The Indoor Concentration and Size Distribution of Airborne Bacteria in a Typical Traditional Market

Yi-Tang Chang1*, Wen-Te Liu2, I-Chun Chen3*, Cheng-Che Chiang1, Sai Hung Lau1

1 Department of Microbiology, Soochow University, Taipei 11102, Taiwan
2 Department of Tourism, Tungnan University, New Taipei City 22202, Taiwan
3 Department of Land Resources, Chinese Culture University, Taipei 11114, Taiwan

ABSTRACT

Bioaerosols generated by fresh foods in traditional wet markets (TWMs) in Taiwan are classified as significant biological contaminants and can lead to biological hazard risk. This study aims to explore the distribution of the different particle sizes of airborne bacteria (AB) present at typical public indoor TWM. The concentrations during operations at the chosen indoor TWM were measured to be 1.55 × 10³ CFU m⁻³ of AB. The presence of AB in the environment was influenced by the specific products being sold, particularly in areas where vendors sold fresh poultry and livestock meat. These areas exhibited a higher concentration of AB. Although the AB concentrations were comparable to Taiwan indoor air quality standards, the size distribution of AB indicated a potential biological hazard for the staff working with poultry and livestock meat. The size distribution analysis revealed that the highest frequency of bioaerosols was observed within the range of 2.1 to 3.3 µm, accounting for 25.76% of all AB during TWM operation and 22.79% after operations were completed. The main bacterial source came from the excrement produced by the live poultry around these sampling points. The second highest proportion was in the range of 1.1 to 2.1 µm. These two sizes of particles are highly likely to deposit in the bronchial tubes of the respiratory tract after being inhaled by humans. Among the AB sampled, isolated and identified were Kocuria marina, K. carniaphila, and Staphylococcus sciuri, which were frequently found in a typical indoor TWM. We recommend using these representative AB as biological indicators for monitoring public indoor TWMs in Taiwan. It is important to enhance the effective management of environmental sanitation during and after operations in TWMs. This can be achieved through makes such as proper waste collection and disposal, as well as the installation of mechanical ventilation systems.

Keywords: Traditional wet market, Airborne bacteria, Particle size distribution

1 INTRODUCTION

Taiwan is located in a subtropical region and has a high average annual temperature as well as often having a relative humidity (RH) of between 70% to 80%. Biological pollutants are easily produced indoors and these largely consist of bioaerosols containing bacteria and fungi. A variety of items are sold in Taiwan's traditional wet markets (TWMs), and these include fresh poultry raw meat, fresh livestock raw meat, fresh raw seafood, vegetables, fruit, and various grains as well as chicken eggs, duck eggs, etc. Many customers in Taiwan still enjoy shopping at TWMs in their local neighborhoods because it allows them to negotiate prices with the vendors. However, many public indoor TWMs have failed to effectively manage environmental sanitation during their business operations. Poor indoor air quality (IAQ) may bring about a high health risk to consumers and staff at indoor TWMs (Cincinelli and Martellini, 2017). They often have poor ventilation systems that not only cause off-smells to drift into the air but also allow the creation of significant bioaerosols within the indoor environment (Wei et al., 2021). The biological hazards are caused...
by crowded footpaths and the close contact between staff and customers in TWMs. Past studies have shown that the presence of a high concentration of bioaerosols in a closed room can easily lead to a range of symptoms including asthma, allergies, and coughs, as well as related respiratory diseases such as influenza and Legionnaire’s disease (Du et al., 2018; Grewling et al., 2019). Hence, the IAQ of TWMs has become a major concern.

In recent years, emerging infectious diseases such as the avian influenza virus, SARS-CoV, and COVID-19 have been reported to be airborne disease infections and all are believed to have been transmitted in indoor public buildings (Guzman, 2021). Investigation and planning for controlling the particle size distribution of airborne bioaerosols plays an important role in providing occupational safety and a healthy indoor environment. The bioaerosols that form the indoor microbial community are present at different levels depending on the human activities that take place in the building. Some studies have investigated the distribution of different particle sizes of indoor bioaerosols, and these include Byeon et al. (2008) at a municipal composting facility; Ferguson et al. (2021) at a green-waste composting site; Górny et al. (1999) and Górny and Dutkiewicz (2002) in general dwellings; Gryzb and Lenart-Boroń (2019) in animal enclosures at a zoological garden; and Gormley et al. (2021) at poorly designed sanitation systems. Furthermore, the size distribution of bioaerosols is known to be affected by various biotic and abiotic factors, which include the microorganisms present, the environmental conditions of the building, and the type of human activity taking place.

Bioaerosols of different sizes will reach different parts of the respiratory system and cause significantly different health risks. For example, bioaerosols, which range in size from 0.1 to 10 µm (aerodynamic diameter), can be inhaled directly into the respiratory system (Cox and Wathes, 2020). However, little information is available on the distribution of different-sized bioaerosols in traditional markets (Guo et al., 2004; Reanprayoon and Yoonaiwong, 2012; Jagzape et al., 2013; Gao et al., 2016; Tavakoli, 2020). A previous study examining two TWMs in Taiwan identified a significant difference (p < 0.05) between large particle bioaerosols (LPBs) and small particle bioaerosols (SPBs) concentrations before and after indoor TWMs were operating (Wei et al., 2021). One of the two TWMs was found to have percentages of bacterial LPBs and SPBs of 22% and 78% before operations commenced and 25% and 75% after work finished, respectively. However, the distribution of bacterial LPBs and SPBs in the other TWMs was significantly different. The percentages of LPBs and SPBs before operations started were 76% and 24%, respectively. After the market operation had finished, the percentages of bacterial LPBs and SPBs had changed obviously to 27% and 73%, respectively. The highest concentration of bacterial SPBs was found after market operation and existed in the livestock fresh raw meat districts of the TWM. These findings suggest that the distribution in different indoor TWMs may vary on a case-by-case basis and will be dependent on the locations within the market and the goods being sold.

