ORIGINAL ARTICLE

Evaluation of Indoor Bacterial Load and Its Association with Respiratory Symptoms among Children in Kuantan Early Childhood Centres

Nur Aliya Maisarah Mohammad Maliki[1], Nurul Falah Mohamad Zaky[1], Noor Fatihah Mohamad Fandi [1*], Norsyuhada Alias[1], Maryam Zahaba[2], Norashikin Sopian[3]

[1]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Pahang, Malaysia.
[2]Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia, Pahang, Malaysia.
[3]Faculty of Science and Industrial Technology, University Malaysia Pahang, Malaysia.

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ABSTRACT

Background: Indoor air quality (IAQ) plays a crucial role in ensuring a healthy and comfortable environment, particularly in early childhood education centers (ECEs). Children are especially vulnerable to poor IAQ due to their developing immune and respiratory systems. This study investigated the indoor bacterial load and the associated respiratory symptoms among children in selected ECEs in Kuantan, Pahang. **Methods:** A cross-sectional study was conducted in two urban ECEs from March to April 2025 to assess indoor bacterial counts and physical parameters (temperature and relative humidity), which were measured with the Surface Air System SAS IAQ 100 and the Testo Air Meter 440, respectively. Potential respiratory symptoms (cough, wheezing, phlegm, and breathlessness) were assessed in 30 children aged from 3 to 6 years using a parent-administered questionnaire adopted from the American Thoracic Society. **Results:** Both ECEs A and B recorded total bacterial counts exceeding the Industry Code of Practice (ICOP) limit, with concentrations of 617.22 CFU/m^3 and 647.22 CFU/m^3, respectively. The recorded temperatures (27.41℃ and 27.96℃) also surpassed the ICOP comfort range. Among the children, cough was the most commonly reported symptom (23.3%), followed by wheezing (16.7%), and phlegm (10.0%). No case of breathlessness symptom was reported in children. Statistical analysis indicated no significant association between the indoor bacterial load and the respiratory symptoms observed. The study was limited by the small sample size and the short monitoring duration, which may not fully represent variations in bacterial levels across different times or seasons. Additionally, the high bacterial counts observed might have been influenced by occupant density, cleaning practices, and ventilation patterns, which were not controlled in this study. However, the present findings provide valuable baseline data on indoor bacterial concentrations, where the local evidence remains limited. **Conclusion:** Overall, the findings indicate early signs that IAQ in ECEs may fall below recommended standards, underscoring the need for better cleanliness and ventilation practices to improve indoor environments and protect children's health. These results also provide useful direction for future research, monitoring efforts, and targeted interventions.

INTRODUCTION

Indoor air quality (IAQ) plays a crucial role in human health, as individuals spend almost 90% of their time indoors (Khamal et al., 2019). The World Health Organization (WHO) reported that indoor air pollution (IAP) is responsible for 3.8 million deaths annually, including 570,000 children under five who die from respiratory infections linked to air pollution and second-hand smoke (WHO, 2017). Monitoring IAQ is therefore essential in early childhood education centres (ECEs), where children are very young, typically between one and six years old, and highly vulnerable due to their immature respiratory and immune systems (Ezinwanne & Okoro, 2023). In addition, children in ECE spend long hours in enclosed indoor environments with high occupant density and having frequent close interactions. Moreover, their behavioural patterns, such as close-contact play, frequent touching of surfaces, and floor-level activities, further increase their susceptibility to airborne bacteria and other indoor biological contaminants.

Indoor pollutants originate from multiple sources, including both indoor and outdoor environments. Physical contaminants such as particulate matter ($PM_{2.5}$ and PM_{10}), dust, and smoke can accumulate in enclosed spaces with inadequate ventilation, aggravating respiratory conditions in children (Pumkaeo et al., 2020). Biological contaminants, including bacteria, fungi, viruses, pollen, and dust mites, are equally significant (US EPA, 2024). Studies in Malaysian schools have reported *Staphylococcus spp.*, *Pseudomonas spp.*, and *Bacillus spp.* as dominant bacterial genera, while *Aspergillus*, *Penicillium*, and *Fusarium* were among the common fungi (Hussin et al., 2011). The presence of these biological pollutants indoors can significantly affect occupants'

