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Study and characterization of airborne microbial communities in indoor air of an urban polyclinic

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Bioaerosols in the medical environment have been identified as suspected agents for the transmission of nosocomial infections, and the COVID-19 pandemic serves as a concrete example. This study aims to provide a qualitative and quantitative estimation of bioaerosols within a polyclinic located in the northern part of Algiers, Algeria. It also involves analyzing the influence of the sampling duration and period on the variability of bioaerosols within different rooms of the polyclinic. The passive sampling technique carried out the measurement of airborne bacteria in five rooms of the polyclinic. Two sampling times were chosen, 30 and 60 min with three sampling periods of two days each. The bacterial bioaerosols were characterized by MALDI TOF-MS.The bacterial bioaerosol concentration was notable in the five rooms of the polyclinic, reaching a medium-risk level. There was no significant difference (P =0.847-1.116) observed in the sampling duration, and similarly results for the sampling period (P=0.093 -0.798). *Staphylococcus*, *Bacillus* and *Raoultella* were the most dominant defined bacteria. These bacteria can have a harmful effect on the health of patients and workers of the polyclinic.

Keywords: Bioaerosol, Bacteria, Passive sampling, MALDI-TOF MS, Polyclinic

In the contemporary context, optimizing indoor air quality across various settings stands as a global challenge with far-reaching societal implications. The COVID-19 pandemic has starkly illustrated this challenge, emphasizing the urgent need to regulate and minimize airborne biological pollution on a global scale. Bioaerosols, defined as airborne particulate matter carrying a biological fraction, whether living or inert microorganisms[1], present a complex amalgamation of allergens, toxins, and assorted components[2]. Research endeavours in this domain are directed toward uncovering their origins and sources[3–6], evaluating their impact on human health[7–11], advancing sampling and analytical methodologies[12–18], and scrutinizing specific professional environments[19–27]. In recent years, the exploration of bioaerosols has garnered significant attention in medical settings. This field of investigation encompasses a blend of qualitative and quantitative studies focused on comprehending bioaerosols and their associated health implications[6–8,27–31]. As reported in bibliography, numerous studies have underscored the pivotal role played by bioaerosols in disease transmission within medical environments[32]. Inhalation of these particles significantly contributes to the development and severity of various health problems, including infectious diseases, respiratory illnesses, and allergic reactions[33,34]. More particularly, in the wake of the COVID-19 pandemic, research has taken a new direction aimed at shedding light on the presence of this virus in air, its transmission patterns and methods for its elimination[35–37]. However, existing gaps persist in understanding anthropogenic behavior, validating sampling, analysis techniques, and formulating legislation concerning bioaerosols[14]. Particularly noteworthy are the transmission patterns and risks associated with airborne antibiotic resistance genes carried by bioaerosols, a phenomenon susceptible to weather conditions[9]. Recent report by[31] highlighted that *Staphylococcus hemolyticus* isolated in different wards of educational hospital, exhibited the highest concentration among isolated bacteria. Notably, the majority of the bacteria demonstrated pronounced resistance to gentamicin. An investigation in urban hospitals in China conducted by[38] found that samples of isolated *E. coli* from three wards displayed resistance to multiple antibiotics — quinolones, aminoglycosides, sulfonamides, and third-generation cephalosporin.Such resistance poses a direct threat to patients and medical staff due to inhalation. A multi-drug resistance (> 3 antibiotics) among cultivable *Staphylococcus* in five urban hospitals noted by[39]. Similarly,[40] identified methicillin-resistant *Staphylococcus* genus in hospital in Mexico.

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Polyclinics serve as public healthcare facilities that provide care and follow-up for patients. These facilities ensure versatile medical activities and manage large patient and personnel flows. The activities within polyclinics along with the environmental factors, result in the emission of airborne particles, including bioaerosols. These public healthcare predominantly rely on natural ventilation, primarily through window openings. Studies have indicated the limited efficacy of this natural ventilation in eliminating indoor bioaerosols, leading to higher concentrations of human-related and potentially harmful bioaerosols[41]. Particularly noteworthy is the role of toilet plume bioaerosols as a significant pathway that can increase exposure to enteric and airborne pathogens[42].