This study aims to analyze the size distribution of airborne bacteria (AB) in this TWM and to identify the frequently occurring representative microbial species present in the aerosols. The research outcomes contribute to evaluating the impact of these biological pollutants on market workers.

2 METHODS

2.1 Indoor TWM Selection

This study selected the public indoor TWM in Miaoli County, Taiwan, as the research target. This indoor TWM served a single town whose population was about 98,000 in 2011. The market building, located downtown, is a three-story reinforced concrete building built in 1980. It is the most well-known public indoor traditional market in the district. There are 66 stalls in total on the ground floor and they sell a wide variety of products including animal meat, poultry, fish and seafood, grains, vegetables, and fruits, as well as there being cooked food stalls present too. The operation duration per day is about six hours (6:00–12:00) and the average visit time by customers is about fifteen minutes in the studied market. Because there is no mechanical ventilation system on the ground floor where the fresh and raw food stalls are found, this research will have a greater impact on worker health than on the health of visitors to the market.
2.2 Sampling Procedure

The aerobic plate counts of AB present in the indoor TWMs were analyzed using the culture-based approach: The Sampling Indoor Bacteria Method (NIEA E301.10C) of Taiwan’s EPA (https://www.epa.gov.tw/niea/). There were 12 sampling points (point a to point l) and an outdoor control (Fig. 1). The indoor sampling points covered entire paths in the indoor market area. Three sampling points, namely j, k, and l, were outside the market but next to the building in a narrow alley, and there were stalls close to point j and point k on both sides. There were rain covers for the stalls that make the alley semi-indoor. A distance meter (Trimble Laser HD 50) was used to measure the distance between each booth and aisle, and a configuration diagram was created, which is shown in Fig. 1 and Table S1. Table 1 shows the vendors around each sampling location, the items sold by the vendors, and the coordinates of the site. The sampling was conducted on May 13, 2010. The sampling time consisted of the period during which the market was in operation (09:00–12:15) and the period after which the market had closed (12:30–18:45). At the time of sampling, the indoor temperature of the market was 28°C, the humidity was 81% (RH) and there was a wind speed was 0.0 ms⁻¹.

This study used an Andersen six-stage sampler (Tisch Environmental TE-10-860) that was wiped and completely sterilized using 70% alcohol at each stage before and after each sampling period. The sampling flow rate was 28.3 L min⁻¹ and this was adjusted using dry flow calibration equipment (DefenderL520). Six stages were used to collect particles of six different diameters, namely (1) ≥ 7.0 μm (1st stage); (2) 7.0–4.7 μm (2nd stage); (3) 4.7–3.3 μm (3rd stage); (4) 3.3–2.1 μm (4th stage); (5) 2.1–1.1 μm (5th stage); and (6) 1.1–0.65 μm (6th stage). Each stage in the sampler consisted of uniformly distributed precision-drilled holes. The sampler was placed at a height of 150 cm above the floor surface at each sampling location in each of the districts being tested; this is where the human breathing zone is situated in Asia. The flow rate of the sampler during the collection of the AB samples was set at 28.3 L min⁻¹. The agar media used were tryptone soy agar (TSA) to allow the growth of bacteria. The bioaerosols sampling time was determined using Fig. 1.

![Fig. 1. AB present at the sampling locations (star dots) collected on the footpaths of the twelve different regions of TWM. Position coordinates: ●; Outdoor sampling location (control): ◆: A: Fresh raw pork; B: Fresh raw beef; C: Fresh raw lamb; D: Fresh raw chicken; E: Fresh raw duck; F: Fresh raw seafood; G: Hot pot ingredients; H: Groceries; I: Refrigerated storeroom; J: Vegetables and fruit; K: Pork floss and pork/beef jerky.](image-url)
Table 1. Information on the public indoor TWM investigated during this study.

