is recommended. For example, in the daily use of a refrigerator, food being adequately covered before being put into the refrigerator may contribute to improving the sanitation status of domestic refrigerators[5].

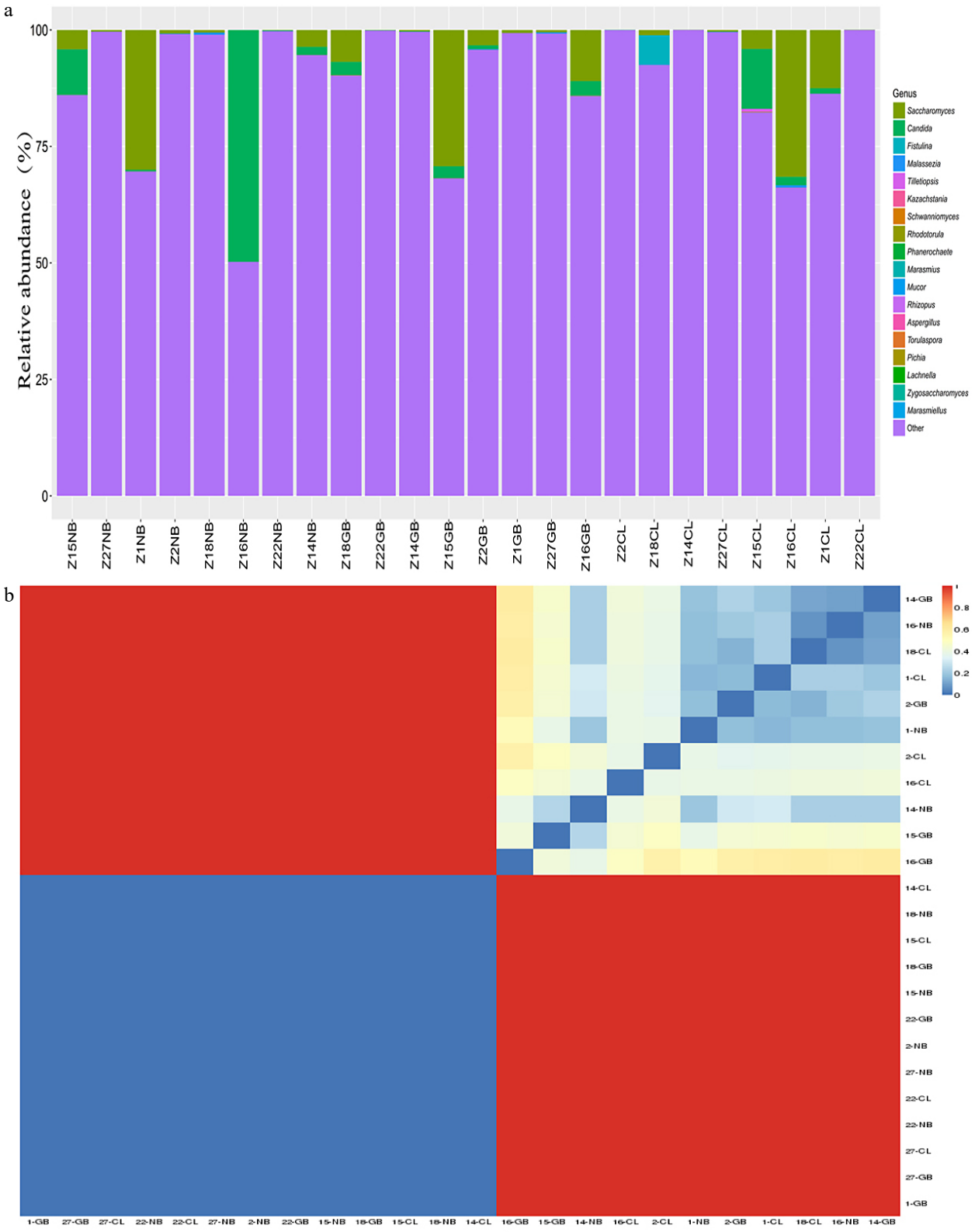

Fig. 2 Composition and diversity of fungal community on three different surfaces within domestic refrigerators (NB: wall, GB: shelf, CL: basket). (a) The relative abundance of fungi on different contact surface of eight users. (b) Diversity of fungal species on different surfaces.