This study aims to assess the microbiological quality of indoor air within a chosen polyclinic in the northern region of Algiers, by determining the concentration and types of airborne bacteria present in the air. Furthermore, we seek to estimate the impact of sampling duration and specific periods on bioaerosol concentration via this study.

Materials and Methods

Sampling site and procedure

The study was carried out at a polyclinic in the northern part of the capital Algiers. Four rooms (R1-4) exclusively designated for patient examination along with a waiting room (WR) were selected. Fig. 1 illustrates the internal layout of the polyclinic, highlighting the locations of the sampling rooms, along with the doors and windows, and the positions of the toilets. This polyclinic receives patients from 8 a.m. to 4 p.m., the human presence in the sampling rooms ranged from 2 to 15, typically peaking in the morning. Cleaning and disinfection of the premises were usually done at the end of the day. Bacterial

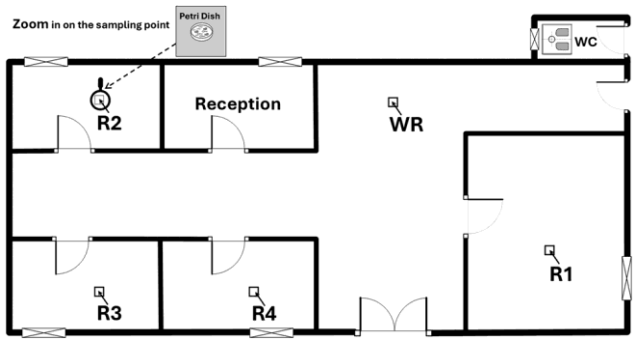

Fig. 1 — Internal layout of the studied polyclinic. R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room.

bioaerosol levels in these various rooms were assessed using a passive air sampling technique employing 9 cm diameter Petri dishes containing nutrient agar as the culture medium. Sampling was conducted at a height of 1 meter above the floor and at the room's central point. Sampling was performed during three distinct periods, with samples taken at 10 a.m. on two consecutive days. The time of exposure was 30 and 60 min. After exposure, the Petri dishes were sealed with parafilm, stored and then transported to the laboratory for incubation at 37°C for 2 days. A manual colony counting was performed under a light source. The quantification of CFU/m^3 was determined using the equation 1, outlined by Hayleeyesus & Manaye (2014).

$$N = 5a * 10^4 (bt)^{-1} \quad \text{... (1)}$$

[Where: N = CFU/m^3; a = Number of colonies per Petri dish; b = Petri dish surface (cm^2) ; t = Exposure time (min)]

After manual counting and observation of the appearance and colour of the colonies, a Gram stain was performed on each colony followed by identification by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS).

Bacterial identification by MALDI-TOF MS

The instrument used for the bacterial identification was the MALDI-TOF MS Microflex LT mass spectrometer (Bruker Daltonik, Germany) with FlexControl software (version 3.4) and Biotyper 2.0 RTC 4.0 software. The analysis was based on fingerprints of the mass spectra in the mass range 0–100000 Da. The spectra were compared to a reference database (Bruker Taxonomy BDAL containing 3995 library entries). The extended method was used for the identification of pure bacterial colonies according to the manufacturer's instructions. For this, one or more subcultures of fresh colonies were utilized to crystallize their proteins. In this method, a fresh colony was applied onto the target surface, overlaid with 1 µL of 70 % formic acid dried at room temperature. Subsequently, 1 µL of matrix solution [4-hydroxy-a-cyanocinnamic acid (HCCA)] was added, and a secondary air-drying was performed before introducing the sample for analysis using MALDI-TOF MS. Data acquisition was performed using an in linear mode detector set at 2558 V and 2555 V for the monitor. The mass range analyzed spanned from 1986-20137 Da. A UV laser served as the source operating at a frequency of 60.0 Hz with 40 shots, employing high voltage and positive polarity.

The bacterial test standard (BTS) utilized was *E. coli* ATCC 25922 THL in dehydrated form. The interpretation of the identification score is based on the scale recommended by the manufacturer, where a score between 2.300-3.000 indicates highly probable species identification; [2.000-2.299] signifies secure genus identification and probable species identification; [1.700-1.999] suggest probable genus identification; while score falling between [0.000-1.699] indicates not reliable identification.

