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REVIEW ARTICLE

A Systematic Review of the Concentration of Bacteria in Daycare Centers (DCCs)

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ABSTRACT - Microbes in indoor buildings have gotten a lot of interest from the scientific community and the public because of their direct relationship to human health. However, there is still a shortage of thorough data on bacterial concentration in daycare centers air. This research systematically reviews the influencing factor of bacteria growth, the concentration of bacteria and proposes a further research direction. The research article was selected from year 2011 to 2022, 11 original research that met the qualifying criteria were assessed in the major databases. The highest indoor bacteria content was identified in a study from Portugal (5256 CFU/m3), while the lowest was found in an Iranian study (453.93 CFU/m3). The concentration of bacteria indoors are varies in different seasons and the higher indoor bacteria concentrations were found in winter and spring seasons. In addition, researchers also find a positive correlation between bacterial growth with the exposure of particulate matter (PM1, 2.5, PM10). This review reveals that a short sampling period is not enough to explain and evaluate the concentration of bacteria in daycare centers. Thus, research suggests that further research be done on bacteria exposure in daycare centers because it can raise awareness among parents and staff about the need for clean air for children and provide ideas for improving daycare facilities.

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1.0 INTRODUCTION

The term "indoor air quality" (IAQ) describes the air quality inside buildings and other structures, focusing on how it affects building occupants' comfort and well-being. Poor indoor air quality is often caused by inadequate ventilation, off-gassing from construction materials, and increased occupant density and time spent indoors. According to the U.S. Bureau of Labor Statistics and National Institute of Environmental Research, indoor air quality has become an important issue since people spend most of their time inside [1][2]. Indoor air contaminants caused 4.3 million of the 7.3 million annual deaths worldwide [3]. Poor indoor air quality negatively impacts vulnerable groups like the elderly, pregnant women and children, who inhale more contaminants per kilogram of body weight. The tissues and organs in children's bodies are actively growing and hence vulnerable to harmful environmental hazards [4][5]. Headaches, fatigue, shortness of breath, and dizziness are common symptoms linked to poor indoor air quality, which can contain various physical, chemical, and biological pollutants.

In recent decades, the number of children attending daycare centers (DCCs) has risen sharply worldwide because more women are working. As a result, DCCs have become a major indoor environment for infants and young children, as well as a key place for social interaction [6]. Therefore, providing healthy indoor air quality in DCCs is critical for child development and well-being. Studies have shown that poor indoor air quality has a greater impact on these vulnerable populations than on adults. Failure to recognise and establish IAQ status can lead to both short- and long-term health issues. Prolonged exposure to biological air contaminants has been linked to allergies, asthma, cough, wheezing and other respiratory problems [7]. DCCs rank second among the most used indoor places after residential buildings [8][9]. According to [10], children who attend daycare facilities and primary schools have higher environmental exposure than adults. Their daily activities, such as eating, playing, learning, and diaper changing, when combined with poor indoor air quality at DCCs, can increase the infections to bioaerosol contaminants, especially bacteria, which thrive under certain temperatures, pH, salinity and pollutant concentrations [11]. In 2017, the World Health Organization (WHO) reported that pneumonia, often caused by bacteria like Streptococcus pneumoniae, killed 808 694 children under the age of five.

Assessing the bacteria present in DCCs is therefore important to understand transmission risks, especially of common respiratory and gastrointestinal pathogens and inform effective infection control strategies. This review systematically examines recent studies that have determined bacterial concentration in DCCs. The aims are to synthesize current knowledge on which bacteria are prevalent in DCCs and influencing factors in terms of occupant density, climate factors and seasonal variation and provide recommendations for future research priorities to better promote child health.

2.0 METHODOLOGY

This research used the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) search methodology strategy. Electronic databases were searched for published literature with a concentration of bacteria in indoor buildings. Articles published from 2011 to 2021 were searched in Scopus, PubMed, Google Scholar and

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ScienceDirect databases. The key search terms used were “concentration of bacteria in daycare center”, day care centers "indoor airborne bacteria", “bacterial concentration in indoor” and ((bacteria AND species) AND (daycare center OR indoor)). The article search query was “TITLE-ABS-KEY ((indoor AND air AND quality AND bacteria AND children) AND (school OR kindergarten OR nurseries))” AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT- TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) AND LIMIT- TO (LANGUAGE, “English”) and ((((airborne) AND (bacteria)) AND (species)) AND (indoor)) OR (daycare center)))). The Boolean Operators such as AND, OR and bracket were used as conjunctions to combine in a search resulting in more focused and productive results. A hand search of publications and a review of relevant references were conducted to ensure all related articles were not missed from the terminology used. After removing duplicate articles from the retrieved papers, the remaining publications were evaluated for research relevance based on their abstracts, provided that the selection criteria were satisfied. Full-text articles were then retrieved, reviewed and included for final review.