*Corresponding author.
E-mail address: fatihahfandi@iium.edu.my

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health by causing allergic reactions, respiratory problems, and infections, particularly among children whose immune and respiratory systems are still developing (Ezinwanne & Okoro, 2023; Kumar et al., 2021; EPA, 2024). Continuous exposure to bacteria and fungi in poorly ventilated environments may lead to symptoms such as coughing, wheezing, and phlegm, which could interfere with children's learning and overall well-being (Kumar et al., 2021; Gorny, 2019). Human activities such as coughing, sneezing, and cleaning, as well as the presence of damp or moldy spaces, further contribute to elevated microbial loads indoors (Kumar et al., 2021; Jabeen, 2023).

The Industry Code of Practice on Indoor Air Quality (ICOP IAQ, 2010) provides reference limits for indoor workplace spaces, specifying acceptable ranges for temperature (23-26°C), relative humidity (40-70%), air movement (0.15-0.50 m/s), and biological thresholds of 500 CFU/m^3 for bacteria and 1000 CFU/m^3 for fungi (DOSH, 2010). However, compliance with these standards in indoor playschools and early education settings has not been well documented. Exposure to biological aerosols can cause allergic rhinitis, hypersensitivity, pneumonitis, and communicable diseases such as measles, influenza, and chickenpox (Kumar et al., 2021). Symptoms such as coughing, wheezing, shortness of breath, and lethargy may impair children's well-being and ability to learn (EPA, 2024; Ezinwanne & Okoro, 2023).

Despite the recognised risks, research on IAQ in playschools, particularly among children aged one to six years, remained limited. Prior studies in Kuantan and Pekan either did not report age groups (Hizzri et al., 2018), focused on older children (Hazrin et al., 2018; Madureira et al., 2018), or examined only particulate matter (Maryam et al., 2018). These areas were selected because they represent rapidly developing urban and industrial regions in Pahang, where increased human activity and traffic emissions may influence indoor and outdoor air quality (Nazri et al., 2017; Maryam et al., 2018). Considering that children have higher respiratory rates compared to adults (Choupiliadis & Bhardwaj, 2022), they are more susceptible to bioaerosol exposure. However, empirical evidence linking microbial contamination to health outcomes in this age group is still scarce. This study aims to address this knowledge gap by assessing the IAQ of selected early childhood education centres in Kuantan, Pahang and examining the association between indoor bacterial load and respiratory symptoms among children at ECEs.

MATERIALS AND METHODS

Study Design and Study Location

This cross-sectional study was conducted at two private ECEs in Kuantan, Pahang, Malaysia, referred to as ECE A and ECE B. ECE A is located in Bandar Indera Mahkota, a suburban area of Kuantan that has undergone urban development and mixed residential–commercial land use. Meanwhile, ECE B is located in a mixed commercial–residential area along Jalan Tun Ismail, a major arterial road of Kuantan known for high traffic density and busy commercial activity (Phoo Ken Seng, 2011; Vun Hon Yuan, 2016). The selection of the study sites (Figure 1) was based on accessibility and the centres' willingness to participate.

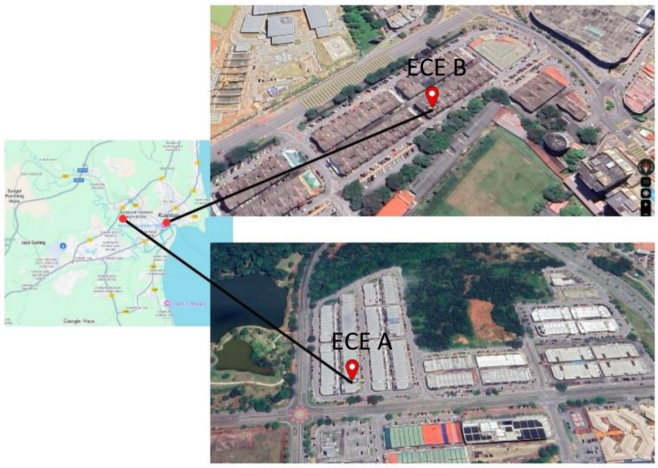

Figure 1: Location of the studied ECE in Kuantan, Pahang. ECE A is located in Bandar Indera Mahkota, while ECE B is situated along Jalan Tun Ismail.

