atmosphere

MDPI

Article

Diversity of Bioaerosols in Selected Rooms of Two Schools and Antibiotic Resistance of Isolated Staphylococcal Strains (Bydgoszcz, Poland): A Case Study

Marta Małecka-Adamowicz [1], Beata Koim-Puchowska [2] and Ewa A. Dembowska [1,*]

[1] Department of Microbiology and Immunobiology, Faculty of Biological Sciences, Kazimierz Wielki University, ul. Al. Powstańców Wielkopolskich 10, 85-090 Bydgoszcz, Poland; marmal@ukw.edu.pl
[2] Department of Biotechnology, Faculty of Biological Sciences, Kazimierz Wielki University, ul. Poniatowskiego12, 85-090 Bydgoszcz, Poland; koimpuch@ukw.edu.pl
* Correspondence: dembow@ukw.edu.pl; Tel.: +48-52-376-79-27

Received: 18 September 2020; Accepted: 13 October 2020; Published: 15 October 2020

Abstract: The present study is aimed at evaluating microbiological air pollution in libraries, cafeterias and selected classrooms of two schools in Bydgoszcz city, northern Poland and determining the antibiotic resistance of Staphylococcal strains isolated from the indoor air. One of the investigated schools (School A) is located in the very center of the city, in the vicinity of a park, among old houses and stone-lined streets, while the other (School B), among modern residential buildings, close to a street with heavy traffic. In each school, air samples were collected in the morning, always from all three sampling sites, using the MAS-100 sampler. Selective growth media were used for bacteria and mold isolation and quantifying analysis. The antibiotic resistance of the isolated mannitol-positive staphylococci was assessed using the disc diffusion method in accordance with EUCAST recommendations. The highest mean concentration of heterotrophic bacteria was recorded in the cafeterias: 884 CFU m^{-3} in School A and 1906 CFU m^{-3} in School B. Molds were the most abundant in the library and cafeteria in School B, where their average concentration exceeded 300 CFU m^{-3}. *Cladosporium* and *Penicillium* species prevailed, while *Fusarium*, *Acremonium* and *Aspergillus* were less abundant. Airborne mannitol-positive staphylococci were recorded at low concentrations, ranging from 6 to 11 CFU m^{-3} on average. According to the taxonomic analysis, *Staphylococcus aureus* isolates were the most abundant in both schools, followed by *S. xylosus*, *S. haemolyticus* and *S. saprophyticus*. The antibiograms indicated that resistance to erythromycin was common in 62.5% of the isolated staphylococcal strains. Levofloxacin and gentamicin were the most effective antibiotics. No multidrug-resistant strains were identified.

Keywords: indoor air quality; microbiological contamination; heterotrophic bacteria; antimicrobial resistance; mannitol-positive staphylococci; fungi

1. Introduction

Air quality in public facilities, including schools, is of growing concern. Numerous reports indicate that indoor air pollution is worse than outdoor air pollution and that the composition of indoor air microflora is more stable [1,2]. Although the concentrations of the majority of indoor air pollutants are so low that they cannot be considered harmful, long-term exposure may have negative effects on human health [3].

Indoor air contaminants come from different sources. Some of them, such as building materials, furnishings, ventilation systems and household products (e.g., air fresheners) can release pollutants

almost continuously. Other sources, related to activities, such as smoking, cleaning or redecorating, release pollutants occasionally [4]. According to Andualem et al. [5] overcrowded classrooms, inadequate fresh air supply, poor construction and maintenance of school buildings negatively affect air quality in schools. Since students and teachers spend most of their day inside school buildings, the concentration of airborne microorganisms is an important parameter with a large impact on their performance, mental health and even physical condition [6]. Moreover, the absence of a student due to health issues may affect their academic achievements [7,8].

Particulate matter is considered an air pollutant of the greatest concern mainly due to its acute and chronic effects on children's health [9]. According to Du et al. [10] bacterial and fungal particles (PM 2.5) significantly affect air quality, contributing to the spread of infectious diseases and allergies [11]. Asthma is common in children with allergies. Since children spend most of their time in school, indoor air contamination can affect their pulmonary health [12].