2.1 Selection Criteria

The inclusion criteria for this review were: (i) the original research articles published from the year 2011 to 2021, (ii) presented at least one quantitative data on the concentration of bacteria in term of mass and number concentration of particles, total colony or species, and (iii) the articles written in English. Articles were excluded if they were: (i) research conducted in a building other than the daycare centers, kindergarten or nursery, (ii) only presented data on chemical and physical parameters, and (iii) review articles, books or book chapters. If the articles were outside the scope of this evaluation, they were discarded during the initial screening.

2.2 Synthesis of Finding

PRISMA flowchart was completed to summarize the research flow of the research after finalizing the relevant articles. The included full-text articles were analyzed using a thematic analysis approach to identify key concepts and coded them into related non-mutually exclusive themes. The themes were synthesized by comparing the discussion and conclusion of the included articles.

3.0 RESULTS AND DISCUSSION

3.1 Search Finding

A total of 666 related articles were found after the initial literature search. Therefore, after the article was exported to Mendeley, the duplicated articles were automatically removed. 548 articles were excluded (Figure 1) because they were marked as ineligible by automation tools, and some of the articles were duplicated. Another 120 articles went through the screening stage, and 94 articles were excluded. Then, 26 articles were assessed for eligibility, which involved reviewing abstracts and full-text articles, resulting in 15 more articles being removed due to different study locations (n = 5), outcomes not related (n = 2), and discussion of other parameters (n =8). Finally,11 studies met the criteria as shown in Figure 1.

3.2 Influencing Factor on The Concentration of Bacteria in The Daycare Centre

The main influencing factor in determining concentration of bacteria in daycare centres is occupant density and activity. [12] indicated that daycares' relatively high indoor activity levels may contribute to the high concentration distribution of airborne bacteria. The concentration of airborne bacteria collected at 6:30 without children's presence was 390 to 440 CFU/m^3, which is five times lower than the data obtained during children's activities [13]. Similar to a study from [14], when the daycare centres are closed, the external environment has a greater influence on the bacterial community because fewer humans are around. [15] said that there is a positive correlation between indoor CO2 concentration and the concentration of bacteria due to occupancy, but this is different from [16] results, which show that there is no association was found between population density and indoor bacteria.

Other than that, temperature and relative humidity are the two most important physical parameters that influence microbe viability (RH). Bacteria and fungus may grow faster if the temperature and relative humidity are higher [17]. According to [18], temperature is an important environmental component that can influence organism growth. The majority of infections thrive at 37 degrees Celsius (body temperature). In a prior study from Iran, high-traffic regions like Yekiyedoneh (459.93 and 179.39 CFU/m^3 for indoor and outdoor, respectively) and Gologoldon (458.7 and 189.14 CFU/m^3 for indoor and outdoor, respectively) had the highest mean concentrations of airborne bacteria with temperatures ranging from 23.1 to 23.8 degrees Celsius for indoor and 23.4 to 24.0 degrees Celsius for outdoor. The results obtained from a study in Poland show that indoor humidity levels were correlated with indoor bacteria, but studies from Tehran, Iran, show that indoor bacterial bioaerosols had no significant connection with relative humidity ($p < 0.66$ and $r < 0.05$) or temperature ($p < 0.23$ and $r < 0.12$). Similarly, there was no significant association between outdoor bacterial bioaerosols and relative humidity ($p < 0.68$ and $r < 0.04$) or temperature ($p < 0.79$ and $r < 0.027$). Bacterial concentration is also influenced by seasonal variation. According to a study from Poland, the maximum overall bacterial concentration (2588 CFU/m^3) was discovered in the spring, both inside and outside a building located 50 m from the highway. Agricultural activities (during non-heating months) and biomass and fossil fuel burning for domestic purposes are the main activities at the locations (during heating periods). Therefore, the highest and lowest monthly mean concentrations of culturable airborne bacteria in Iran were recorded in February (812 CFU/m^3) (winter season) and November (188 CFU/m^3) (fall season), respectively. This is owing to two severe dust storms that occurred over Ahvaz over the winter, and these dusty lands indicate the reason for repeated dust event days, as well as the primary cause of increased bioaerosols in both indoor and outdoor environments. Lastly, a study from Korea shows that airborne bacteria and particulate matter have a positive relationship (PM10, PM2.5). In the case of PM10, the correlation coefficient ranged from 0.29 to 0.70, whereas in the case of PM2.5, the correlation value ranged from 0.01 to 0.43. PM10 was more prevalent in most of the daycare centres evaluated than in the others. Similar to Iran, it also found a positive correlation between airborne bacteria, PM, PM2.5, and PM10 concentrations in the DCCs during the study period.

