ISBN: 978-628-95207-4-3. ISSN: 2414-6390. Digital Object Identifier: 10.18687/LACCEI2023.1.1.756

Evaluation of the concentrations of cultivable airborne bacteria present in a Higher Education Institution in Cartagena de Indias (Colombia)

Carolina Rubiano Labrador[1*], Ludys Baena De Avila[2], Rosa Acevedo Barrios[1], Dayana Doria Posada[2], Juan Rebollo Perez[1], Damaris Jimenez Uribe[2], Jorge Villalba Acevedo[1] and Jorgelina Pasqualino[2]
[1]Facultad de Ciencias Básicas. Universidad Tecnológica de Bolívar. Cartagena de Indias, Colombia, drubiano@utb.co, racevedo@utb.edu.co, jrebollo@utb.edu.co, jvillalba@utb.edu.co
[2]Facultad de Ingeniería. Universidad Tecnológica de Bolívar. Cartagena de Indias, Colombia, lmbda20@gmail.com, ddoria-97@hotmail.com, jpasqualino@utb.edu.co

Abstract* - *Bacteria are an important biological component present in bioaerosols and can cause disease in humans and animals and damage vegetation. In the city of Cartagena de Indias there is limited information on the environmental bacteria present in the air and the microbiological quality in that environment. Therefore, the purpose of this study was to evaluate the concentration of airborne bacteria present in an HEI in the city of Cartagena de Indias and to determine the influence of weather conditions on the concentration of bacterial bioaerosols. 128 outdoor air samples were collected with a cascade impactor over 4 months. Total bacteria count, and parameters such as temperature, relative humidity, and wind speed were monitored during the 16 sampling campaigns. The results showed that the average concentration of bacteria was 24.84 ± 8.02 CFU/m^3. Furthermore, they indicated that these bacterial bioaerosols are mainly constituted by gram-positive bacteria (92%), the dominant genera being Staphylococcus and Bacillus. The concentration of bacteria in the air of the IES was mainly influenced by the wind speed.

Keywords*—*air quality, bacteria, bioaerosols, meterorological factors, outdoor environment.

I. Introduction

Bioaerosols are a set of particles that are released directly into the atmosphere, and include living and dead organisms, fungal spores, plant pollen, and fragments or excretions [1]. The bacteria that can be found in the air are allochthonous microorganisms, coming from the soil and water by resuspension. The presence, concentration and diversity of aerial bacteria will depend on the season, region, meteorological parameters, and climatic conditions of each ecosystem [2]. Bioaerosols can be emitted from various types of natural sources, typical of the dynamic processes of environmental systems; or of anthropogenic origin, associated with urban activities carried out in areas with high levels of population density [3]. The emission of bioaerosols can be passively, removed from the surface of the source by turbulence (mechanical or thermal), or actively through ejection processes [4].

Digital Object Identifier: (only for full papers, inserted by LACCEI).
ISSN, ISBN: (to be inserted by LACCEI).
DO NOT REMOVE

Bioaerosols of bacterial origin have an influence on human health, since they are retained in the upper part of the respiratory system and can develop allergenic symptoms, they can penetrate the respiratory system to the alveoli and cause allergic alveolitis [5]. In recent years, exposure to bioaerosols in indoor and outdoor environments has received more attention due to its impacts on human health and biota, it is estimated that they are approximately responsible for air pollution between 5 and 34% [6]. However, there is a lack of knowledge of the exposure limit to them, epidemiological and toxicological studies are needed to establish a level of adverse effect due to exposure to bioaerosols [7].

For the context of Cartagena de Indias, studies on air quality in external and internal environments are limited. Therefore, the objective of this study is to identify and quantify bacteria present in the external environment of an HEI to determine a profile of microorganisms to which the educational community is exposed daily. The data found in this investigation can serve as a basis for future epidemiological and toxicological studies, as well as help to take the necessary preventive measures, providing relevant information in local, regional, and national investigations on air pollution.