The condition of educational facilities is a global problem because exposure to indoor air of poor quality may affect children's respiratory and cardiovascular functions [13], particularly since they breathe in more air in relation to their body weight than adults [14] and their immune system and internal organs are not yet fully developed [15]. In addition, young (and still growing) organisms are more susceptible to damage than mature ones [8]. Therefore, in institutions with an increased risk of pathogenic microbiota development, there is a need for monitoring air quality and evaluating the ability of pathogenic bacteria to acquire antibiotic resistance. According to Labi et al. [16] multidrug-resistant strains pose a serious threat to human health and are a challenge for modern medicine.

Ensuring good air quality in schools is of paramount importance for work efficiency, good health and mental wellbeing [17] and, as stated in WHO recommendations, access to air of acceptable quality is a fundamental human right.

In the available literature, there are numerous reports on air quality in classrooms in countries all over the world. However, since this type of research had been conducted only in the southern and central Poland prior to this study, the authors decided to investigate microbiological air contamination in schools in Bydgoszcz, the city located in northern Poland. Air samples were collected not only from classrooms (standard approach) but also from libraries and cafeterias. The analyses focused on three aspects. The main objective was to determine bioaerosol abundance in selected rooms and the influence of several factors (e.g., room type, room size, room temperature and season) on the concentration of individual microbial groups. Another objective was to determine the genera of microorganisms and their taxonomic affiliation. Finally, antibiotic resistance profiles of opportunistic staphylococci were prepared.

Based on the above objectives, the following hypotheses were formulated:

- The number of microbial groups is higher in smaller rooms and differs depending on the month of sampling;
- Room temperature affects the concentration of microorganisms;
- Opportunistic multidrug-resistant staphylococci are found in the air of the investigated school rooms.

2. Experiments

2.1. Sampling and Sampling Sites

Microbiological tests were carried out in two schools in Bydgoszcz City, Poland. School A is located in the city center, in the vicinity of a park, old residential buildings and cobbled streets, while School B is located among modern blocks of flats in a street with heavy traffic.

Air samples were collected in three parallel repetitions once a month from September to February in two schools (School A and School B) from three sampling sites in each school: I—classroom; II—library; III—cafeteria (Table 1). During each sample collection (always in the morning hours), the schools were open and filled with students.

Air (in the amount of 100 L) was collected 1.5 m above the ground with an MAS-100 sampler (Merck, Germany) with a dual-flow turbofan, which sucks the air stream through a metal head with 400 × 1 mm holes and directs it onto the surface of a sterile Petri dish with the substrate. A centrifugal fan, controlled by a flow sensor, regulates the air flow. The flow rate was 100 L per minute.

Samples were transported to the laboratory, placed in a thermostat and incubated for a specific time at an appropriate temperature. After that, grown colonies were counted. The results were corrected using the table of statistical corrections according to Feller [18] and expressed as colony-forming units per cubic meter of air (CFU m^{-3}).

Table 1. Description of sampling sites.

School Characteristics	**School A**			**School B**		
School location	53°07′37.3″ N 18°00′23.6″ E			53°06′38.1″ N 18°02′53.3″ E		
Year of construction	1878			1955		
Floor covering	Vinyl PCV, tiles			Vinyl PCV, tiles		
Number of children	760			600		
Age of children	14–19			14–19		
Sampling Site Characteristics	**School A**			**School B**		
	Classroom	**Library**	**Cafeteria**	**Classroom**	**Library**	**Cafeteria**
Sampling site location	Ground floor	First floor	Ground floor	Ground floor	Basement	Ground floor
Ventilation system	Natural	Natural	Natural, gravity	Natural	Natural	Natural, gravity
Surface area, m^2	45	83	14	48	72	20
Indoor temperature, °C (average)	23	22	23	22	22	22

2.2. Microbial Research

The total concentration of heterotrophic bacteria was determined using Trypticase Soy Lab Agar medium (BTL, Poland). The bacteria were incubated at 37 °C for 48 h; then, grown colonies were counted and their concentration was expressed as colony-forming units per cubic meter of air (CFU m^{-3}).