3.3 Comparison of Bacteria Concentration with Guidelines or Standards

According to research undertaken by a WHO expert panel on assessing biological agents' health risks in indoor environments, overall microbial concentrations should not exceed 1000 CFU/m^3. Therefore, most of the bacteria concentrations indoors exceed the WHO 2019 standard limit. The highest indoor concentration of bacteria was in Portugal 5256 (CFU/m^3); the higher occupancy and active behavioural pattern/activity level of children in relatively compact areas could explain this observation. The second-highest indoor bacteria concentration was recorded in Poland with 2588 (CFU/m^3). This bacteria concentration was high during spring seasons both inside and outside the daycare centres labelled "S", located nearer the highway because spring is better for the survival and growth of culturable bacteria in Poland than summer [23]. As for Malaysia, indoor bacteria concentration in sub-urban areas is 3330 (CFU/m^3) and 1310 (CFU/m^3) in urban areas. Both bacteria concentrations recorded in the studies from Malaysia exceeded the WHO 2019 standard limit and the Industrial Code of Practice on Indoor Air Quality 2010, which is 500 CFU/m^3 for bacteria concentration indoors [24]. Indoor and outdoor concentrations in both areas in Iran do not exceed the standard guidelines by WHO, and the highest indoor bacteria concentration was recorded in Ahvaz with 453.93 (CFU/m^3), which is in the high-traffic regions. Figure 2 illustrates the culturable bacterial concentration in daycare centres by country.