II. Material and Methods

A. Site description

Air samples were collected from the roof of an outdoor academic building on the campus of a higher education institution (HEI) located in the city of Cartagena de Indias, Colombia (10° 22' 13.6 "N - 75° 27' 55.5" W). Cartagena is a port city on the Caribbean coast of Colombia. The city has a warm semi-arid climate, an average annual temperature of 29 °C, a relative humidity of 90%. The rainy season is typically between December and March, and the dry season between August and November [8]. The HEI is located on the outskirts of the city of Cartagena, in the "Carlos Vélez Pombo"

Industrial and Technological Park, where there are companies related to the supply of materials and services related to the energy industry and is characterized by the presence of vegetation (Fig. 1). The sample point was in the most vegetated area of the institution.

The temperature, precipitation, wind speed and humidity were taken from the record of the main synoptic station located at the Rafael Núñez airport supplied by the IDEAM (Institute of Hydrology, Meteorology and Environmental Studies).

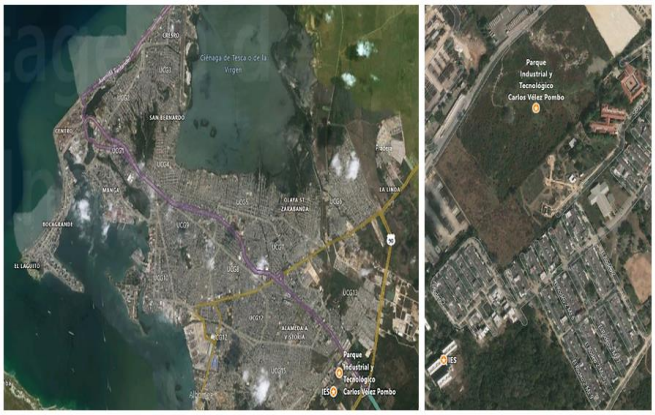

Fig 1: Geographic location of the sampling sites evaluated in this study

B. Sampling methods

A two-stage Andersen-type cascade impactor with a flow rate of 28.3 L/min was used to isolate culturable bacteria from the atmosphere. Each stage of the impactor captures a range of particles with a different size range. Stage 1 captures particles with a diameter > 7µm (area of the nasal cavity of the human respiratory system) and stage 6 captures particles with a diameter of 2 µm (area of the primary bronchi or alveoli of the respiratory system) [9].

The impactor was placed on a tripod at an approximate height of 1.5 m, to establish average human breathing conditions [10]. The flow rate used in the sampling was 28.3 L/min and the sampling time was 8 min. Duplicate samples were taken once a week between the months of June to October 2018. During the day, two sampling days were carried out, one in the morning hours (9h00 to 10h00) and another in the afternoon hours (15h00 to 16h00) [11]. The sampling time corresponded to 16 sampling campaigns and 128 samples were collected.

For each sampling, the impactor was loaded with 9.0 cm Petri dishes containing LB agar to culture the collected bacterial samples. The exposed culture plates were incubated at 37 °C for 24 h. After the incubation time, bacterial growth was verified and counted. Results were expressed as colony-forming units per cubic meter of air (CFU/m^3). CFU/m^3 was calculated as [12]:

CFU/m^3 = Colony forming units/Air volume

The air volume is calculated as the product between the pumping flow rate and the sampling duration time.

C. Identification of bacteria

Single colonies were successively subcultured on LB agar to obtain pure cultures. The phenotypic characterization was based on the description of morphology, Gram staining, endospore formation, motility, and catalase and oxidase activity [13]. Bacterial colonies were identified using the BBL Crystal kit following the manufacturer's instructions.

D. Statistical analysis

All the experimental data were evaluated statistically using Statgraphics software (version 16). The significance level was $P < 0.05$. The correlation between the concentration of bioaerosols and the meteorological variables was determined using the Pearson correlation coefficient (r).

III. Results

A. Culturable bacteria concentrations

During the sampling period, the average concentration of bacteria was 24.84 ± 8.02 UFC/m^3. The minimum concentration values of bioaerosols occurred in campaign 12 (1.1 UFC/m^3) on both time and the maximum value occurred in campaign 10 during the morning session with a value of 66.25 UFC/m^3 (Fig. 2).