The presence of mannitol-positive staphylococci was detected using Chapman's nutrient medium (BTL, Poland). Bacterial cultures were incubated at 37 °C for 48 h; then, grown colonies were counted. Bright yellow zones around a grown colony indicated a positive result. Additionally, the strains were Gram stained and identified by as Gram-positive cocci. Taxonomic analysis of the strains was performed using API tests (API Staph bioMerieux, Craponne, France).

Antibiotic resistance of the identified Staphylococcal strains was determined using the disc diffusion method. Paper discs containing antibiotics were placed on Mueller-Hinton medium (BioMaxima, Lublin, Poland) inoculated with randomly selected strains of mannitol-positive staphylococci. Seven different groups of antibiotics of specified concentration (cefoxitin—FOX 30 µg; gentamycin—CN 10 µg; erythromycin—E 15 µg; tetracycline—TE 30 µg; chloramphenicol—C 30 µg; levofloxacin—LEV 5 µg; and rifampicin—RD 5 µg) were used to assess the full spectrum of resistance of the all strains. For *Staphylococcus aureus*, one more antibiotic—i.e., penicillin (P 1 unit)—was used in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19]. After an 18-h incubation at 37 °C, we measured zones of inhibited growth formed around the discs. Subsequently, the investigated strains were divided into two groups: susceptible and resistant to antibiotics.

The concentration of molds was determined using Sabouraud's nutrient medium (BTL, Warszawa, Poland). These microorganisms were incubated at 26 °C for 5 days, after which time grown colonies were counted and their concentration was expressed as colony-forming units per cubic meter of air (CFU m^{-3}). For their identification, molds were transferred on Czapek-Dox medium and incubated at

26 °C for 5 days. Subsequently, microscope slides were prepared and stained with Shear's medium. Molds were identified on the basis of their macroscopic (colony diameter; colony color—reverse; colony structure; colony edge; colony center) and microscopic (vegetative mycelium, vegetative spores, chlamydospores) features according to Samson et al. [20].

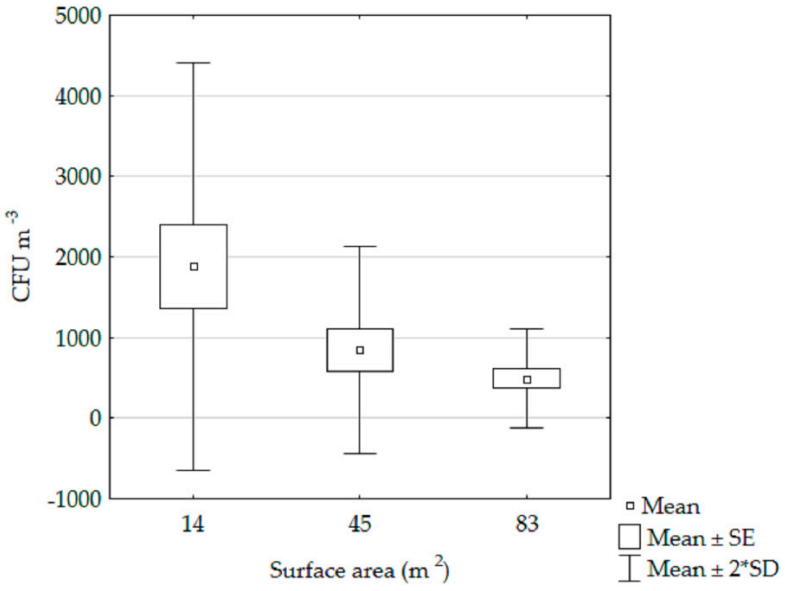
2.3. Statistical Analysis

The Statistica 13.1 software was used for statistical analysis of the results. After log transformation, the results in the analyzed groups were normally distributed, which was confirmed using the Shapiro–Wilk test. In order to assess the impact of independent factors—i.e., time and place of sampling—on dependent variables—i.e., the concentrations of particular microbial groups—the ANOVA test was used. Tukey's multiple comparison analysis method (the posthoc Tukey's test) was conducted to compare the differences in the studied variables between the groups. In order to analyze relationships between the examined parameters, the Spearman correlation coefficient was determined. Statistical analyses were performed at the significance level of $p \leq 0.05$.