Statistical analysis

To determine if there is significant difference in bioaerosol concentration (CFU/m^3) between sampling duration of 30 min and 60 min., a student's *t-test* was employed. Additionally, the test was utilized to assess the mean evolution of bioaerosol concentration (CFU/m^3) mean evolution over the three-week period. The level of significant value was set at $P < 0.05$ for all the tests performed. The raw data was analyzed using JAMOVI statistical software (Version 2.3.21.0).

Results and Discussion

Airborne microbial load

The bacteria concentration in internal air in various rooms of the polyclinic across the three sampling periods and two sampling duration are presented in Fig. 2. For the first sampling period, the concentration ranged from 26 to 1441 (CFU/m^3), with three values exceeding 500 CFU/m^3 (WHO standard for internal air). The highest value was recorded in Room 4 after 60 min of sampling. During the second sampling, the bacteria concentration ranged from [13-2745] (CFU/m^3), with only one value exceeding the WHO standard. Room 1 exhibited the maximum value after 30 min of sampling. While, for the third sampling period, concentration ranged from [39-8922] (CFU/m^3) with four values exceeding the WHO standard. The maximum value was recorded in Room 1 after 30 min of sampling (8922 CFU/m^3).

Table 1 and Fig. 3 present a statistical analysis of the mean bacterial bioaerosol concentration (CFU/m^3)