Subject Selection

The study population comprised children aged 3 to 6 years who were enrolled in the selected ECEs in Kuantan. Eligible participants met the following inclusion criteria: children who had attended the ECEs for at least three months to ensure adequate exposure to the ECE's indoor environment, attended the ECE for a full eight-hour day, had no diagnosed chronic respiratory or immunocompromising conditions, and whose parents or guardians provided written informed consent for participation. The inclusion criteria for parents or guardians required that they be the legal parent or guardian of the participating child, be able to comprehend and respond in either Bahasa Melayu or English, be willing to participate in a health-related questionnaire, and reside in Kuantan, living in the same household as the child.

Walkthrough Survey and Questionnaire

A walkthrough survey was conducted to observe the overall conditions of the ECEs, identify suitable sampling locations, and document the types of air-conditioning systems used. Information on children's respiratory symptoms was obtained using a translated version of the American Thoracic Society (ATS-DLD-78-A) questionnaire, originally developed by the American Thoracic Society (Helsing et al., 1979; Smith et al., 2008). The questionnaire also included sections on socio-demographic background and potential home exposure factors. This instrument has been previously validated in local studies conducted by Juliana et al. (2001, 2014) and Sopian et al. (2021). Data were analysed using descriptive statistics to report the prevalence of respiratory symptoms, while Fisher's exact analysis was performed to examine associations between total bacterial count and respiratory health outcomes. All analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) Version 20.

Indoor Air Quality Assessment

After measuring the floor areas of both ECEs, the total measurement area for each centre was calculated. The number of sampling points was then determined by referring to the Industry Code of Practice on Indoor Air Quality (ICOP, 2010). According to the ICOP, at least one real-time air sampling point is recommended for every 500 m^2. The measurement area of each ECE was less than 300 m^2, which meets the requirement for at least one real-time air sampling point per centre. In each ECE, the main activity area and dining area were chosen as sampling sites, as these spaces were commonly used for teaching, learning, and eating activities. During the air sampling, the ECEs were maintained under their normal operating conditions, with air-conditioning units running and all windows kept closed, to accurately reflect the typical indoor environment of the centres.

Testo Air Meter 440 was used to measure physical parameters (temperature and relative humidity). Surface Air System (SAS) IAQ 100 air sampler was used to collect airborne microorganisms by using an agar plate, allowing quantitative total bacterial count analysis. Both instruments were placed at a height of 65-80 cm above the ground (children's breathing zone) and positioned at the main activity area, at least 0.5 m away from walls, corners, and windows. They were oriented away from air-conditioning units and were kept out of direct sunlight, in accordance with ICOP (2010) guidelines. For the SAS IAQ 100 air sampler, the impaction method was employed, whereby air was drawn through the perforated lid at a controlled rate of 100 L/min, thus directing airborne microorganisms onto the surface of the agar plate. After sampling, the agar plate is incubated to allow microbial colonies to grow, and the results are expressed as colony-forming units per cubic meter (CFU/m^3) using the formula shown below (Anua, Haris and Mazlan, 2020):

$$\text{CFU/m}^3 = [T \times 1000) / [t(min) \times F(L/min)]$$

Where:

T: Number of colonies counted from the agar plate

1000: Conversion of a factor of litres to cubic meters

t: Duration of sampling (in minutes)

F: Pump flow rate (L/min)

For physical parameters, data were recorded at 9:00 a.m. to 10:00 a.m., 10:00 a.m. to 11:00 a.m., and 11:00 a.m. to 12:00 p.m. Within each hour, six readings were taken with a 1-minute interval. From this, the average value was determined. Meanwhile, for bacterial load in the indoor ambient, the triplicate technique was used with the device, with 2-minute intervals set for each sampling. The sampling procedure utilised approximately 12 plates between 9.00 a.m. and 12 p.m., with 4 plates used each hour. This IAQ sampling was conducted in March and April 2025, during the normal school operation period. This IAQ sampling was conducted on two separate days within the same week. These devices were only handled by the researcher teams and were not applied to children in schools. Therefore, the safety of children was maintained.