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>Sampling locations using Cartesian coordinates (x, y)</th>
<th>Representative products sold on their stalls¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>(−1.5, 16)</td>
<td>A: Fresh raw pork; D: Fresh raw chicken</td>
</tr>
<tr>
<td>b</td>
<td>(−1.5, 28.5)</td>
<td>A: Fresh raw pork; D: Fresh raw chicken; E: Fresh raw duck</td>
</tr>
<tr>
<td>c</td>
<td>(−1.5, 42)</td>
<td>A: Fresh raw pork; F: Fresh raw seafood</td>
</tr>
<tr>
<td>d</td>
<td>(−9.5, 16)</td>
<td>D: Fresh raw chicken; H: Groceries; I: Refrigerated storeroom; K: Pork floss and pork/beef jerky</td>
</tr>
<tr>
<td>e</td>
<td>(−9.5, 28.5)</td>
<td>D: Fresh raw chicken; A: Fresh raw pork; I: Refrigerated storeroom</td>
</tr>
<tr>
<td>f</td>
<td>(−9.5, 42)</td>
<td>A: Fresh raw pork; D: Fresh raw chicken; E: Fresh raw duck; I: Refrigerated storeroom; J: Vegetables and fruit</td>
</tr>
<tr>
<td>g</td>
<td>(−20, 16)</td>
<td>A: Fresh raw pork; D: Fresh raw chicken; I: Refrigerated storeroom; K: Pork floss and pork/beef jerky; F: Fresh raw seafood; G: Hot pot Ingredients</td>
</tr>
<tr>
<td>h</td>
<td>(−20, 28.5)</td>
<td>B: Fresh raw beef; C: Fresh raw lamb; F: Fresh raw seafood; H: Groceries</td>
</tr>
<tr>
<td>i</td>
<td>(−20, 42)</td>
<td>H: Groceries; I: Refrigerated storeroom; J: Vegetables and fruit</td>
</tr>
<tr>
<td>j</td>
<td>(−28, 16)</td>
<td>F: Fresh raw seafood; G: Hot pot Ingredients; J: Vegetables and fruit</td>
</tr>
<tr>
<td>k</td>
<td>(−28, 28.5)</td>
<td>A: Fresh raw pork; D: Fresh raw chicken; E: Fresh raw duck; F: Fresh raw seafood; H: Groceries; J: Vegetables and fruit</td>
</tr>
<tr>
<td>l</td>
<td>(−28, 42)</td>
<td>B: Fresh raw beef; C: Fresh raw lamb; D: Fresh raw chicken; J: Vegetables and fruit</td>
</tr>
<tr>
<td>◆</td>
<td>(−15, −8)</td>
<td>: Outdoor control</td>
</tr>
<tr>
<td>Zero end of distance survey</td>
<td>(0, 0)</td>
<td>※ Origin (datum point) for the Cartesian coordinate plane used for the distance survey</td>
</tr>
</tbody>
</table>

¹ A: Fresh raw pork; B: Fresh raw beef; C: Fresh raw lamb; D: Fresh raw chicken; E: Fresh raw duck; F: Fresh raw seafood; G: Hot pot Ingredients; H: Groceries; I: Refrigerated storeroom; J: Vegetables and fruit; K: Pork floss and pork/beef jerky.

precise timers designed to work with our bioaerosols collecting equipment, and the equipment was set up. A preliminary study was carried out that used a series of sampling times (namely 30, 25, 20, 15, 10, 5 mins) in the TWM in order to identify the requirements of the standard method. The collected sampling time was fixed at 5 mins on a standard 9-cm Petri dish, measuring as the range of 30–300 CFU (colony-forming unit) m⁻³ colonies in the MWN. If the sampling time was ≥5 minutes in the MWN, excessive colonies on a standard 9-cm Petri dish was not measured. Before sampled air in indoor markets, the 70% ethanol was used as sterilizing completely each stage of the sampler. The two-dimensional contour plot of the AB concentration distributions within the TWM was analyzed using contour lines with the help of Surfer12 software.

AB sampling was carried out in triplicate at each sampling location. The sampled Petri dishes were incubated at 30 ± 1°C in an incubator for 48 ± 2 hours, and the numbers of colonies were then counted for each plate. A positive hole correction factor also was used to adjust CFUs based on Anderson’s conversion table (Macher, 1989).

2.3 Strains Identification

The single colonies of representative bacterial strains that appeared at a high frequency (defined as above 50%) on the Petri dishes were isolated and purified completely by the streaking method using growth on TSA media at 30 ± 1°C. The colony characteristics, their Gram stain status, and various microscopic observations were recorded. Full-length 16S rDNA was then amplified by PCR from each of the chosen strains using the universal primers 27F and 1492R (Lau et al., 2022b); the amplification used an annealing temperature of 55°C for 30s. Finally, the amplified DNA was sequenced by Mission Biotech (Taipei, Taiwan) and the results were used to search the NCBI

2.4 Principal Component Analysis (PCA)

It is a difficult and complicated process to know the distribution of different particle sizes of AB at the public TWM. The application of identifying the typical characteristics of environmental problems using principal component analysis (PCA) was often used. The PCA methodology is a powerful tool for processing environment-monitoring data, such as air pollutants, water parameters, bacterial community-level physiological profile in soil, etc. (Yang et al., 2020; Lau et al., 2022a). It was utilized to clarify the relationship of various size particles of AB and products sold on their stalls in this study. The principle of the PCA method is composed of the following operation steps: (1) to construct the original data matrix, composed of the measured concentrations of AB in all sampling points at different stages; (2) to standardize the original data with the Z-score standardization formula to eliminate the impact of dimension; (3) to calculate the correlation coefficient matrix, R, with standardized data and determine the correlation between indicators; (4) to calculate the eigenvalues and eigenvectors of the correlation coefficient matrix to determine the number of principal components (PCs) and illustrate scree plot for data visualization; (5) to obtain principal components are weighted and summed to obtain a comprehensive evaluation function (Yang et al., 2020). In this study, the software R language (X64, version 4.2.1) was used to analyze and visualize the relationships between the various characteristics of the different sizes of AB at the various sampling points in the public indoor TWM using PCA. The raw data analysis was carried out by the built-in PCA command of the R language, and the visualization of analyzed data used the package “factoextra”. The correlation plot was analyzed and illustrated by the package “corrplot” in the R language.