3. Results

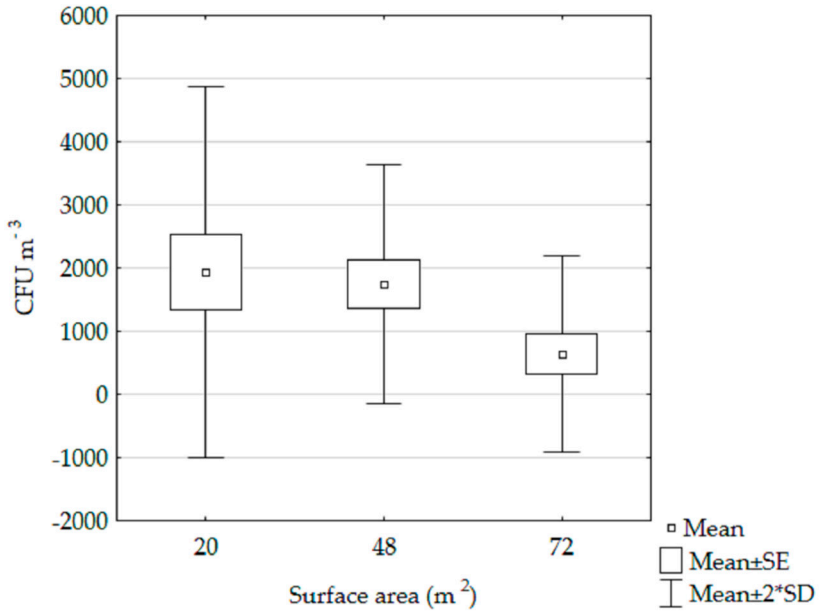
In terms of surface area (size), libraries in both schools were the biggest studied rooms, followed by classrooms and cafeterias. The air temperature in School B was at a constant level—i.e., 22 °C—while in School A it was one degree higher in the classroom and the cafeteria than in the library (Table 1).

In both schools, the concentration of heterotrophic bacteria was correlated with the size of the studied rooms (Figure 1a,b). In rooms with a smaller surface area (the cafeterias), the concentration of heterotrophic bacteria was higher (School A: r = −0.63, $p = 0.005$; School B: r = −0.52, $p = 0.025$). A negative relationship was also observed for staphylococci (School A: r = −0.14; School B: r = −0.36), but the results were not statistically significant. On the other hand, the concentration of molds was not correlated with the size of the rooms in neither of the schools ($r < 0.1$, $p > 0.05$). The air temperature was similar in all the investigated rooms in School A and the same, in School B. (Table 1). Therefore, this factor did not significantly affect microbial concentration.



(**a**)

Figure 1. *Cont.*



(**b**)

Figure 1. The relationship between (**a**) the size of the areas in School A and the total concentration of heterotrophic bacteria and (**b**) the size of the areas in School B and the total number of heterotrophic bacteria. SE—standard error; SD—standard deviation.

3.1. Concentrations of Bacterial Bioaerosol

In the air of the studied rooms, heterotrophic bacteria were the most abundant, and their concentrations ranged from 93 to 6070 CFU m^{-3} (Table 2).

Table 2. Number of heterotrophic bacteria in air (CFU m^{-3}).

Sampling Sites		Month of Sampling						M ± SD
		September	October	November	December	January	February	
School A	I—classroom	1607	1357	1266	546	219	93	848 ± 643
	II—library	430	480	1050	333	520	133	491 ± 307
	III—cafeteria	2120	3047	3593	1397	527	620	1884 ± 1265
M ± SD		1386 ± 866	1628 ± 1305	1970 ± 1410	759 ± 563	422 ± 176	282 ± 293	
School B	IV—classroom	347	1613	2776	2762	899	1949	1724 ± 982
	V—library	387	2143	190	393	160	150	571 ± 778
	VI—cafeteria	373	880	3077	4080	6070	2353	1906 ± 1499
M ± SD		625 ± 189	1545 ± 634	2014 ± 1587	2412 ± 1868	576 ± 378	1484 ± 1173	

M—mean; SD—standard deviation.