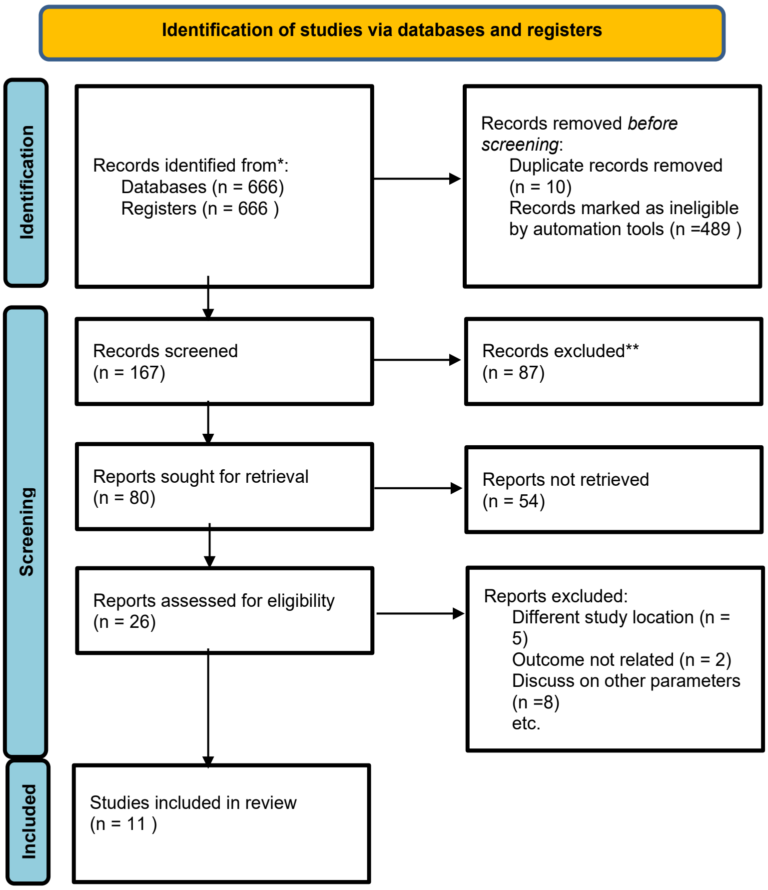

Figure 1. Flow chart of article search strategy according to the PRISMA

Table 1. The characteristics of daycare centres by country

Country	Region	Ventilation types	Number of occupants	Indoor bacteria concentration
Iran [19][20]	Industrial	Natural (windows)	15-30 children, 4-10 staff	143 CFU/m3
Poland [13]	Industrial	Natural (buildings' air duct system)	9-25 children, 1-2 staff	2588 CFU/m3

Table 1. (cont.)

Country	Region	Ventilation types	Number of occupants	Indoor bacteria concentration
Korea [21]	Residential Commercial Construction	Natural (windows) Natural & Mechanical (HVAC system)	13-20 children	898.5 CFU/m3
Portugal [6]	Urban	Natural & Mechanical	17 children	5256 CFU/m3
Hong kong [16]	Residential Business district Large community	Natural (windows)	30 children	N/A CFU/m3
Malaysia [22]	Urban Sub Urban	Natural (doors & windows) Mechanical (air conditioners)	23-24 children 10-32 children	3330 CFU/m3
America	N/A	Mechanical (HVAC system)	200 children	N/A CFU/m3

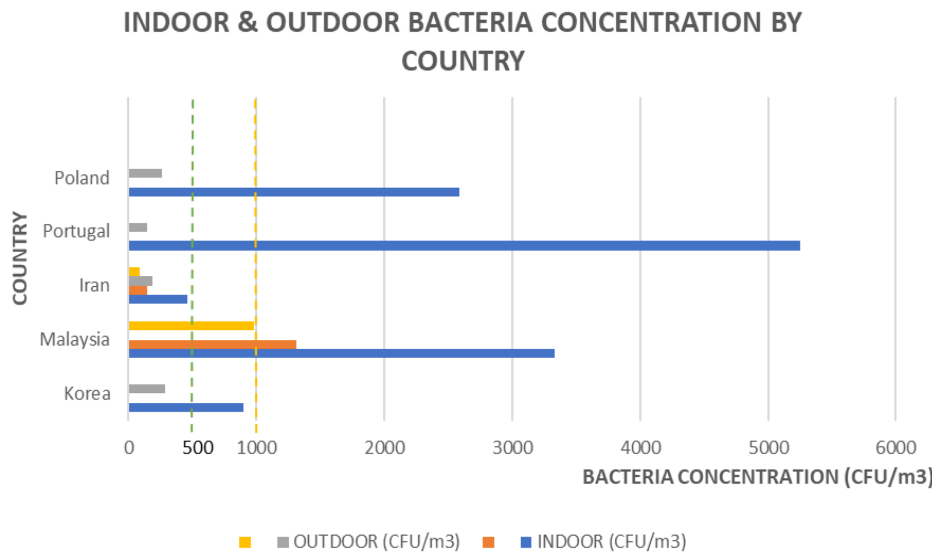

Figure 2. The culturable bacterial concentrations in daycare centers by country

*The yellow line represents WHO 2019 standard 1000 CFU/m^3 for bacteria exposure in indoor and the green line represents the Malaysia Code of Practices 2010 standard 500 CFU/m^3

Table 2. Summary on the instrumentation, standard method, study duration and seasons of studied article

Country	Instrumentation	Standard method	Study duration	Seasons
Hong kong	- MO-BIO PowerSoil DNA isolation kit - Gel electrophoresis and micro-ultraviolet spectrophotometry - MyCycle PCR instrument - Automated sequencer	- Quantitative polymerase chain reaction (PCR) was used to determine the concentration of bacteria - Terminal restriction fragment length polymorphism method was used to predict the phylogenetic airborne bacterial community structures.	Short term	N/A
Korea	- Single stage Anderson samplers - Tryptic Soy Agar - Petri dishes	- Anderson principle	Short term	Summer, Autumn, Winter

Table 2. Summary on the instrumentation, standard method, study duration and seasons of studied article