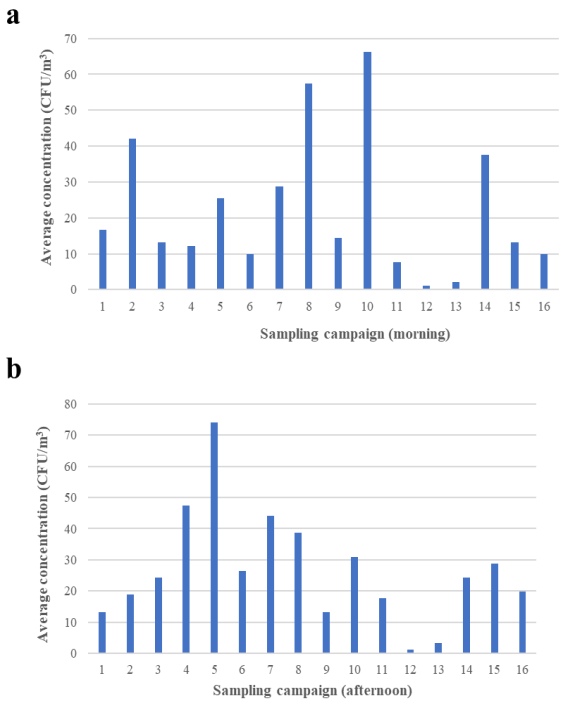

Fig 2: Average concentration of culturable bacteria at a HEI in Cartagena. a) morning time and b) afternoon time

B. Characterization and identification of culturable bacteria from air samples

A total of 13 bacterial strains were isolated from the 128 samples analyzed. The characterization of the identified strains is presented in Table 1. The morphological characterization showed the presence of colonies with pigmentation. Approximately 54% of the 13 species presented a characteristic pigmentation reflected in a specific color in their colony. On the other hand, it was determined that most of the isolated bacterial strains are Gram positive (92%), and 85% are sporoformers.

TABLE I
PHENOTYPIC CHARACTERIZATION OF THE IDENTIFIED BACTERIA

Strain code	Colony description	Morphology	Gram stain	Presence of spores	Catalase	Oxidase
B1	Orange colony, flat, entire, circular, small	Cocci	Gram(+)	-	+	+
B2	Colony yellow, flat, entire, circular	Cocci	Gram(+)	-	+	+
B3	Orange colony, flat, entire, circular. 2 mm	Cocci	Gram(+)	-	+	+
B4	Colony white, flat, lobed, irregular, large. 50 mm	Cocci	Gram(+)	-	-	-
B5	Colony yellow, raised, wavy, irregular. 5mm	Cocci	Gram(+)	-	+	-
B6	White, elevated, entire, circular colony. 1mm	Cocci	Gram(+)	-	+	-
B7	White, flat, wavy, irregular colony. 7mm	Bacilli	Gram(+)	+	+	+
B8	Colony yellow, raised, wavy, irregular. 5mm	Bacilli	Gram(+)	-	-	+
B9	Colony pink, flat, entire, circular. 4mm	Bacilli	Gram(-)	-	-	+
B10	White, elevated, entire, irregular colony. 10mm	Estreptococci	Gram(+)	-	+	-
B11	Colony white, convex, entire, circular. 2mm	Bacilos	Gram(+)	+	+	+
B12	Colony orange, flat, serrated, irregular. 4mm	Estreptococci	Gram(+)	-	+	+
B13	White, umbellate, wavy, irregular colony. 5mm	Cocci	Gram(+)	-	+	+

Five genera of culturable bacteria were identified. The dominant genus was *Staphylococcus* (70%), followed by *Bacillus* (15%) and *Enterococcus* (7%), and *Streptococcus* (5%) and *Pseudomonas* genera (3%) were identified in a smaller proportion (Fig. 3). On the other hand, the frequency of appearance of the identified bacteria is similar on both times.