The highest average concentrations of airborne heterotrophic bacteria were recorded in the cafeterias—i.e., 1884 (School A) and 1906 CFU m^{-3} (School B)—and in the classrooms—i.e., 848 (School A) and 1724 CFU m^{-3} (School B). The lowest average concentration of airborne heterotrophic bacteria in the studied schools was noted in the libraries: 491 (School A) and 571 CFU m^{-3} (School B) (Table 2). In the present study, the concentration of heterotrophic bacteria increased in the period from November to February in the cafeteria in School B, with maximum values recorded in January. In November, December and February, relatively high concentrations of these bacteria were recorded in the classroom in the same school (Table 2).

There were statistically significant differences in the concentration of heterotrophic bacteria depending on the sampling sites in School A ($F = 4.47$, $p = 0.03$) and School B ($F = 4.59$, $p = 0.027$). The concentration of these microorganisms in School A was significantly higher in the cafeteria than in

the library ($p = 0.029$). On the other hand, in School B, the concentration of heterotrophic bacteria in the class and cafeteria was statistically significantly higher than in the library, with $p = 0.046$ and $p = 0.049$, respectively. The concentration of heterotrophic bacteria was not correlated with the sampling month (Table 3).

Table 3. Statistical differences in microbial concentrations depending on a sampling site and sampling month.

	Microorganisms	F	*p*	Differences	F	*p*	Differences
		Sampling Sites			**Month of Sampling**		
School A	Heterotrophic bacteria	**4.47**	**0.030**	II:III *	1.74	0.2	ns
	Fungi	0.02	0.984	ns	**23.43**	**<0.001**	S:D **; S:J **; S:F *;O:D ***; O:J ***; O:F ***; N:D **, N:J **; N:F **
	Staphylococci	1.31	0.299	ns	1.57	0.242	ns
School B	Heterotrophic bacteria	**4.59**	**0.028**	V:IV *; VI:IV *	1.22	0.358	ns
	Fungi	0.12	0.889	ns	**13.66**	**<0.001**	S:D *; S:J **; S:F **; O:D *; O:J **; O:F **; N:J *
	Staphylococci	1.57	0.241	ns	1.59	0.236	ns

F—statistic ratio for ANOVA analysis; *p*—probability value. The bold *p* indicates a significant difference ($p < 0.05$) between the numbers of microorganisms in dependent of sampling sites (School A: I—classroom, II—library, III—cafeteria; School B: IV—classroom; V—library, VI—cafeteria) and month of sampling (September (S), October (O), November (N), December (D), January (J), February (F)). Differences—multiple comparisons (Tukey's HSD test); ns—differences statistically non-significant; *—$p \leq 0.05$; **—$p \leq 0.01$; ***—$p \leq 0.001$.

3.2. Concentrations of Fungal Bioaerosol

In the air of the studied schools, the concentration of molds ranged from 3 to 840 CFU m^{-3} (Table 4). The average concentrations of molds in all the investigated school rooms (classrooms, libraries, cafeterias) were similar and amounted to above 160 CFU m^{-3} in School A and around 300 CFU m^{-3} in School B (Table 4). There were no statistically significant correlations between mold levels and the sampling site.

Table 4. Number of fungi in air (CFU m^{-3}).

Sampling Sites		Month of Sampling						M ± SD
		September	October	November	December	January	February	
School A	I—classroom	283	297	230	30	13	113	161 ± 126
	II—library	220	460	290	40	3	37	175 ± 181
	III—cafeteria	263	283	270	80	27	107	172 ± 113
M ± SD		255 ± 32	347 ± 98	263 ± 31	50 ± 26	14 ± 12	86 ± 42	
School B	IV—classroom	487	457	380	297	37	97	293 ± 188
	V—library	840	690	310	57	37	117	342 ± 345
	VI—cafeteria	547	660	483	310	56	137	366 ± 239
M ± SD		625 ± 189	602 ± 127	391 ± 87	221 ± 142	43 ± 11	117 ± 20	

M—mean; SD—standard deviation.

In the present study, there were statistically significant differences in mold level depending on the sampling month ($p < 0.001$). In School A, average mold concentration was significantly higher ($p < 0.01$–$p < 0.001$) in autumn months—i.e., September, October and November—compared to December, January and February. A similar pattern was observed in School B: statistically significantly lower average mold concentrations ($p < 0.016$–$p < 0.001$) were also recorded in winter months—i.e., December, January and February—as compared to September and October. Mold concentration in November differed significantly ($p = 0.027$) only in relation to January (Table 3).