Microbiological Analysis

Nutrient agar was used for environmental monitoring of bacterial sampling. After the data collection, each plate was incubated for 24 to 48 hours at 37°C. Upon the incubation period, the visible colonies of bacteria were counted to determine the total bacterial load. With adjustments made according to the correction table from SAS, the result was expressed in colony-forming units per cubic meter (CFU/m^3).

Ethical Approval

This study was conducted upon the approval of the IIUM Research Ethics Committee (IREC), as it involves human participants with the approval number IREC 2025-KAHS/DBMS15. The ECE staff, parents, and guardians were informed firsthand by explaining the procedure of this study. The permission letter was distributed to parents along with the questionnaire by the teachers. Instructions to use the device were followed by the researcher and data collection remained anonymous.

RESULTS

Sociodemographic Data of Respondents

A total of 33 parents from both ECEs were approached, but only 30 met the eligibility criteria and participated in the study. Among these 30 children, 17 (56.7%) were male and 13 (43.3%) were female, and all were Malay. More than half of the participants, 17 (56.7%), were enrolled in ECE A, while the remaining 13 (43.3%) were from ECE B. In terms of age distribution, children aged four made up the largest group (36.7%), followed by those aged five (30.0%), six (23.3%), and three (10.0%). No students aged one or two years were enrolled in either of the two ECEs. Most children had been enrolled in their respective ECE for three months to one year (43.4%), while others had been enrolled for two years (10.0%) and three years (3.3%).

Comparison of Building Characteristics of the Studied ECEs

The floor area of ECE A (134 m²) is smaller than that of ECE B (234 m²). Both centres have natural ventilation through windows and mechanical ventilation via air-conditioning; however, only the air-conditioning systems were used in both ECEs. ECE A is equipped with a cassette-type air conditioner, while ECE B uses a split-type air conditioner. During sampling, the air-conditioning units were switched on, and the fans were turned off. Walkthrough inspections revealed that although windows were installed in both ECEs, they remained closed while the air-conditioning was operating. Overall, the general cleanliness in both centres was well maintained. Additionally, both ECEs had a toilet located near the main activity and dining area. Both centres are located in concrete, double-storey shop-lot buildings, with ECE A operating on the upper floor and ECE B on the ground floor.

Total Bacterial Count and Physical Parameters at the Selected ECEs

Table 1 shows that ECE B recorded a slightly higher total bacterial count than ECE A. The bacterial concentrations at ECE A and B were 617.22 CFU/m³ and 647.22 CFU/m³, respectively, corresponding to 1.2 and 1.3 times above the ICOP standard limit (DOSH, 2010). Despite these differences, the total bacterial counts between the two ECEs were not statistically significant (p>0.05). Meanwhile, the measured temperature for both ECEs exceeded the ICOP (DOSH, 2010) guideline, with the mean values of 27.41 and 27.96, respectively. Relative humidity levels were within the acceptable range, recorded at 65.62% for ECE A and 69.38% for ECE B. However, both values were close to the upper permissible limit of 70%.

Table 1: Total Bacterial Count (CFU/m^3) and level of Physical Parameter at the Studied ECEs in Kuantan, Pahang.

IAQ Parameter	ECE A Mean ± SD	ECE B Mean ± SD	DOSH ICOP (2010)
Total Bacterial Count (CFU/m^3)	**617.22 ± 352.23***	**647.22 ± 338.09***	≤500
Temperature (°C)	**27.41 ± 1.51***	**27.96 ± 1.53***	23-26
Relative Humidity (%)	65.62 ± 9.33	69.38 ± 5.43	40-70

(*) Above the ICOP acceptable limit

Prevalence of Respiratory Symptoms Experienced by Children

Table 2 shows that the respiratory symptoms observed in children at the two ECEs included cough, phlegm, and wheezing, with no case of breathlessness documented. The prevalence of cough was comparable between the centres, at 23.5% in ECE A and 23.1% in ECE B. Phlegm was reported in 11.8% of children in ECE A and 7.7% in ECE B, giving an overall prevalence of 10.0%. Similarly, wheezing was observed in 17.6% of children in ECE A and 15.4% in ECE B, with an overall prevalence of 16.7%. In the overall population of studied children, cough (23.3%) was the most commonly reported symptom, followed by wheezing (16.7%), and lastly phlegm (10.0%).