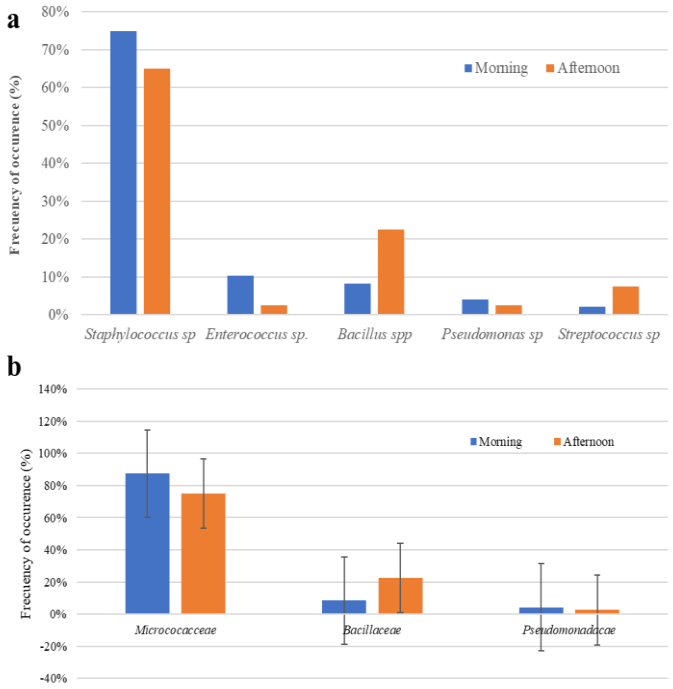

Fig 3: Frequency of bacterial bioaerosols occurrence in the morning and afternoon. According to a) genus and b) family.

C. Correlation of meteorological variables and concentration of bioaerosols

According to the information recorded by the meteorological station during the sampling time, a relative humidity between 0% and 90% was recorded, a temperature between 28-33 °C and the wind speed fluctuated between 5 and 20 km/h. Fig. 4 shows the behavior of the meteorological variables during the 16 sampled campaigns.

On the other hand, it was determined that a low correlation between temperature and bacteria concentration (r = 0.17). In addition, a low correlation was found between wind speed and bacteria concentration (r = 0.18), likewise a negative correlation was identified between relative humidity and concentration (r = -0.3). During the sampling campaign, the highest correlation was found between wind speed and bacteria concentration.

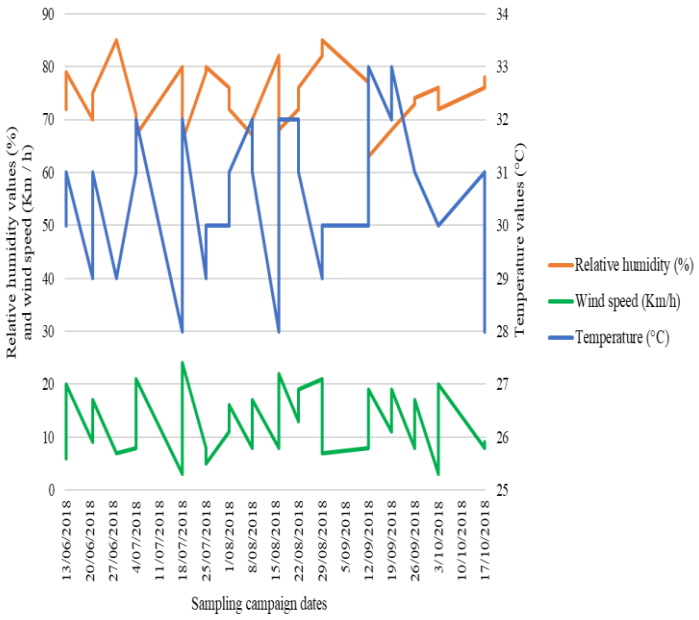

Fig 2: Meteorological variables during the 16 sampled campaigns

IV. Discussion

In this study, the presence of airborne bacteria present in a Higher Education Institution - IES in the City of Cartagena de Indias, and its correlation with meteorological variables, was evaluated. The results revealed that the concentrations of bacteria are strongly influenced by the conditions of the day, since they present a greater dispersion in the afternoon hours, which can be related to the increase in wind speed during these hours of the day, and changes in temperature and humidity. [11] reported that the concentrations of bacteria in air samples taken from a beach in Cartagena were not different between morning and afternoon, which may be related to the influence of meteorological variables on the concentration of bioaerosols. The counts of bacterial bioaerosols in coastal regions reported in previous studies are higher than those registered in this study (146 – 340 CFU/m^3) [11], however, factors such as meteorological variables and natural and variable emissions may influence these differences.

In the evaluated study area, a total of 13 bacterial strains were identified, where the majority presented pigmentation of their colonies. This pigmentation is considered a strategy used by some bacteria to survive environmental stress conditions, uch as exposure to UV rays [14]. These pigments have a protective effect against ultraviolet radiation in bacteria that develop in habitats subjected to light [15]. These results are similar to those reported by [16] and [17] who determined that the majority of bacteria presented pigmentation. These studies were carried out in the city of Cartagena de Indias and in coastal areas, with atmospheric conditions similar to those found in this investigation, verifying that the bacteria use this characteristic as a defense system.