3 RESULTS AND DISCUSSION

3.1 Distribution of Different Particle-size Airborne Bacteria during Market Operation

During market operations (business hours), a two-dimensional representation of the AB shows the bacterial aerosol concentration of the twelve sampling points in the market, measured to be $1.85 \times 10^3$ CFU m$^{-3}$, and the range was $7.18 \times 10^2$ CFU m$^{-3}$ (sampling point l) to $2.71 \times 10^3$ CFU m$^{-3}$ (sampling point d) (Fig. 2(a)). The concentrations at six of the sampling points (namely a, d, e, f, h, and i) exceeded the IAQ standard ($1.5 \times 10^3$ CFU m$^{-3}$) of Taiwan. Table 1 shows the sampling points covering the 1st aisle (a, b, c) and the 2nd aisle (d, e, f) were mainly selling poultry and livestock fresh meat (A, D, E) and that there was refrigeration equipment present (l). Regarding the concentration in the 2$^{nd}$ aisle, this was highest at $2.71 \times 10^3$ CFU m$^{-3}$ at point d, which was followed by point e ($2.20 \times 10^3$ CFU m$^{-3}$) and then point f ($2.05 \times 10^3$ CFU m$^{-3}$). The vendors on the third aisle (sampling points g, h, i) sell a variety of items including fresh meat products such as poultry and livestock (A, B, C, and D), fish and seafood (F), and groceries (H). The bacterial aerosol concentrations were $1.18 \times 10^3$ CFU m$^{-3}$ (point g), $1.60 \times 10^3$ CFU m$^{-3}$ (point h), and $1.72 \times 10^3$ CFU m$^{-3}$ (point i). The goods sold at stalls along the fourth aisle (sampling points j, k, l) include fish and seafood (F), poultry and livestock, as well as other fresh meat products (A, B, C, and D), fresh vegetables and fruits (J), cooked food (G), and groceries (H). The bacterial concentrations in this area were $1.31 \times 10^3$ CFU m$^{-3}$ (point j), $1.08 \times 10^3$ CFU m$^{-3}$ (point k), and $7.18 \times 10^2$ CFU m$^{-3}$ (point l), which were the lowest concentrations among all of the sampling points. The reason for this is that this area is close to a residential area (j, G, H), and there are no other commercial activities in the surrounding area. The results (points d and e) are similar to the results of a previous study of TWMs’ bioaerosol distribution in Taiwan, which showed that the bacterial concentrations near vendors selling animal meat, poultry, fish, and seafood are relatively higher compared to other products (Wei et al., 2021).

The outdoor control point is an open space with vendors (G, H) side by side, and here the crowds are concentrated at the entrance and exit, while also shopping at the same time. Interestingly, the bioaerosol concentration at the control point was measured to be $4.5 \times 10^3$ CFU m$^{-3}$, which
Fig. 2. The contour plot of the total AB concentration (CFU m\(^{-3}\)) present in the indoor TWM: (a) during operations and (b) present after operations had finished. The X and Y axis are the distance survey using Cartesian coordinates (Unit: mm). White square dots with red English words represent the sampling locations.

is even higher than point d. One possible reason for this is the presence of 350–400 active outside stalls around the TWMs during public indoor TWM operation. This creates a crowd of customers and staff present together and this is likely to result in a high concentration of AB to be generated in the outdoor space of TWM, which includes the control point).

3.2 The Different Particle-size Airborne Bacteria during Market Operation

A two-dimensional representation of the AB concentration (percentage) in the TWM was measured at 1.55 \(\times\) 10\(^3\) CFU m\(^{-3}\), including 2.99 \(\times\) 10\(^2\) CFU m\(^{-3}\) (19.35%) in the 1st stage (particle size > 7.0 \(\mu\)m), 2.12 \(\times\) 10\(^2\) CFU m\(^{-3}\) (13.71%) in the 2nd stage (4.7–7.0 \(\mu\)m), 2.83 \(\times\) 10\(^2\) CFU m\(^{-3}\) (18.27%) in the 3rd stage (3.3–4.7 \(\mu\)m), 3.99 \(\times\) 10\(^2\) CFU m\(^{-3}\) (25.76%) in the 4th stage (2.1–3.3 \(\mu\)m), 3.00 \(\times\) 10\(^2\) CFU m\(^{-3}\) (19.41%) in the 5th stage (1.1–2.1 \(\mu\)m), and 54 CFU m\(^{-3}\) (3.50%) in the 6th stage (0.65–1.1 \(\mu\)m) during market operation (Fig. 3). The median of AB concentration in each particle size presents the same trend as the concentration (Table S2), namely 2.83 \(\times\) 10\(^2\) CFU m\(^{-3}\) in the 1st stage (particle size > 7.0 \(\mu\)m), 1.24 \(\times\) 10\(^2\) CFU m\(^{-3}\) in the 2nd stage (4.7–7.0 \(\mu\)m), 2.83 \(\times\) 10\(^2\) CFU m\(^{-3}\) in the 3rd stage (3.3–4.7 \(\mu\)m), 3.60 \(\times\) 10\(^2\) CFU m\(^{-3}\) in the 4th stage (2.1–3.3 \(\mu\)m), 3.07 \(\times\) 10\(^2\) CFU m\(^{-3}\) in the 5th stage, and 24 CFU m\(^{-3}\) in the 6th stage (0.65–1.1 \(\mu\)m). Moreover, the AB size distribution, Fig. 3, shows the highest proportion is collected by the 4th stage and the lowest proportion is collected by the 6th stage. There are one to two orders between the 4th stage and the 6th stage. Fig. 3 shows the box plot in 6th stage (0.65–1.1 \(\mu\)m) is the lowest and comparatively short, which means lower uncertainty occurs when monitoring the smallest particles of AB both during and after market operation and this has the lowest variance compared to other sampling points. Nevertheless, the total concentrations for small particle sizes of AB in 4th stage (2.1–3.3 \(\mu\)m) and 5th stage (1.1–2.1 \(\mu\)m) had higher variances at all sampling locations and the different concentration distributions for these sizes were found. In addition, the highest value (4.78 \(\times\) 10\(^3\) CFU m\(^{-3}\) in 4th stage; 3.60 \(\times\) 10\(^3\) CFU m\(^{-3}\) in 5th stage) compared to other particle sizes implies that improved control strategies are required in indoor TWMs, particularly during the market operation in order to avoid significant exposure hazards to workers. This size distribution means that more than 80% of these AB are likely to be able to enter the respiratory system and 70% will deposit in the trachea or the lungs.