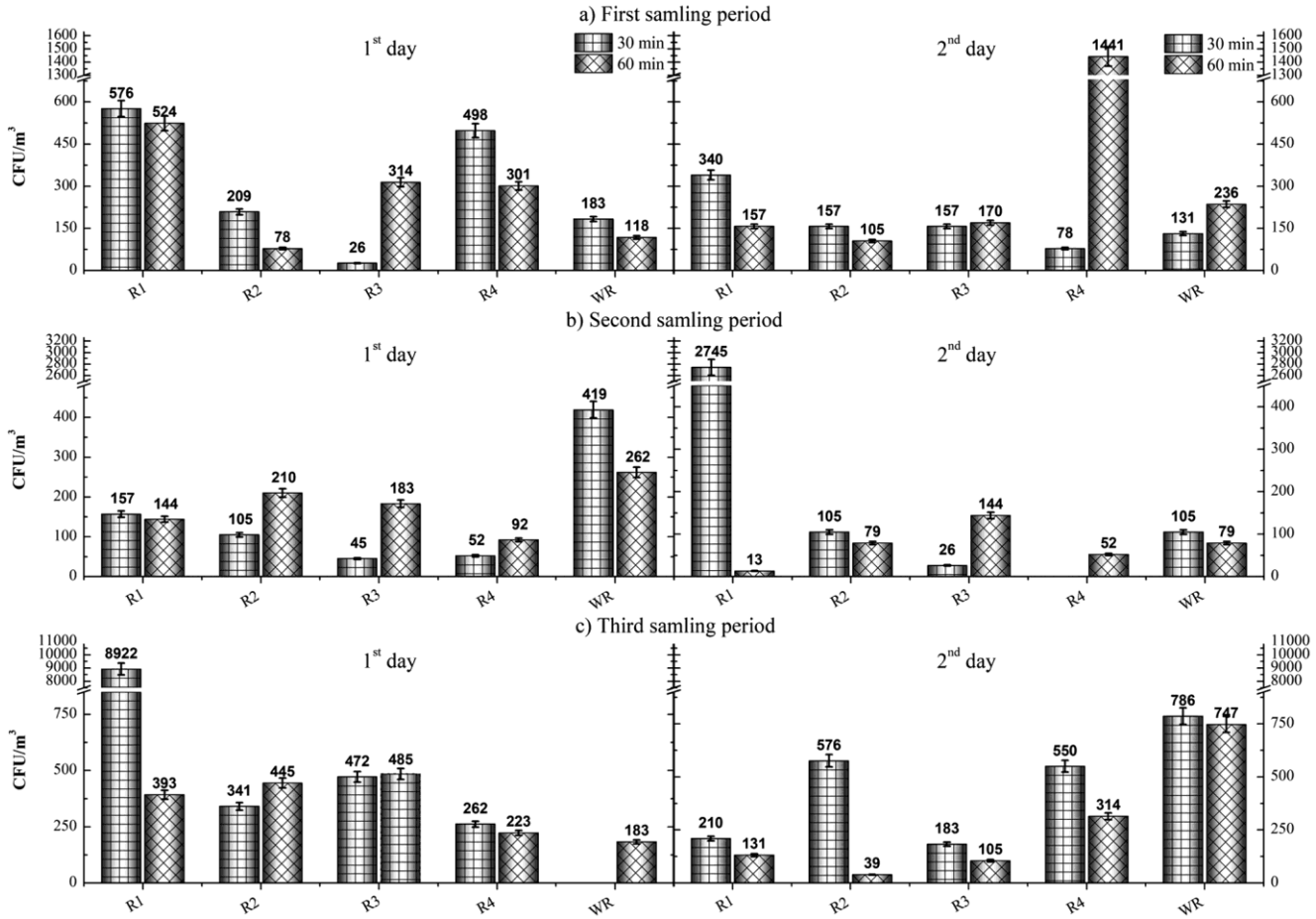

Fig. 2 — Bacterial bioaerosol concentration (CFU/m^3) in different rooms, during the three-sampling period separately at the Polyclinic. R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room.

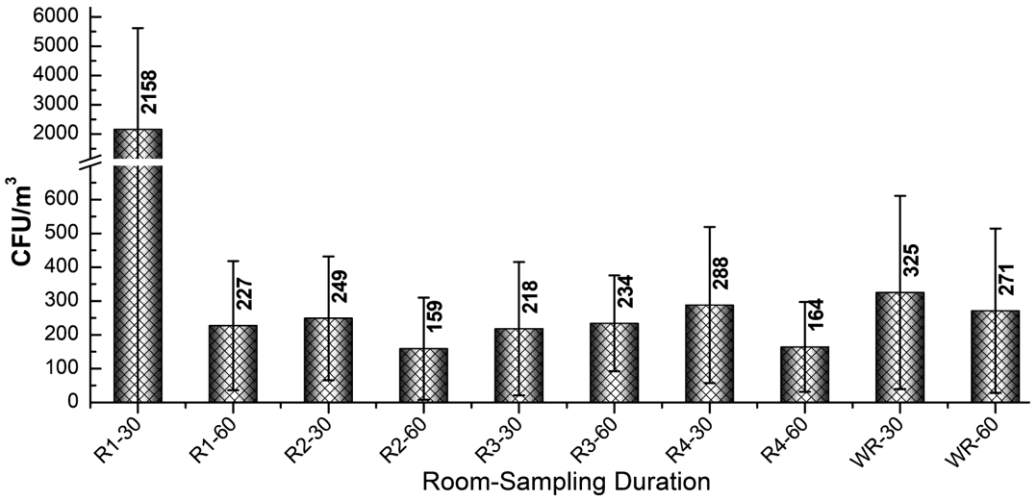

Fig. 3 — Bacterial bioaerosol concentration (CFU/m^3) average according to sampling duration (30 min *vs* 60 min) in the five rooms (R1 to R4 and WR: Waiting Room) during the six days.

Table 1 — Statistics for bacterial bioaerosol concentration (CFU/m^3) mean according to sampling duration effect (30 min & 60 min)

Room-Duration	R1-30	R1-60	R2-30	R2-60	R3-30	R3-60	R4-30	R4-60	WR-30	WR-60
N	6	6	6	6	6	6	5	6	5	6
Mean	2158	227	249	159	218	234	288	164	325	271
SD	3456	191	183	151	197	142	231	133	286	243
Minimum	157	13	105	39	26	105	52	1	105	79
Maximum	8922	524	576	445	472	485	550	314	786	747

[R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room, SD: Standard Deviation]

based on the effect of sampling duration (30 min *vs* 60 min). The lowest recorded value was observed in Room 4 after 60 min of sampling, with a value of 1 CFU/m^3. Conversely, the highest recorded value is in Room 1 after 30 min of sampling, reaching 8922 CFU/m^3. The average is ranged between 159-2158 (CFU/m^3). This significant variation primarily driven by outliers. Notably a peak concentration of 8922 CFU/m³ recorded in room 1 on the first day of the third period at 30 min of exposure and another high value of 2745 (CFU/m^3) observed on the second day of the second period also at 30 min of exposure (Fig. 3). Several conditions can influence this variation, such as ventilation, occupancy and cleaning frequency. Non-significant difference (P>0.05) between sampling duration of 30 min or 60 min for all site sampling P=0.116-0.847 (Table 2).