3.3. Predominant Genera of Airborne Fungi

In the investigated schools, *Cladosporium* prevailed in fungal bioaerosol at the majority of the sampling sites. Their highest concentrations were recorded in the libraries of both schools: School A

(59%), School B (54%), in the classroom in School B (47%), and in the cafeteria in School A (50%). Lower concentration—i.e., 35%—was noted in the classroom in School A and in the cafeteria in School B (Figure 2). The exception was the cafeteria in School B, dominated by *Penicillium* spores accounting for 42% of the fungal bioaerosol. They had a slightly smaller share in the cafeteria in School A (30%), in the classroom in School A (28%) and in the classroom in School B (22%). In the library in School A, *Penicillium* had a share of 12% and in the library in School B (10%) (Figure 2).

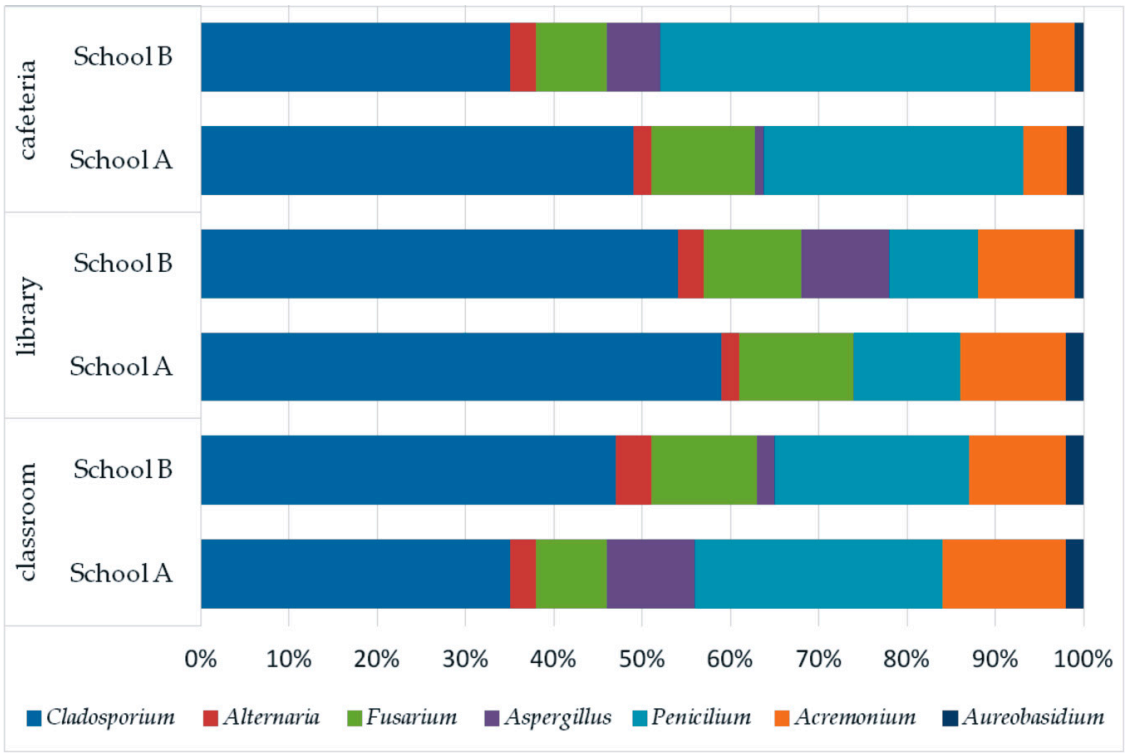


Figure 2. Predominant genera of airborne fungi at all sampling sites.