Among the twelve sampling points in the two-dimensional distribution (Fig. 4), six points (namely a, c, d, h, i, k) showed the highest AB number (proportion) of 4th stage particles (particle size 2.1–3.3 \(\mu\)m), these accounted from 4.12 \(\times\) 10\(^2\) CFU m\(^{-3}\) (25.72%) to 6.01 \(\times\) 10\(^2\) CFU m\(^{-3}\) (34.94%) of all particles at these sampling points in Fig. 4(d). Three sampling points (g, j, l) made
Fig. 3. Box plot of different particle sizes AB collected during market operation and after market operation cease. Legends: dash (-): median; cross (×): means; dots (•): outliers; *: \( p < 0.05 \) (means). Detailed information is referred to Table S2.

up the highest AB number (proportion) of 1st stage particles (particle size > 7.0 \( \mu m \)), accounting from \( 2.00 \times 10^2 \) CFU m\(^{-3} \) (27.86\%) to \( 5.54 \times 10^2 \) CFU m\(^{-3} \) (42.32\%) of all particles at these sampling points in Fig. 4(a). Two sampling points (e, f) gave the highest AB (proportion) of 2nd stage particles (particle size 4.7–7.0 \( \mu m \)), accounting from \( 4.48 \times 10^2 \) CFU m\(^{-3} \) (21.85\%) to \( 6.95 \times 10^2 \) CFU m\(^{-3} \) (31.55\%) of all particles at these sampling points in Fig. 4(b). Byeon et al. (2008) reported that bioaerosols, when measured at a municipal composting facility, had about \( 10^4 \) CFU m\(^{-3} \) at each stage. Gryzb and Lenart-Boron (2019) reported that the bioaerosols’ fraction of 2.1–3.3 \( \mu m \) (4th stage) had the largest share when premises housing animals in a zoological garden were examined. Li et al. (2021) found that particles with diameters of 2.1–4.7 \( \mu m \) carried the most airborne bacteria when a high-density layer operation for chickens was examined. Ferguson et al. (2021) measured the AB in a green-waste compost facility and found that the concentrations decreased from the smallest size (0.65–1.1 \( \mu m \)) to the largest size (> 7.1 \( \mu m \)) and that the difference was an order of magnitude. Based on the findings in this study, the dominant bioaerosol particle size from the public indoor TWMs during the market operation is also 2.1–3.3 \( \mu m \) (4th stage).

### 3.3 The Principle Components Analysis of AB during Market Operation

Further analysis of the particle size distribution at different sampling points in the present study showed that the different commodities sold by the various market vendors and the activities of the staff/consumers affected the size distributions. PCA was used to comprehensively evaluate the AB size distribution across twelve sampling points and analyze the sources of AB generation. The eigenvalues of each principal component are shown in Fig. S1. The scree plot helps us to choose the PC and understand the basic data structure. It was observed that the slope became noticeably flattered after the three components. The first two principal components were preserved, which explained 79.80\% of the total variance in the indoor TWM during operations. The analysis of PCA allows the identification of groups that have taken similar eigenvalues for certain analysis parameters. We defined a total of three groups that contributed to correlations between analysis parameters on the PCA profile as shown in Fig. 5(a). Factor scores are listed in Table S3. The results showed that all samples could be analyzed by two main components. The AB particle size distributions of the sampling points were explored. Firstly, Group III in Fig. 5(a): Points e and d were similar. Taking point d as a further example in Fig. 4, the 4th stage particle concentration was \( 8.72 \times 10^2 \) CFU m\(^{-3} \), accounting for 32.18\% of all particles and the 5th stage particle concentration is \( 6.95 \times 10^2 \) CFU m\(^{-3} \), accounting for 25.65\% of all particles. On the other hand, for point e in Fig. 4, the 2nd stage particle concentration was \( 6.95 \times 10^2 \) CFU m\(^{-3} \), accounting
Fig. 4. The contour plot of the AB concentration (CFU m⁻³) in the public indoor TWM during operations commenced collected by the various different stages: (a) 1st stage; (b) 2nd stage; (c) 3rd stage; (d) 4th stage; (e) 5th stage; (f) 6th stage present. The X and Y axis are the distance surveyed using Cartesian coordinates (Unit: mm). White square dots with a red English title represent the sampling locations.
Fig. 5. The PCA profiles of the various size particles of AB in the public indoor TWM: (a) during operations. The cumulative proportion (PC1 + PC2) of this result was 79.80%. (b) after operations. The cumulative proportion (PC1 + PC2) of this result was 87.70%. Sample No.: a to k and NC (outdoor control).