Likewise, it was also determined that Gram-positive bacteria predominated in the samples analyzed. This may be related to the composition of their cell wall, which is made up of peptidoglycan, which allows them to be more resistant to adverse conditions such as desiccation and solar radiation [11] and [18]. These results coincide with what was reported by [1], [16-17] where Gram positive bacteria predominate compared to Gram negative bacteria, demonstrating that in the air of coastal regions this type of airborne bacteria predominates due to the main characteristic of peptidoglycan in its structure.

The bacterial strains isolated from the sampling area were related to the genera *Staphylococcus*, *Bacillus*, *Enterococcus*, *Streptococcus* and *Pseudomonas*. These results are comparable with other bioaerosol studies that have found the presence of these genera in different outdoor environments including coastal regions [11], urban environments [20-22], farms [23-24], landfills [25], and schools [26]. *Staphyloccus* spp. It was the gender that had the maximum concentration and frequency in the days evaluated. Species of the genus *Staphyloccus* are saprophytic, facultative aerobic, and colonize the surface of the skin and mucous membranes of humans and animals and are the causative agent of many severe acute and chronic infections [19]. The dominance of this genus in outdoor and indoor environments has been reported in previous studies [11] and [20-26], which is probably related to its ability to survive in unfavorable conditions, and because its presence in the environment increases through the skin, mouth, and nostrils. noses, human hair, and pets [26]. The genus *Bacillus* is a diverse group of bacteria that are characterized by being Gram-positive, sporoforming, and strictly aerobic or facultative anaerobic. Some species of this genus are the cause of respiratory diseases and food poisoning [27]. Different studies have reported that this genus is ubiquitous in bioaerosol samples [11], [20-23] and [25-26], which is related to its ability to produce spores in hostile conditions such as those prevailing in the atmosphere [21].

Species of the genus *Enterococcus* are gram-positive, non-sporoforming cocci, facultative anaerobes, and obligate fermentative chemoorganotrophs [28]. This genus is ubiquitous and has been isolated from water, soil, plants, fermented food products, and is part of the intestinal microbiota of both vertebrates and invertebrates [28]. Some clinical infections are caused by *Enterococcus* spp. including urinary tract infections, bacteremia, bacterial endocarditis, meningitis, and bacterial peritonitis [29]. This genus has been reported in previous studies as an airborne bacterium [11], [21], [23] and [25]. The genus *Streptococcus* is made up of gram-positive cocci and most are facultative anaerobes. Some species of this genus are responsible for infections such as conjunctivitis, meningitis, bacterial pneumonia, endocarditis,

and necrotizing fasciitis [30]. Previous studies have shown the presence of this genus in bioaerosols from outdoor environments [11], [20-21] and [23-24]. *Pseudomonas* spp. are Gram-negative rods and strict anaerobes and includes species that are commensals of plants and pathogens of insects, animals, and humans [31]. Some species of this genus can cause infections in animals and immunocompromised patients [32]. The presence of this genus in external environments has been previously reported [20-21] and [23-25].

In this study, a correlation was determined between wind speed and the concentration of bacteria. Wind speed is associated with the release and dispersal of spores; however, this effect varies with intensity and height [33]. The bacteria concentrations showed a low positive relationship with the wind speed since the concentrations were higher in the hours when the wind speeds were higher. According to [34], this is because the increase in wind speed can cause an increase in the elimination of bacteria from different sources. Furthermore, according to [17] higher wind speeds can cause bacteria to be resuspended in the atmosphere, suggesting that the dispersal of terrestrial bacteria increases during strong winds.

V. Conclusions

Outdoor concentrations of bacterial bioaerosols were evaluated in a higher education institution in a coastal region (Cartagena de Indias, Colombia). We found that the prevalent bacterial genera in the sampled area were *Staphylococcus* and *Bacillus*, and that wind speed can influence the concentration of bacteria in the air in this area. This study provides useful information to generate policies for the benefit of the academic and administrative community of the analyzed university. Additional studies are required to determine the air quality in indoor environments to determine the impact of bioaerosols on the population of this institution.