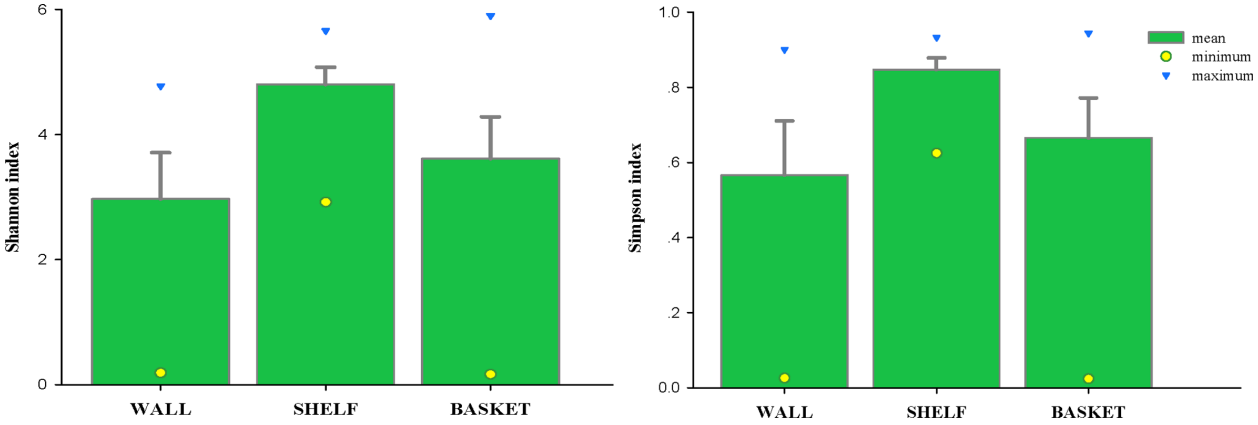

Fig. 3 The diversity indexes of bacterial community on three surfaces within domestic refrigerators (mean, maximum, minimum).

The bacterial community composition on three surfaces within domestic refrigerators

Figure 4 shows the composition of bacteria on three surfaces and each sample at a family level. It can be seen that *Bacillaceae* (49.3%), *Aeromonadaceae* (25.9%) and *Shewanellaceae* (12.5%) were dominant on the inner wall; *Bacillaceae* (43.1%), *Aeromonadaceae* (20.9%) and *Moraxellaceae* (11.3%) held preponderance on the shelf and *Bacillaceae* (31.9%), *Enterobacteriaceae* (23.2%) and *Moraxellaceae* (16.3%) had an advantage in the basket. Particularly, compared with the basket and shelf, the inner wall owned the simpler bacterial community structure. Specifically, there were only six species with relative abundance higher than 0.5% of the total OTUs, while for shelf and basket, there were 12 and 11 species constituted their bacterial community respectively. This was consistent with the lowest bacterial diversity of the inner wall mentioned above.

Although three surfaces within domestic refrigerators shared the high relative abundance of *Bacillaceae*, the difference of position within refrigerators lead to a difference in respect to the dominant bacterial community. Moreover, the result that the inner wall owned simpler bacterial composition indicated a good agreement with the bacterial diversity mentioned above. Bassey et al. had identified the bacterial isolates of different refrigerator compartments basing on the morphological and biochemical characteristics[4], but due to the limitation of the detection method, only the frequency of target bacteria can be given, the structure of bacterial composition on three surfaces within refrigerators was insufficient. In this study, the main bacterial composition was comprehensively analyzed by high-throughput sequencing technology, which could provide the theoretical basis for the selection of sanitation technology in domestic refrigerators.

Correlation between bacterial community richness and air quality of three surfaces within domestic refrigerators

Figure 5 showed the correlation between the chao1 index and $PM_{2.5}$ values in three surfaces within domestic refrigerators. The chao1 index can be used to indicate the number of species in the community. Results showed that there was a significant negative correlation between the chao1 index and $PM_{2.5}$ values in the basket ($P < 0.05$), but no significant correlation in the shelf or wall ($P > 0.05$). Moreover, the correlation coefficient of $R^2 = 0.940$ in the basket indicated a high linear correlation between the chao1 index and $PM_{2.5}$ values.