for 31.55% of all particles; the 3rd stage particle concentration was $4.24 \times 10^2$ CFU m$^{-3}$, accounting for 19.25% of all particles. The main sources of bacterial contamination observed in this area are the microorganisms from the poultry and livestock, from the excrement of live poultry, from blood, and from the sewage from slaughtering poultry and livestock. In addition, the size distributions of the connected sampling points b, c, g, j, k, h, and i (Group I in Fig. 5(a)) are similar, and the proportion of larger-size particles is higher. Taking point j as an example in Fig. 4, the 1st stage particle concentration was $5.54 \times 10^3$ CFU m$^{-3}$, accounting for 42.32% of all particles. This particle size distribution may be related to the products being sold near these sampling points, which are fish and seafood (F) and cooked food (G); furthermore, it should be noted that these stalls are connected to outdoor roads. Due to the crowds created as people choose goods from these narrow market aisles, consumers tend to stay in this area for a long time. The source of bioaerosols is
mainly from microorganisms carried by fish and seafood, which are released as the ice used while selling the fish and seafood melts. In addition, when the slaughtering and washing of livestock and fish take place, the drainage of these facilities often becomes blocked by fish scales and internal organs, which results in the retention of sewage and significant splashing. Furthermore, it can be seen that sampling points $f$, $a$, and $j$ of Group II in Fig. 5(a) are connected to the stairs leading to the second floor, and their particle size distributions were found to be similar. Taking point $a$ as an example (Fig. 4), the 4th stage particle concentration was $6.01 \times 10^5$ CFU m$^{-3}$, accounting for 31.29% of all particles and the 3rd stage particle concentration was $4.12 \times 10^5$ CFU m$^{-3}$, accounting for 21.45% of all particles. The source of pollution in this area seems to be related to the activity around the three refrigerators for vegetables, fruits, and groceries (I) and the proximity of the stairwell. During business hours, the staff continuously carry inventory items up and down the stairs and many consumers also use the stairs; this results in highly disturbed air in this area.

3.4 Distribution of Different Particle-size Airborne Bacteria after Market Operations Cease

After the market ceases to operate, there is a reduction in the crowd density, and cleaning is commenced by the vendors. At this point, the concentration of AB was $9.56 \times 10^5$ CFU m$^{-3}$, which is a decrease of 38.18% compared with during the operation of the market. Furthermore, the range of bacterial aerosol concentrations at twelve sampling points now ranged from $2.83 \times 10^3$ CFU m$^{-3}$ to $2.98 \times 10^3$ CFU m$^{-3}$ (Fig. 2(b)). A similar decrease in AB concentrations after closure was also found in a previous study (Wei et al., 2021). Two sampling points (j, k) had very high concentrations of $2.42 \times 10^3$ CFU m$^{-3}$ and $2.98 \times 10^3$ CFU m$^{-3}$ respectively, which exceed the IAQ standard of Taiwan. Furthermore, the concentration at the outdoor control point (point $p$ in Fig. 1) also decreased by 23% to $9.43 \times 10^2$ CFU m$^{-3}$ compared to during market operation.

3.5 The Different Particle-size Airborne Bacteria after Market Operations Cease

Fig. 3 shows the highest contribution was now the 1st stage at $2.42 \times 10^2$ CFU m$^{-3}$ (accounting for 25.25% of all particles), followed by the 4th stage at $2.18 \times 10^2$ CFU m$^{-3}$ (22.79%), the 3rd stage at $2.15 \times 10^2$ CFU m$^{-3}$ (22.48%), the 2nd stage at $1.54 \times 10^2$ CFU m$^{-3}$ (16.13%), the 5th stage at $1.12 \times 10^2$ CFU m$^{-3}$ (11.69%), and the 6th stage at $16$ CFU m$^{-3}$ (1.66%). The concentration of the 6th stage was the lowest and one order lower than the other stages. This was similar to that during market operation hours. Integrating the size distribution data from 12 sampling points showed that the concentration at all six stages was reduced compared with operating hours ($p < 0.05$). Among them, the 6th stage was reduced to 30% remaining, which was the largest decrease; the 1st stage decreased to 81% remaining, which was the least decrease. Thus, the concentrations of all six stages decreased but remained in the same order of magnitude after market operation ceased. In addition, the median of different particle-size AB were reduced compared with operating hours ($p < 0.05$) in Table S3. The first stage was measured at $1.18 \times 10^3$ CFU m$^{-3}$ (reduced 58.3%), followed by the fourth stage at $1.42 \times 10^2$ CFU m$^{-3}$ (reduced 37.9%), the third stage at $1.41 \times 10^2$ CFU m$^{-3}$ (reduced 50.1%), the second stage at $77$ CFU m$^{-3}$ (reduced 60.5%), the fifth stage at $83$ CFU m$^{-3}$ (reduced 72.9%), and the sixth stage at $12$ CFU m$^{-3}$ (reduced 50%).