Acknowledgment

This work was supported by a grant from Universidad Tecnológica de Bolívar.

References

[1] J. Fröhlich-Nowoisky et al., “Bioaerosols in the Earth system: Climate, health, and ecosystem interactions”, Atmos. Res., vol. 182, pp. 346–376, 2016.

[2] X. Zhong, J. Qi, H. Li, L. Dong, y D. Gao, “Seasonal distribution of microbial activity in bioaerosols in the outdoor environment of the Qingdao coastal region”, *Atmos. Environ. (1994)*, vol. 140, pp. 506–513, 2016.

[3] A. E. Haddrell y R. J. Thomas, “Aerobiology: Experimental considerations, observations, and future tools”, Appl. Environ. Microbiol., vol. 83, núm. 17, 2017.

[4] C. F. Perez, M. L. Gassmann, N. E. Tonti, & L. Curto. “Panorama sobre la producción, el transporte y depósito de aerosoles de origen biológico”, Meteoro-logica, pp. 1–24, 2020.

[5] B. Audrain, S. Létoffé, y J.-M. Ghigo, “Airborne bacterial interactions: Functions out of thin air?”, Front. Microbiol., vol. 6, p. 1476, 2015, doi: 10.3389/fmicb.2015.01476.

[6] K.-H. Kim, E. Kabir, y S. A. Jahan, “Airborne bioaerosols and their impact on human health”, *J. Environ. Sci. (China)*, vol. 67, pp. 23–35, 2018, doi: 10.1016/j.jes.2017.

[7] S. M. Walser *et al.*, “Evaluation of exposure-response relationships for health effects of microbial bioaerosols - A systematic review”, *Int. J. Hyg. Environ. Health*, vol. 218, núm. 7, pp. 577–589, 2015.

[8] CIOH, Centro de Investigaciones Oceanográficas e Hidrográficas del Caribe (2010). Clima Cartagena. Retrieved from https://www.cioh.org.co/meteorologia/Climatologia/ResumenCartagena2.php

[9] C. Rubiano-Labrador, L. B. De Avila, D. Doria Posada and R. Acevedo-Barrios, "Concentrations of Airborne Cultivable Fungi at a Higher Education Institution in Cartagena de Indias, Colombian Caribbean", *Aerosol Science and Engineering*, vol. 6, no 4, p. 381-390, 2022.

[10] Y. C. Camargo, A. Velez-Pereira. Emisiones atmosféricas de origen biológico, Santa Marta, Colombia. 2011.

[11] M. E. Huertas, R. L. Acevedo-Barrios, M. Rodríguez, J. Gaviria, R. Arana, and C. Arciniegas, “Identification and quantification of bioaerosols in a tropical coastal region: Cartagena de Indias, Colombia”, *Aerosol Sci. Eng.*, vol. 2, núm. 4, pp. 206–215, 2018.

[12] L. Caicedo, G. Giusiano, Alvarez M (2015) Estudio de la micota ambiental de tres edificios de laboratorios de la Universidad del Valle, Colombia, y su relación con los síntomas de alergias respiratorias que manifiestan trabajadores. Universidad Nacional del Nordeste, p 100.

[13] C. Rubiano-Labrador, C. Díaz-Cárdenas, G. López, J. Gómez, y S. Baena, “Colombian Andean thermal springs: reservoir of thermophilic anaerobic bacteria producing hydrolytic enzymes”, *Extremophiles*, vol. 23, núm. 6, pp. 793–808, 2019.

[14] B. Saviola, “Pigments and Pathogenesis”, *Mycobact. Dis.*, vol. 04, núm. 05, 2014.

[15] J. R. Jaramillo Cisterna, “Caracterización fisico química de pigmentos de origen bacteriano aislados de sedimentos marinos de la región de Los Lagos”, Universidad Austral de Chile, 2007.

[16] Y. Marrugo Mejia and J. Uribe Caro, “Caracterización bacteriologica del material particulado proveniente de un ambiente marino de la ciudad de Cartagena de Indias - Caribe Colombiano”, 2017.

[17] C. Rodriguez-Gomez *et al.*, “Characterization of culturable airborne microorganisms in the Yucatan Peninsula”, *Atmos. Environ. (1994)*, vol. 223, núm. 117183, p. 117183, 2020.