Although many kinds of literature have studied how air quality affects microorganisms[15–17], there is currently no literature to analyze the correlation between air quality and bacterial richness in refrigerators. Domestic refrigerators are a closed space, the air in them may affect microbial contamination. In this study, the correlation between the chao1 index and $PM_{2.5}$ values of the basket, shelf and inner wall were analyzed. The results showed that there was no correlation on the wall or shelf, but a significantly negative correlation was found in the basket. This difference may be caused by the fact that frequent opening and closing of the refrigerator doors made inner walls and the shelves exchange air with the exterior, but the basket can keep a relatively independent and closed air environment, so a significant correlation can be observed. Although the total number of species decreased with the increase of $PM_{2.5}$, Liu et al. found with the increase of AQI (Air Quality Index), the proportion of pathogenic bacteria will increase[15]. So it is still necessary to keep the air in the basket within the domestic refrigerator clean. According to the above results, different surfaces of refrigerators have different levels of microbial complexity. Therefore, keeping each surface of refrigerators clean and tidy plays an important role in household food safety.

CONCLUSIONS

In this study, the bacterial contamination on three different surfaces within the domestic refrigerator was determined. Almost half of the samples on the shelves and in the baskets had coliform counts of more than 3 log MPN cm^{-2}, but all samples on the inner walls were below this number. The results showed that compared to the shelf and basket, the inner wall has the best sanitary status. And the total bacterial counts and psychrophilic bacterial counts on three internal surfaces in the same refrigerator tended to be consistent. The inner wall had the lowest bacterial diversity and simplest bacterial composition structure. High-throughput sequencing results showed that different positions within the refrigerator will lead to differences in the predominant bacterial community. At the genus level of fungi, the dominant flora of