Table 2: Prevalence of Respiratory Symptoms Experienced by Children ECEs in Kuantan, Pahang.

Respiratory Symptoms	ECE A N = 17(%)	ECE B N = 13(%)	Total N= 30(%)
Cough			
Yes	4 (23.5%)	3 (23.1%)	7 (23.3%)
No	13 (76.5%)	10 (76.9%)	23 (76.7%)
Phlegm			
Yes	2 (11.8%)	1 (7.7%)	3 (10.0%)
No	15 (88.2%)	12 (92.3%)	24 (90.0%)
Wheezing			
Yes	3 (17.6%)	2 (15.4%)	5 (16.7%)
No	14 (82.4%)	11 (84.6%)	25 (83.3%)
Breathlessness			
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)
No	17 (100.0%)	13 (100.0%)	30 (100.0%)

Association between Total Bacterial Count and

Respiratory Symptoms Experienced by Children in both ECEs

Table 3 presents the association between total bacterial count levels (high > 617.2 CFU/m³ and low ≤ 617.2 CFU/m³) and respiratory symptoms among children in both ECE using Fisher's Exact Test. The analysis showed no statistically significant association between total bacterial count categories and the presence of respiratory symptoms (p > 0.05). These findings indicate that, within the sampled ECEs, variations in total airborne bacterial levels did not correspond to differences in reported respiratory symptoms among the children.

Table 3: Association between Total Bacterial Count (CFU/m^3) and Respiratory Symptoms Experienced by Children in both ECEs

Variables	Total Bacteria Count (CFU/m^3)		*p*-value
	High (> 617.2)	Low (≤617.2)	
	n (%)		
Cough			
Yes	4 (57.1)	3 (42.9)	1.000
No	13 (56.5)	10 (43.5)	
Phlegm			
Yes	2 (66.7)	1 (33.3)	1.000
No	15 (55.6)	12 (44.4)	
Wheezing			
Yes	3 (60.0)	2 (40.0)	1.000
No	14 (56)	11 (44.0)	
Breathlessness			
Yes	0 (0)	0 (0)	1.000
No	17 (56.7)	13 (43.3)	

N=30, Fisher's Exact test
(*) Significant at p < 0.05

DISCUSSION

Total Bacterial Count in Playschools

In comparing the two ECEs, ECE B demonstrated a higher total bacterial count than ECE A. Although this difference was not statistically significant, it is noteworthy that the total bacterial counts in both centres exceeded the ICOP standard limit by approximately 1.2–1.3 times. The elevated bacterial concentration observed in ECE B could be partly attributed to higher occupancy during sampling, as approximately 35 individuals were present compared with fewer than 30 in ECE A. Hospodsky et al. (2012) reported that human presence can increase airborne bacterial genome concentrations by up to 66-fold in an occupied classroom compared to vacant conditions, contributed largely due to resuspended dust and direct shedding from hair, skin, and nostril passages.

The present findings recorded a slightly higher average temperature in ECE B (~27.96 °C) compared to ECE A (~27.41 °C), which may support enhanced bacterial growth. Moreover, the average relative humidity in ECE B (69.38%) was higher than in ECE A (65.62%), and elevated humidity is known to promote bacterial survival and activity. The combined effect of increased temperature and relative humidity likely contributed to the elevated total bacterial count observed in ECE B, consistent with previous findings showing rapid bacterial growth in hot and humid indoor environments (Albers, 2023; Anualem et al., 2019; Qiu et al., 2022). High relative humidity, in particular, can produce a damp environment that promotes the survival and growth of harmful microorganisms, including bacteria, mold, and viruses (Guarnieri et al., 2023). Additionally, studies in Malaysian laboratories found a positive association between increased indoor temperature and relative humidity and higher bacterial contamination (Yogeswaran et al., 2023).