There were five sampling points (namely $c$, $e$, $f$, $j$, and $l$) that showed the highest proportion of 1st stage particles and these accounted for 31.56%–54.64% of all particles. Furthermore, four sampling points ($a$, $g$, $h$, and $i$) showed the 4th stage as the highest proportion, accounting for 29.67%–33.96% of all particles. Finally, there were three sampling points ($a$, $b$, $k$) that had the 3rd stage as the highest proportion, and these accounted for 25.70%–30.99% of all particles at each sampling point in the two-dimensional distributions (Fig. 6). The characteristics of size distribution could be inferred that the stalls related to selling products, including raw pork, raw beef, raw chicken, and seafood. The previous study presented the major size distribution of AB particles is ascribed to $7.0$ µm (1st stage), $7.0$–$4.7$ µm (2nd stage), $4.7$–$3.3$ µm (3rd stage), and $3.3$–$2.1$ µm (4th stage) in a specific environment, such as livestock husbandry and food and feedstuff industry (Clauß, 2015). Based on the outcomes in this study, the highest proportion in 3rd stage should be controlled at the selling the livestock fresh raw meat districts after the market operation.
Fig. 6. The contour plot of the AB concentrations (CFU m⁻³) in the public indoor TWM present after operations collected by the different stages: (a) 1st stage; (b) 2nd stage; (c) 3rd stage; (d) 4th stage; (e) 5th stage; (f) 6th stage. The X and Y axis are the distance surveyed using Cartesian coordinates (Unit: mm). White square dots with a red English title represent the sampling locations.
3.6 The Principle Components Analysis of AB after Market Operation

Further analysis found that the particle size distribution could be classified into three categories based on the PCA analysis (Fig. 5(b)). The eigenvalues of each principal component are shown in Fig. S2. The first two PC were preserved, which explained 87.70% of the total variance in the indoor TWM after operation. Factor scores were listed in Table S3. The first category (Group I in Fig. 5(b)) is sampling points: a, b, c, e, and l, which showed a high proportion of the 3rd, 1st, and 4th stage particles. Taking point b as an example, the 3rd stage particle concentration was $8.2 \times 10^1$ CFU m$^{-3}$, accounting for 28.98% of all particles, and the 1st stage particle concentration was $7.66 \times 10^1$ CFU m$^{-3}$, accounting for 54.64% of all particles. The main source of bacterial pollution came from the excrement produced during the continuous activities of vendors who were still keeping live poultry (D) around these sampling points. The second category (Group II in Fig. 5(b)), consisted of sampling points: d, f, h, and i. This category exhibits the largest proportion of the 1st stage or 4th stage particles, followed by the 3rd stage particles. Among these sampling points in Fig. 6, point f exhibited the highest concentration of $1.01 \times 10^3$ CFU m$^{-3}$ (all particles), and its 1st stage particle concentration was $5.54 \times 10^2$ CFU m$^{-3}$, which accounts for 54.64% of all particles, while the 3rd stage particle concentration was $1.77 \times 10^3$ CFU m$^{-3}$, accounting for 17.46% of all particles. The main bacterial pollution sources were similar to those of normal business hours. After business hours, the vendors in this district would clear up any unsold poultry and animal meat products (A, D), pack them into boxes, and send them to refrigeration (l). However, it should be noted that some vendors who sold groceries (G) were still in business. The third category (Group III in Fig 5(b)) is the sampling points: g, j, and k. This category exhibits the largest proportion of the 3rd stage particle size, followed by the 1st or 4th stage particle size. Among these sampling points in Fig. 6, point j ($2.47 \times 10^3$ CFU m$^{-3}$) and point k ($2.98 \times 10^3$ CFU m$^{-3}$) were 1.85–2.75 times higher than that during operation. Taking point k as an example, the 3rd stage particle concentration was $7.66 \times 10^2$ CFU m$^{-3}$, accounting for 25.70% of all particles, and the 4th stage particle concentration was $7.54 \times 10^2$ CFU m$^{-3}$, accounting for 25.29% of all particles. Similarly, point l had a 1st stage particle concentration of $2.00 \times 10^2$ CFU m$^{-3}$, accounting for 36.10% of all particles, while the 3rd stage particle concentration was $1.41 \times 10^2$ CFU m$^{-3}$, accounting for 25.45% of all particles. The main sources of bacterial pollution were the stalls still selling various grains (G) and cooked food (H); in addition, vehicles were driving in and out carrying unsold goods and removing waste.

3.7 Airborne Bacteria Identification in an Indoor TWM and their Possible Pathogenicity

There were 38 different bacterial colonies identified by culture consisting of 23 Gram-negative pure strains and 15 Gram-positive pure strains in Table S5. All of the species mentioned above have been frequently observed in the TWMs. Table 2 shows the most frequent appearance (> 50%) of top three bacterial species identified during operation hours of the market. *Kocuria marina* (Strain No. I-B-2) was measured as having a 100% appearance frequency in all sampled locations. *Kocuria carniphila* (Strain No. I-B-4) was measured as having a 50% appearance frequency in all sampling locations. *Staphylococcus sciuri* (Strain No. I-B-5) was measured as having a 75% appearance frequency in all sampling locations. These representative strains of AB could be suggested to be useful as possible biological indicators at public indoor TWMs in Taiwan.

*Kocuria* sp. is Gram-positive, aerobic, coccoid, non-encapsulated, non-halophilic, and non-endospore-forming species of bacteria. Their normal habitat includes a wide range of ecological niches, such as soil, the rhizoplane, fresh water, mammalian skin, and the oral cavity (Kim et al., 2004). There has been a rise in the incidence of infections caused by *Kocuria* spp. and these result in both superficial infections and deep-seated/invasive infections (Kandi et al., 2016). One species, *K. marina* has been reported to have been isolated from marine sediment and can grow in a temperature range of 4–43°C and in the presence of up to 15% NaCl (Kim et al., 2004). Another species, *K. carniphila* has a diameter of 1.0–1.5 µm and shows good growth in the ranges 28–37°C and pH 7.0–9.1; it has been reported to have been isolated from meat (Kroppenstedt, 2005).