Table 3 shows a statistical analysis of the variation in bioaerosol concentrations across the three sampling periods. The minimum recorded value was observed in Room 4 during the first week of sampling, with a concentration of 1 CFU/m^3. Conversely, the maximum recorded value was in Room 1 during the third week, reaching 8922 CFU/m^3. The average concentration ranged between 65.3 and 572 CFU/m^3

Table 2 — *t-test* for sampling duration of 30 min or 60 min

Compared means	df (degree of freedom)	*P* value
R1-30 / R1-60	5	0.223
R2-30 / R2-60	5	0.399
R3-30 / R3-60	5	0.847
R4-30 / R4-60	4	0.116
WR-30 / WR-60	4	0.436

[R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room]

(Fig. 4). Non-significant difference (P>0.05) for the mean evolution during the three weeks according to site (room) sampling. P was in the range 0.093-0.798 (Table 4).

Bacterial identification

The identification of airborne bacteria at the polyclinic for the first sampling gives the following: *Staphylococcus* (*hominis, haemolyticus*) (72%), *Raoultella ornithinolytica* (14%) and *Bacillus cereus* (14%) with an identification score ranged between 1.81- 2.194. For the second sampling, the identification reveals *Staphylococcus* with various species (*hominis, haemolyticus, warneri*) and an identification score ranging from 1.766-2.281. The third sampling indicates *Staphylococcus haemolyticus* at 63%, *Bacillus cereus*

Table 3 — Statistics for Bacterial bioaerosol concentration (CFU/m^3) mean evolution during the three weeks

Room-Wi	R1-Wi			R2-Wi			R3-Wi			R4-Wi			WR-Wi		
Week (i)	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
N	4	4	4	4	4	4	4	4	4	4	3	4	4	4	3
Mean	399	78.8	184	137	125	350	167	200	311	220	65.3	337	167	216	572
SD	191	83.2	164	58	58.1	229	118	177	196	225	23.1	147	53.9	157	337
Minimum	157	1	1	78	79	39	26	26	105	1	52	223	118	79	183
Maximum	576	157	393	209	210	576	314	445	485	498	92	550	236	419	786

[R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room, Wi: Week of sampling, SD: Standard Deviation]

Table 4 — *t-test* for mean evolution during the three weeks according to site (room) sampling

Room-Wi	R1-Wi			R2-Wi			R3-Wi			R4-Wi			WR-Wi		
Compared mean (i)	1⇆2	2⇆3	1⇆3	1⇆2	2⇆3	1⇆3	1⇆2	2⇆3	1⇆3	1⇆2	2⇆3	1⇆3	1⇆2	2⇆3	1⇆3
df	3	3	3	3	3	3	3	3	3	2	2	3	3	2	2
P-value	0.093	0.34	0.176	0.798	0.207	0.153	0.752	0.554	0.286	0.601	0.1	0.527	0.59	0.11	0.15

[R1: Room 1, R2: Room 2, R3: Room 3, R4: Room 4, WR: Waiting Room. Wi: Week of sampling]