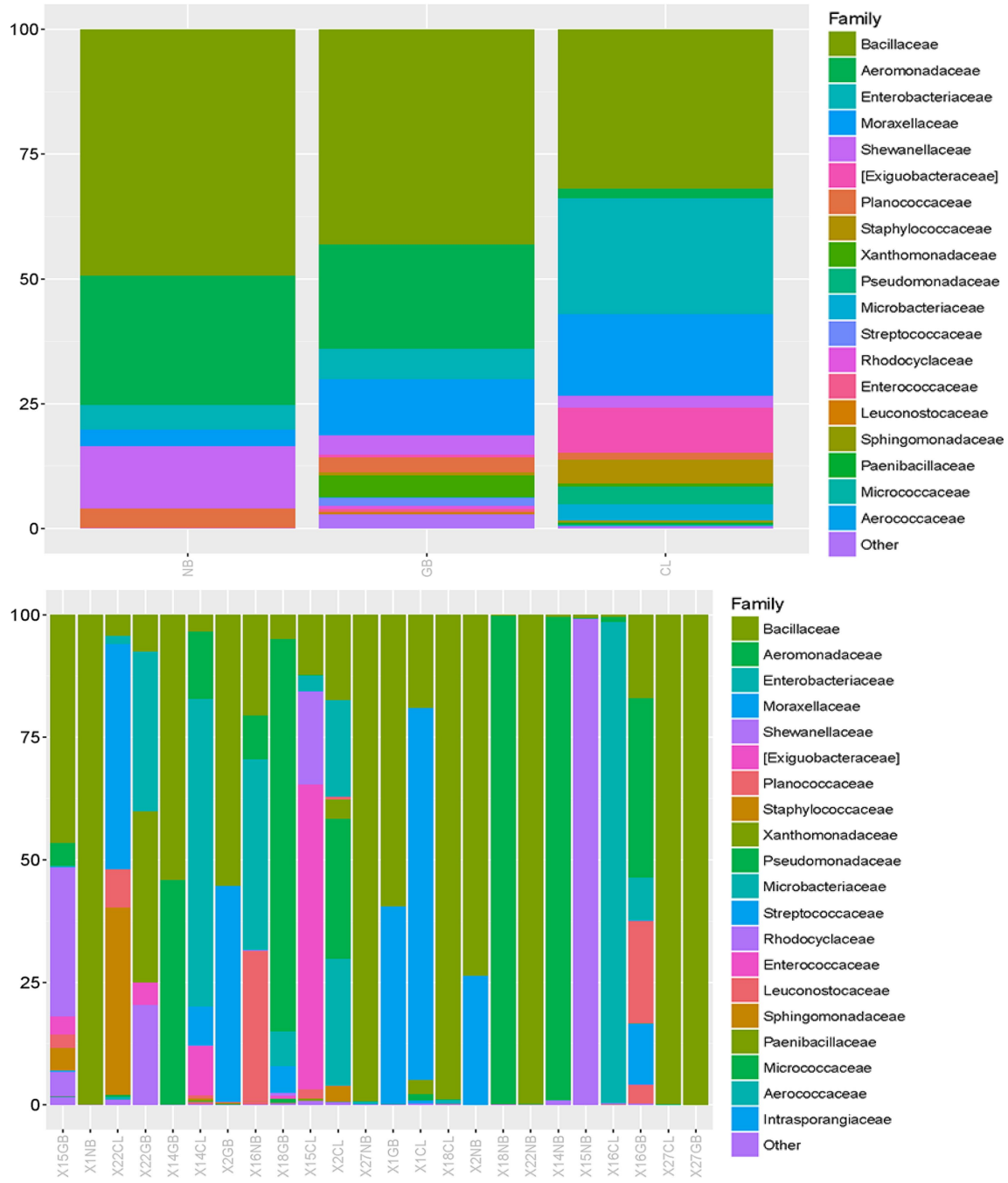

Fig. 4 The bacterial composition of three surfaces within eight domestic refrigerators at the family level (NB: wall, GB: shelf, CL: basket).

both the inner wall and shelf were Saccharomyces spp. and Candida spp., while Saccharomyces spp., Candida spp. and Fistulina spp. took superiority in the basket. The UniFrac values of fungal composition on three different internal surfaces of domestic refrigerators at the genus level showed that the diversity of fungal species was rather polarized, and the influence of different internal surfaces on the diversity of fungal flora is not clear. In addition, it was found that the air quality in the basket was significantly correlated with the total number of bacterial species. The results of this study could help understand the bacterial contamination and species on three surfaces within domestic refrigerators, and provide theoretical guidance for the sanitation of refrigerators. It may help to recommend the adequate packaging of foods stored in domestic refrigerators.

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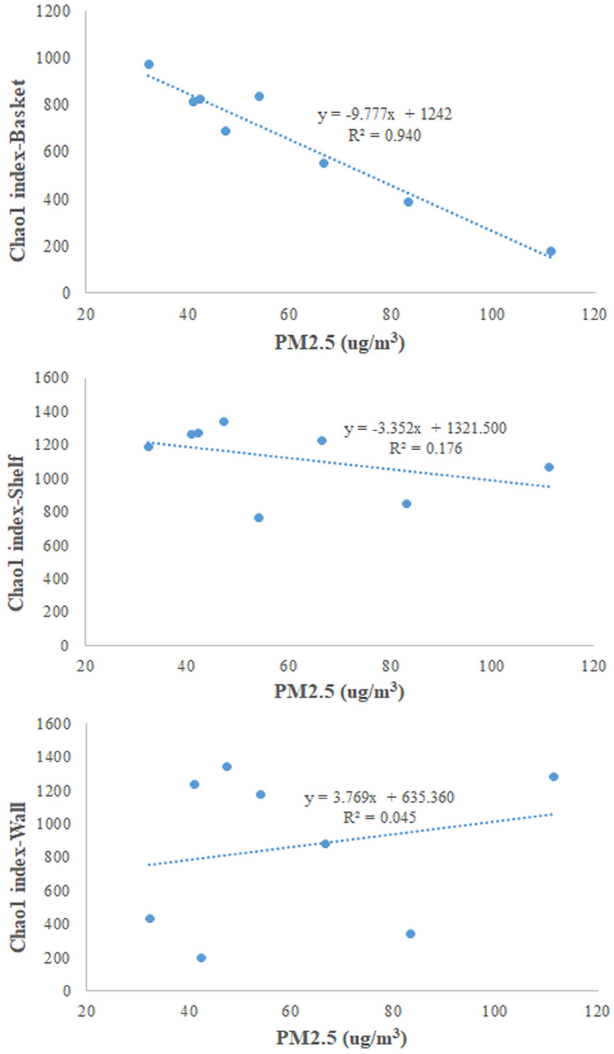

Fig. 5 The correlation between the chao1 index and $PM_{2.5}$ of three surfaces within eight domestic refrigerators.

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Conflict of interest

The authors declare that they have no conflict of interest.

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