Country	Instrumentation	Standard method	Study duration	Seasons
Malaysia	- Nanodrop, gel electrophoresis - Merck Mas-100 Eco, a microbial air sampler - Media plates	- Airborne microbial sampling using NIOSH method and Surface Air System Indoor Air Quality (SAS IAQ). - Anderson principle for biological sampling - Bacterial identification using PCR	Short term	N/A
Saudi Arabia	- Culture medias [Blood agar, MacConkey and Chocolate agar]	- Bacterial species differentiation was done by gram stain, biochemical tests, API for *Staphylococci* and API 20E - Oxidase, Catalase, Coagulase & IMViC, were performed to confirm bacterial isolates	Short term	N/A
Poland	- Six-stage Andersen cascade impactor - calibrated pump - agar plates (bacterial aerosols) - Automatic portable monitors - Petri dishes	- Bacteria identification (morphology, Gram staining and endospore formation) - Bacteria differentiation using analytical profile index (API) biochemical tests	Short term	Winter, Spring
Iran	- Microbial air sampler - Tryptic soy agar (TSA) - Culture media - Petri dishes - Agarose gel electrophoresis.	- US EPA sampling guideline - Bergey's Manual and biochemical tests were applied for identification of bacteria species - Detection of isolated bacteria based on PCR	Short term	Winter, Spring, Autumn, Summer
Portugal	- Single-stage microbiological air impactor - Tryptic Soy Agar (TSA)	- NIOSH method 0800 - European Standard, 2000 (EN 13098)	Short term	Winter

3.4 Concentration of Bacteria in the Daycare Centre

Bacteria can be classified in daycare centres based on the number of concentrations, total colony size, and bacteria species. The concentration of bacteria in indoor and outdoor air can be measured using various techniques and conventional methods. As a result, various studies may employ a variety of instruments and relate to various standard procedures. The instruments, standard method for bacteria identification, study term, and study seasons of bacteria at daycare centres in each nation are summarized in Table 2. The majority of research employs single-stage Anderson samplers, tryptic soy agar, media plates, and a calibrated pump for bacteria sampling in daycare centres [6], [13], [19][21][22][25]. Furthermore, the Anderson principle, NIOSH technique, and Bergey method, as well as PCR, gram stain, and morphological characteristics, were employed to determine bacterial concentration and species. In comparison, the instruments used in the Hong Kong and Malaysia studies were slightly different because they employed gel-electrophoresis and a DNA isolation kit to collect microorganisms from airborne particulate matter such as PM10 and PM2.5. The whole study period of the examined article is comprised of short-term investigations. Because of a lack of time, money, and instrumentation, no country has conducted a long-term investigation. Four of the nine studies did not consider seasonal fluctuation in bacterial concentration because some of them are focused on other factors, such as ventilation types or urban and sub-urban regions. Apart from that, Gram-positive cocci, non-sporing Gram-positive rods, sporing Gram-positive rods from the Bacillaceae family, and Gram-negative rods are the most common bacteria genera detected in daycare centres in Poland. Gram-positive cocci are the most common bacteria found indoors, but Gram-positive rods are more common outside. The findings of bacterial species identification for aerosols in the outside environment revealed that Gram-positive bacilli-forming endospores made up the biggest percentage of the entire bacterial flora, accounting for 43% in the winter and 52% in the spring and Gram-positive non-sporing rods were the second most often isolated bacterial, accounting for 29% and 27% of all isolates in winter and spring, respectively. The smaller particles of bacteria are more frequent in the indoor air of daycare centres in Poland according to the size distribution of bacteria within the classrooms.

The particles with the highest number of culturable bacteria were those with a diameter of 4.7 m, which are respirable and so might be deposited in the tracheal, bronchial, or alveolar regions of the lungs. Smaller size ranges were more common, indicating higher concentrations of individual bacterial cells, which could be attributable to higher occupancy and inadequate ventilation in infant schools. In this study, they didn't investigate in detail the specific species of bacteria found at their daycare centres. Instead, they only stated, in general, the bacteria family. Differing from Iran, the most common bacteria found in detected isolates were Gram-positive bacteria, which are Bacillus subtilis and Staphylococcus, which were found in both indoor and outdoor environments in all daycare centres throughout the study. Bacillus species are commonly found in water and soil, whereas Staphylococcus species are commonly found on human and animal skin and mucous membranes [19]. Furthermore, *Bacteroidetes, Bacillus, Ruminococcus, Coprococcus, Enterobacter, Flavobacterium, Staphylococcus, Micrococcus,* and *Corynebacterium* were dominant in Hong Kong daycare centres. Similar to Hong Kong, the dominant bacteria species in Saudi Arabia is also *bacillus, Staphylococcus* spp. including *Staphylococcus. Aureus* and *Staphylococcus CoN*. Then, *staphylococcus haemolyticus, micrococcus luteus, Rothia,* and *Bacillus subtilis* are the successfully identified Gram-positive bacteria in the study from Malaysia.