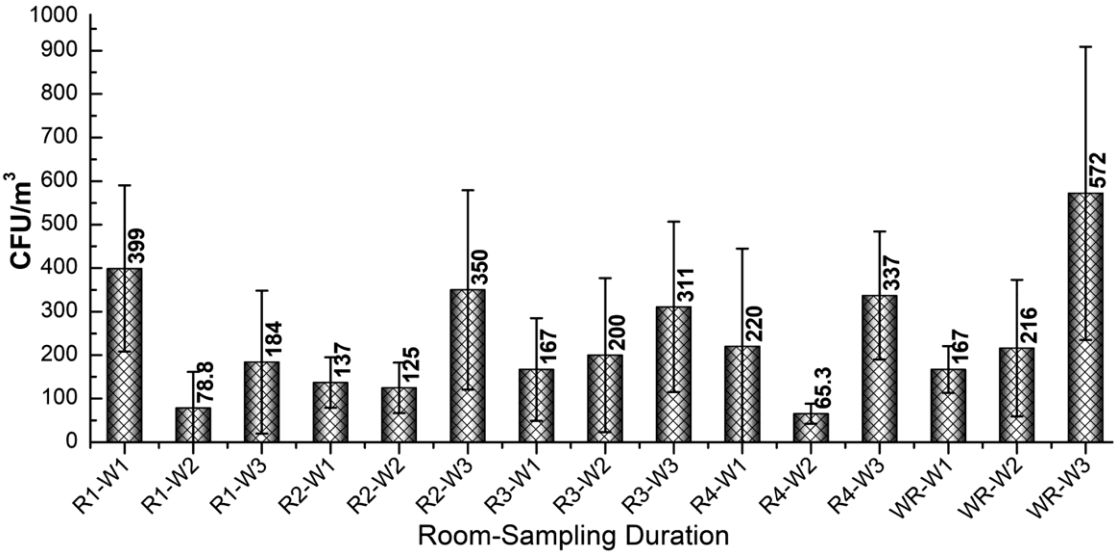

Fig. 4 — Bacterial bioaerosol concentration (CFU/m^3) mean evolution during the three weeks (W1 to W3) according to the five rooms (R1 to R4 and WR: Waiting Room) of sampling.

at 25%, and *Klebsiella pneumoniae* at 12%, with an identification score ranged between 1.842-2.314 (Fig. 5). The identification score for MALDI-TOF MS varies between 1.766 and 2.314, which gives genus identification for the values included in the interval 1.700-1.999 and species identification for the values included in the interval 2.000-2.299.

In a previous study, we carried out in medical emergency of a hospital in north of Algeria, we found a concentration of bacterial bioaerosols raged between 191-2645 CFU/m^3, estimated by the passive method [44]. In their study of the bioaerosols in hospital wards using three sampling techniques,[45], observed that the microbial loads vary with the sampling method (exposed plate: 45–150 CFU/plate; impingement: 1.12E+03–1.6856E+05 CFU/m^3; filtration: 3.788E+03–1.91111E+05 CFU/m^3). A bacterial bioaerosols concentrate was recorded in the range of 65.52 CFU/m^3 to 1179 CFU/m^3 for 60 min of exposure in a study covering indoor air in a tertiary care hospital in India utilizing passive air sampling[46,47], reported in their investigation of microbial air quality in hospitals and ambulances in Poland, bacterial concentrations in the range of 130-4200 CFU/m^3 for hospitals, 130-1400 CFU/m^3 for ambulances, and 42-5000 CFU/m^3 for administration offices. Our study findings are consistent with previously cited studies. However, our results indicate significantly higher concentrations compared to those obtained in a study on bioaerosols in educational hospital in Iran. In that study, the highest and lowest densities of the bioaerosols were observed in lung and operating wards (336.67 and 15.25