In the present study, *Fusarium* and *Acremonium* species were recorded alongside *Cladosporium* and *Penicillum*. At all sampling sites, they had a similar share in the fungal bioaerosol, ranging from 8 to 13% and 5 to 12%, respectively. *Aspergillus* spores were also identified, accounting for 10% of fungal bioaerosol in the classroom in School A and in the library in School B, and for 6% in the cafeteria in School B. They were not identified in the library in School A but constituted 1–2% of the fungal bioaerosol in the classroom in School B and in the cafeteria in School A. The spores of *Alternaria* and *Aureobasidium* constituted the lowest percentage of fungal bioaerosol in the studied school rooms (2–4% and 1–2%, respectively) (Figure 2).

3.4. Concentrations of Mannitol-Positive Staphylococci and Their Identification

In the studied schools, the concentration of staphylococci ranged from 0 to 16 CFU m^{-3}, with the maximum recorded in October in the cafeterias in both schools and the library in School B. No mannitol-positive staphylococci were recorded in September in the cafeteria, in November in the classroom in School B or in January in the classroom in School A (Table 5). There were no statistically significant differences in the concentration of staphylococci depending on the sampling site or sampling month (Table 3).

Table 5. Number of mannitol-positive staphylococci in air (CFU m^{-3}).

Sampling Sites		Month of Sampling						M ± SD
		September	October	November	December	January	February	
School A	I—classroom	10	13	10	3	0	6	7 ± 5
	II—library	6	10	3	6	3	10	6 ± 3
	III—cafeteria	3	16	13	6	13	10	10 ± 5
M ± SD		6 ± 3	13 ± 3	9 ± 5	5 ± 2	5 ± 7	9 ± 2	
School B	IV—classroom	3	10	0	10	13	3	7 ± 5
	V—library	13	16	3	13	6	6	10 ± 5
	VI—cafeteria	0	16	13	13	13	13	11 ± 6
M ± SD		5 ± 7	14 ± 3	5 ± 7	12 ± 2	11 ± 4	7 ± 5	

M—mean; SD—standard deviation.

In the air of the investigated schools in Bydgoszcz, *Staphylococcus aureus* was the dominant species, constituting 43% of all mannitol-positive staphylococci in School B and 26% in School A (Table 6). *Staphylococcus xylosus* (25%) and *Staphylococcus haemolyticus* (17%) had a slightly smaller share in the airborne staphylococcal population in School A, while each of the remaining 4 species—i.e., *S. cohnii* spp. *cohnii*, *S. chromogenes*, *S. lentus* and *S. saprophyticus*—accounted for only 4%. In School B, the species composition of mannitol-positive staphylococci was less diverse with four species identified apart from *S. aureus*: *S. saprophyticus* (25%), *S. xylosus* (16%), *S. haemolyticus* (8%) and *S. capities* (8%) (Table 6).

Table 6. Species diversity of the genus *Staphylococcus*.

Phylum	Class	Order	Family	Genus/Species	Percentage	
					School A	School B
Firmicutes	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus haemolyticus*	17%	8%
				Staphylococcus aureus	26%	43%
				Staphylococcus cohnii spp. *cohnii*	8%	0%
				Staphylococcus chromogenes	8%	0%
				Staphylococcus lentus	8%	0%
				Staphylococcus saprophyticus	8%	25%
				Staphylococcus xylosus	25%	16%
				Staphylococcus capities	0%	8%

3.5. Antimicrobial Resistance of Staphylococci

All isolated mannitol-positive staphylococcal strains (n = 24) were susceptible to levofloxacin and gentamicin (100%), while 62.5% were resistant to erythromycin. Some staphylococcal strains also showed resistance to tetracycline (20.8%) and cefoxitin (25%) (Table 7). Penicillin, which was used only for *Staphylococcus aureus*, turned out to be ineffective. All *Staphylococcus aureus* isolates (n = 8) showed resistance to this antibiotic.

Table 7. Antibiotic resistance of staphylococci, determined by the disc diffusion method.