4.0 LIMITATION AND RECOMMENDATION FOR FUTURE STUDIES

In several areas, information related to this topic is lacking in the current research. First of all, the evaluation period (winter season) was one of the restrictions. Another concern is the short sampling times, which can create significant differences in measurements, resulting in poor consistency and inaccurate comparisons. Other than that, air sampling was used in current research. Air sampling is thought to be adequate to represent exposure since the health impacts of biological parameters are mostly respiratory. Therefore, depending on various environmental conditions, biological aerosols have been discovered to have different patterns in their release and dispersal into the air. Hence, air sampling alone can exaggerate the biological variety in a building [26]. Indoor bioaerosol composition and concentrations are evaluated using culture-based sampling methods with well-known disadvantages. Lastly, some of the research doesn't explain why the indoor-outdoor (IO) ratio of bacteria is higher at certain seasons in their country.

After identifying some shortcomings from previous studies on the topic of the concentration of bacteria in daycare centres, there are some recommendations for future studies and recommendations to improve air quality in daycare centres, which can create a healthy environment for children. To begin, a long-term study of the effects of seasonal weather patterns and varied events on indoor particle and bioaerosol levels, spanning all four seasons and involving a variety of outdoor environments, is required. Aside from that, further clinical and epidemiological research is required because investigations on bacteria that affect children's health are crucial in preventing bacterial diseases and symptoms in daycare centres. Meanwhile, more focused and specific approaches for bacterial sampling methods should be investigated, as well as the differentiation of bacterial concentration in a specific season, to provide a better understanding that could be useful in preventing both long-term and short-term health problems for children and teachers in daycare centres.

Furthermore, enhancing childcare centres with sanitary/modernized engineering solutions such as air treatment equipment and double-glazed windows is one of the numerous measures that can be implemented to control the level of microbial exposure in the indoor air of daycare centres. Aside from that, air purifiers can improve indoor air quality by using exchange filters and internal controls in the system, which can save money and help daycare centres regulate temperature and humidity. The following step is to employ artificial ventilation and air conditioning systems to improve interior air quality and reduce airborne microbes in daycare centres. The final strategy is to increase the air exchange rate, which can be accomplished by opening doors and windows and using filtering devices. If the rate is too low, the air becomes stale and stagnant, which can lead to the accumulation of toxins, viruses, pathogens, and other contaminants. However, rates vary based on the type of structure, the air system in use, the desired air quality, and the structure's efficiency.

5.0 CONCLUSION

This systematic review aims to provide a comprehensive overview of existing knowledge and data on the concentration of bacteria in daycare centers, as well as an analysis of the influencing factors such as occupant density and activity, climate factors such as temperature and relative humidity, seasonal variation, particulate matter present in daycare centers, and common bacteria species in daycare centers. This study clearly demonstrates that the influencing factors of bacteria growth in indoor daycare centers across the country are very similar. The majority of the influencing factors were favorably connected with bacterial concentration, according to the researchers, and one of them is a Korean study that discovered a significant correlation between airborne bacteria and particle matter (PM10, PM2.5). The correlation coefficient varied from 0.29 to 0.70 for PM10 and from 0.01 to 0.43 for PM2.5. Aside from that, bacteria concentrations varied widely based on the season, geographical location, and outdoor air. Apart from that, the bacterial concentration was higher indoors than outside, which could be attributed to children's behaviour, occupancy, or building characteristics itself, including the building's age, furniture, and ventilation system. Micrococcus, Staphylococcus, and Bacillus are the most prevalent bacterial species discovered in daycare centers.

6.0 ACKNOWLEDGEMENTS

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7.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

8.0 AUTHORS CONTRIBUTION

N.F.A. Azman (Writing - original draft; Formal analysis)

M.K. Mokhtar (Writing - review & editing)

Z. Shahri (Writing - review & editing)

A. Norhidayah (Conceptualization; Formal analysis; Visualisation; Supervision)

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