During the walkthrough inspection, it was observed that although windows were present in both ECEs, none were open, as the indoor spaces were fully air-conditioned. This condition may have indirectly contributed to the accumulation of indoor biological aerosols released by occupants, consistent with findings in a Seremban daycare center, which was also fully air-conditioned (Khamal et al., 2019). Similar observations were reported by Onwusereaka et al. (2022), who found that most schools kept windows closed during school hours, limiting adequate ventilation. Air-conditioned centers generally have lower air-exchange rates compared to naturally ventilated facilities (Khamal et al., 2019), leading to poor ventilation, which further contributes to increased contaminant concentrations and potentially hazardous indoor environments (Albers, 2023). Inadequate ventilation reduces the dilution of pollutants released by occupants, resulting in higher indoor pollutant levels (Guo et al., 2007). Collectively, these findings suggest that the combination of inadequate ventilation, high occupancy, and elevated indoor temperature and humidity likely contributed to the elevated bacterial counts observed in the studied ECEs, which both exceed the ICOP limit of 500

CFU/m^3.

Prevalence of Respiratory Symptoms and Its Association with the Total Count of Bacteria

Based on the present findings, no significant differences in respiratory symptoms were observed between children from the two ECEs, even though total bacterial counts in both centres exceeded recommended limits. Among all participating children, cough (23.3%) was the most commonly reported symptom, followed by wheezing (16.7%) and phlegm (10.0%). These findings are consistent with previous studies in Malaysian early childhood settings. Rawi et al. (2015) reported that preschool children in Selangor showed higher odds of experiencing cough (OR = 3.23; 95% CI: 1.24–8.40) and wheezing (OR = 2.56; 95% CI: 1.02–6.47). Similarly, Khamal et al. (2019) documented a wheezing prevalence of 18.9% among children attending a daycare centre in Seremban.

Although no statistically significant associations between total bacterial counts and respiratory symptoms were detected in this study, airborne bacterial exposure remains a plausible contributor to respiratory issues among young children, as supported by previous literature. Elevated indoor bacterial concentrations are commonly linked to inadequate ventilation and high occupancy density, all of which can irritate the respiratory tract and heighten susceptibility to symptoms (Fisk, 2017; Jaben, 2023; Yusof et al., 2025). Furthermore, routine activities performed by children during school hours, such as breathing, talking, laughing, and sneezing, can release and resuspend biological aerosols into the indoor environment, further increasing airborne microbial load (Hospodsky et al., 2012; Pumkaeo & Iwahashi, 2020).

The insignificant association between total bacterial count and reported respiratory symptoms may be explained by several methodological and participant-related factors. First, the relatively small sample size and the short environmental sampling duration (3–4 hours) may not adequately reflect the children's actual day-to-day exposure levels. Second, the reliance on parent-administered questionnaires introduces limitations in accurately capturing children's respiratory symptoms, particularly when symptoms are mild and easily overlooked. This challenge is more pronounced in children aged 3–6 years, who often struggle to clearly express or describe their physical discomfort. Lampi et al. (2018) reported that older children (9–12 years) can reliably describe their symptoms at a level comparable to parental reporting; however, for younger children, symptom information depends entirely on parental observation. This limitation may also explain the absence of reported breathlessness in the present study, as younger children may not recognise or communicate such experiences effectively. Collectively, these factors, especially the reliance on parent-reported symptoms and smaller sample size, may have contributed to the non-significant associations observed between bacterial exposure and respiratory outcomes.

CONCLUSION

This study evaluated the relationship between bacterial load and respiratory symptoms among children in selected ECEs in Kuantan. Both ECEs recorded bacterial counts exceeding the ICOP limit, alongside slightly elevated temperatures and relative humidity near the upper acceptable range, which are conducive conditions to bacterial growth. Although symptoms of cough, phlegm, and wheezing were reported among children, no significant associations were observed between total bacterial count and respiratory symptoms, likely due to the small sample size. Maintaining good ventilation and cleanliness remains essential to improve indoor air quality and safeguard children's health. Larger studies across more ECEs are recommended to increase statistical power, while future research should include additional IAQ parameters such as volatile organic compounds (VOCs), particulate matter, and fungal counts, and longer data collection periods.

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