*S. sciuri* is a Gram-positive, oxidase-positive, coagulase-negative member of the bacterial genus *Staphylococcus* and forms clusters of cocci. *S. sciuri* is widespread and can be isolated from a variety of pet animals, wild/domestic animals, insects, the environment (soil, sand, water, air
### Table 2. AB that appear frequently in the TWM are identified.

<table>
<thead>
<tr>
<th>No.</th>
<th>Speciesb (NCBI Identify %)</th>
<th>Gram stains (Scale: 10 µm)</th>
<th>Shape</th>
<th>Appearance at sampling locations</th>
<th>Appearance of frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria I-B-2</td>
<td><em>Kocuria marina</em> (98%)</td>
<td>G+ stains</td>
<td>bacillus</td>
<td>a, b, c, d, e, f, g, h, i, j, k, l</td>
<td>100% (12/12)*</td>
</tr>
<tr>
<td>Bacteria I-B-4</td>
<td><em>Kocuria carniphila</em> (99%)</td>
<td>G+ stains</td>
<td>cocci</td>
<td>a, e, f, g, h, i</td>
<td>50% (6/12)*</td>
</tr>
<tr>
<td>Bacteria I-B-5</td>
<td><em>Staphylococcus sciuri</em> (99%)</td>
<td>G+ stains</td>
<td>bacillus</td>
<td>a, b, c, d, e, f, g, h, l</td>
<td>75% (9/12)*</td>
</tr>
</tbody>
</table>

* Number of sampling points the bacteria were found/Total number of sampling points (12).

The three species described above have been identified previously as present in many TWMs and this study verifies them as the dominant species present in the various-sized bioaerosols investigated in this study. All three species might present a risk of infection to human beings in TWMs if individuals are exposed to a high concentration of AB. To protect the health of workers in indoor TWMs, it is important to maintain good environmental sanitation across the vendor-selling facilities during operations. It should be noted that the AB bacteria sampled, isolated, and identified included large numbers of *Kocuria marina* (100% appearance frequency), *Kocuria carniphila* (50% appearance frequency), and *Staphylococcus sciuri* (75% appearance frequency). The staff of TWMs and specific types of customers of TWMs, such as the elderly and children, are recommended to wear medical masks or similar equipment in order to prevent biological contamination when they are shopping at an indoor TWMs.

### 3.8 Limitations in this Study

This study evaluates the concentration, size distribution, and stains of airborne bacteria present in a typical Taiwan indoor traditional wet market during operations and after operations. However, this study has some limitations. First, carrying out an experiment in a single day limits the number of samples collected; this was because the operating committee of the TWM only allowed a restricted time for the study in order to minimize inconvenience to customers and market staff. It was impossible to sample the ABs of this TMW during regular opening hours on multiple days. Nevertheless, since selling products on stalls is always fixed at the same location every day, it is reasonable to infer the AB distribution came from similar biological sources and customer activities at the TMW. Secondly, this study only focus on this specific TWM and only compare the AB concentrations during operation and after the operation. It was carried out only one day. The current results thus lack time-series data for this market, and only a limited number of study locations are described.
4 CONCLUSIONS

A descriptive case study examining the size distribution of the ABs present in a typical indoor wet market. The AB concentration during operation hours at the indoor TWMs investigated in this study was found to be $1.55 \times 10^3$ CFU m$^{-3}$. The distribution of bioaerosols was affected by the products sold and the various activities of staff/consumers. The vendors selling fresh poultry and livestock meat were found in areas with a higher concentration of AB. The highest percentage of AB had a size distribution in the range of 2.1–3.3 $\mu$m, and this contributed 25.76% of the AB during market operation. These specific-sized particles are very likely to be deposited in the bronchial tubes of the respiratory tract after being inhaled by humans. After operation hours, the concentration was reduced to $9.56 \times 10^2$ CFU m$^{-3}$ and the largest proportion of bioaerosols were in the range $>7.0$ $\mu$m, which contributed 25.25% of AB present.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This study was supported by the Taiwan MOST project; grant numbers MOST106-2221-E-031-001-MY2.

Conflicts of Interest
The authors declare no conflict of interest regarding the publication of this paper.

Author Contributions
Conceptualization, Y.-T.C.; methodology, C.-C.C. and Y.-T.C.; investigation, C.C.C.; writing, C.-C.C. and Y.-T.C.; original draft preparation, W.-T.L., I.-C.C., Y.-T.C, and S.-H.L.; writing, review and editing, W.-T.L., Y.-T.C., and I.-C.C.; supervision, Y.-T.C.; project administration, Y.-T.C.; funding acquisition, Y.-T.C. All authors have read and agreed to the published version of the manuscript.

Supplementary Materials
Supplementary material for this article can be found in the online version at https://doi.org/10.4209/aaqr.220402

ACKNOWLEDGMENTS

The authors thank Dr. Chou, Hsi-Ling for assisting with the airborne bioaerosols sampling in this study and Dr. Wei, Da-Jiun who helped to visualize the figures in this paper. Part of the results/discussion was presented at the 12th Asian Aerosol Conference (AAC), 2022.

REFERENCES


Reanprayoon, P., Yoonaiwong, W. (2012). Airborne concentrations of bacteria and fungi in...