[18] D. R. Olaya and F. A. Perez Rojas, “Caracterización cualitativa-cuantitativa de bioaerosoles relacionados con factores meteorológicos yl particulado en Puente Aranda Bogotá DC”, Universidad de la Salle, 2006.

[19] G. M. Szafraniec, P. Szeleszczuk, y B. Dolka, “Review on skeletal disorders caused by Staphylococcus spp. in poultry”, *Vet. Q.*, vol. 42, núm. 1, pp. 21–40, 2022.

[20] X. Chen *et al.*, “Concentrations and size distributions of airborne microorganisms in Guangzhou during summer”, *Aerosol Air Qual. Res.*, vol. 12, núm. 6, pp. 1336–1344, 2012, doi: 10.4209/aaqr.2012.03.0066.

[21] Z. Fang, Z. Ouyang, H. Zheng, X. Wang and L. Hu. “Culturable airborne bacteria in outdoor environments in Beijing, China”. *Microbial Ecology*, vol. 54, pp. 487-496, 2007.

[22] C. K. Ruíz-Fonseca y C. Rubiano-Labrador, “Perfil de bacterias y hongos aerotransportados por la lluvia horizontal de la región del Salto del Tequendama (Colombia)”, *Gest. Ambiente*, vol. 24, núm. 1, p. 92479, 2021

[23] R. Liang, P. Xiao, R. She, S. Han, L. Chang, y L. Zheng, “Culturable airborne bacteria in outdoor poultry-slaughtering facility”, *Microbes Environ.*, vol. 28, núm. 2, pp. 251–256, 2013.

[24] A. M. Arfken, B. Song, y J.-S. Sung, “Comparison of airborne bacterial communities from a hog farm and spray field”, *J. Microbiol. Biotechnol.*, vol. 25, núm. 5, pp. 709–717, 2015.

[25] H. A. M. Pagalilauan, C. E. M. Paraoan, y P. G. Vital, “Detection of pathogenic bioaerosols and occupational risk in a Philippine landfill site”, *Arch. Environ. Occup. Health*, vol. 73, núm. 2, pp. 107–114, 2018.

[26] S. Faridi *et al.*, "Indoor/outdoor relationships of bioaerosol concentrations in a retirement home and a school dormitory", *Environ. Sci. Pollut. Res. Int.*, vol. 22, núm. 11, pp. 8190–8200, 2015.
[27] V. M. Baldwin, "You can't B. cereus – A review of *Bacillus cereus* strains that cause anthrax-like disease", *Front. Microbiol.*, vol. 11, 2020.
[28] M. Ferchichi *et al.*, "Enterococcus spp.: Is it a bad choice for a good use-A conundrum to solve?", *Microorganisms*, vol. 9, núm. 11, 2021.
[29] B. Krawczyk, P. Wityk, M. Gałęcka, y M. Michalik, "The many faces of Enterococcus spp.-commensal, probiotic and opportunistic pathogen", *Microorganisms*, vol. 9, núm. 9, p. 1900, 2021.
[30] H. P. S. Dhaked y I. Biswas, "Distribution of two-component signal transduction systems BlpRH and ComDE across streptococcal species", *Front. Microbiol.*, vol. 13, p. 960994, 2022.
[31] J. De Smet, H. Hendrix, B. G. Blasdel, K. Danis-Wlodarczyk, y R. Lavigne, "Pseudomonas predators: understanding and exploiting phage-host interactions", *Nat. Rev. Microbiol.*, vol. 15, núm. 9, pp. 517–530, 2017.
[32] M. A. Llamas, F. Imperi, P. Visca, y I. L. Lamont, "Cell-surface signaling in Pseudomonas: stress responses, iron transport, and pathogenicity", *FEMS Microbiol. Rev.*, vol. 38, núm. 4, pp. 569–597, 2014.
[33] T. Kurkela, "The number of Cladosporium conidia in the air in different weather conditions", *Grana*, vol. 36, num, 1, pp. 54-61. 1997.
[34] A. M. Jones, & R. M. Harrison, "The effects of meteorological factors on atmospheric bioaerosol concentrations—a review", *Science of the total environment*, vol. 326, num, 1-3, pp. 151-180. 2004