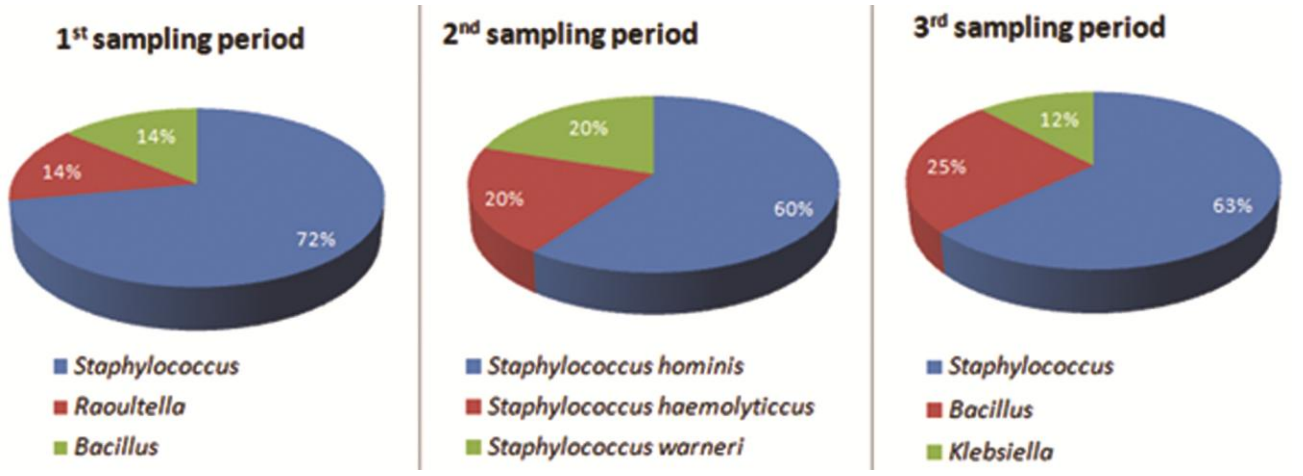

Fig. 5 — Bacteria identified by the MALDITOF MS in the polyclinic.

CFU/m^3)[31]. Our findings also indicated notably higher values compared to those obtained in the study on indoor air at Hajar Hospital in Shahrekord, Iran, where the bacterial bioaerosols ranged between 14-106 CFU/m^3 [37].

The presence of airborne bacteria appeared to be correlated with the occupancy of smaller spaces. Throughout the sampling, the number of occupants ranged between 2 to 15 persons. Moreover, the layout of the building (Fig. 1) features a large circulation area near Room 1, due to its proximity to the main entrance and the passage to the toilets. Variations in bioaerosols concentrations were reported to be influenced by factors such as occupancy and human activity[48,49]. In these rooms, ventilation was achieved naturally through a single window. It has been observed that natural ventilation, specifically via window openings, demonstrated limited efficacy in removing indoor bioaerosols. Indoor environments exhibited higher levels of human-related and potentially harmful bioaerosols, as highlighted by[41]. The release of bioaerosols from toilet plumes is recognized as a significant pathway that can elevate exposure to enteric and airborne pathogens, as indicated by[42]. Environmental factors such as high humidity levels (40-73%) and wind speed contribute to an increased concentration of bacteria in the indoor air of these rooms.

The predominant bacteria identified in the indoor air of the polyclinic were *Staphylococcus* (*hominis, haemolyticus, warneri*) and *Bacillus cereus*, with smaller quantities of *Raoultella ornithinolytica* and *Klebsiella pneumoniae*. These findings are consistent with the results reported by[50], where the highest bacterial population consisted of coagulase-negative *Staphylococci* (32.49%), *Bacillus spp*. (14.74%), *Micrococcus spp*. (13.68%), and *Staphylococcus aureus* (11.34%)[51], indicated a predominance of gram-positive bacteria in the indoor air of surgical rooms at Shariati Hospital. Coagulase-negative *Staphylococci* and *Micrococci* were the primary Gram-positive cocci in both active and passive samples, as noted by[45]. Other studies, including that of[14], have also reported a higher isolation of Gram-positive bacteria, with *Staphylococcus aureus* being predominant. In the research conducted by[47], Gram-positive cocci were the predominant isolated bacteria, with the highest concentration observed for *Staphylococcus hemolyticus* (31.84%) according to[31].

Conclusion

In this work, a comprehensive approach, combining field study and laboratory experiments was adopted to investigate the microbiological quality of indoor air at a polyclinic in northern Algiers. The results indicated a moderate risk level for bioaerosols, influenced by several factors such as the efficiency of air treatment systems, occupant density and activity and the frequency of disinfection. No statistically significant variation in bioaerosols concentrations was observed based on sampling duration or periods. *Staphylococcus* emerged as the predominant bacteria identified by MALDI-TOF MS analysis. However, despite the importance of bioaerosols in our ecosystems, a high rate of BAs can pose significant health risks to humans. This highlights the necessity of implementing strategies to reduce bioaerosol emissions in critical areas such as healthcare establishment. Indeed, applying robust strategies such as aeration, ventilation, enhanced air conditioning,

meticulous cleaning practices, and judicious disinfectant usage becomes imperative to curtail the deleterious effects of high bioaerosol levels on human health. Additionally, we advocate for the regular assessment of microbial air quality as part of polyclinic maintenance system. This study could serve as reference, promote further research focused on understanding biological air contamination in healthcare settings and contribute to the development of Algerian standards in this field.

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Conflicts of interest

The authors declare no conflict of interest.

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