No.	Species	Antibiotic Resistance						
		P1	E15	RD5	CN10	FOX30	TE30	C30	LEV5
1.	*S. saprophyticus*		R	-	-	-	-	-	-
2.	*S. saprophyticus*		R	-	-	-	-	-	-
3.	*S. saprophyticus*		R	-	-	-	-	R	-
4.	*S. saprophyticus*		R	-	-	-	-	-	-
5.	*S. xylosus*		R	-	-	-	-	-	-
6.	*S. xylosus*		-	-	-	-	-	-	-
7.	*S. xylosus*		R	-	-	-	-	-	-

Table 7. *Cont.*

No.	Species	Antibiotic Resistance							
		P1	E15	RD5	CN10	FOX30	TE30	C30	LEV5
8.	*S. xylosus*		R	-	-	-	-	-	-
9.	*S.xylosus*		R	-	-	-	-	-	-
10.	*S. haemolyticus*		-	-	-	R	-	-	-
11.	*S. haemolyticus*		-	-	-	R	-	-	-
12.	*S. haemolyticus*		R	-	-	R	-	-	-
13.	*S. aureus*	R	-	-	-	-	R	-	-
14.	*S. aureus*	R	-	-	-	-	R	-	-
15.	*S. aureus*	R	-	-	-	-	R	-	-
16.	*S. aureus*	R	R	-	-	-	-	-	-
17.	*S. aureus*	R	R	-	-	-	-	-	-
18.	*S. aureus*	R	-	-	-	R	R	-	-
19.	*S. aureus*	R	-	-	-	-	-	-	-
20.	*S. aureus*	R	-	-	-	-	R	-	-
21.	*S.cohnii* spp. *cohnii*		R	-	-	R	-	R	-
22.	*S. chromogenes*		R	-	-	-	-	-	-
23.	*S. lentus*		R	R	-	R	-	-	-
24.	*S. capitis*		R	-	-	-	-	-	-
Percent of resistance		100%	62.5%	4.2%	0%	25.0%	20.8%	8.3%	0%

(R)—resistant; (-)—susceptible; P1—benzylpenicillin; E15—erythromycin; RD5—rifampicin; CN10—gentamicin; FOX30—cefoxitin; TE30—tetracycline; C30—chloramphenicol; LEV5—levofloxacin.

4. Discussion

Bioaerosols are always present in the air and contain contaminant particles including pollen and microorganisms such as bacteria, fungi and viruses [21,22]. As indicators of air quality, they play a key role in air quality and health risk assessment [23–27]. They can reduce air quality in educational institutions [11,28]. According to Brągoszewska [25], indoor air is often polluted by microbial bioaerosols, which are, therefore, a major public health concern.

4.1. Bacterial Bioaresol Concentration

The results presented in the previous section (Section 3.1) indicated that heterotrophic bacteria were the most numerous microorganisms and that their concentrations were similar to those reported by other authors. Madureira [29] in Porto, Portugal recorded a minimum of 268 CFU m^{-3} and a maximum of 8512 CFU m^{-3}, while Andualem et al. [5] in Gondar city, Ethiopia, recorded concentrations ranging from 208 to 9100 CFU m^{-3} in the morning but much higher, ranging from 260 to 23,504 CFU m^{-3} in the afternoon. Slightly lower maximum levels of heterotrophic bacteria were noted by Brągoszewska et al. [3] in rural nursery schools in the Upper Silesia region of Poland (2600 CFU m^{-3}) and by Mainka et al. [30] and Brągoszewska et al. [22] in a nursery school in Gliwice, Poland—i.e., over 3000 CFU m^{-3}. In the studied schools, the highest average concentrations of heterotrophic bacteria were recorded in the cafeterias and classrooms, which tend to be occupied by higher numbers of students and for longer periods of time than libraries. High average concentrations of these microorganisms in these rooms were similar to those reported by Brągoszewska et al. [13] in the primary school (2205 CFU m^{-3}) and nursery school (1408 CFU m^{-3}) in the industrial region of Upper Silesia, Poland. These results differ significantly from those obtained by other researchers, who recorded lower numbers of these microorganisms. In the study by Madureira et al. [31], an average bacterial concentration in schools in Porto, Portugal was 332 CFU m^{-3}. Similar levels were noted by Yang et al. [32] in schools in Seoul, Korea, where the average concentration of heterotrophic bacteria was 451 CFU m^{-3} in the classroom and 297 CFU m^{-3} in the laboratory. Air quality research conducted by Sheik et al. [33] in Saudi Arabia showed a similar average concentration of bacterial bioaerosol in classrooms—i.e., 290 CFU m^{-3}. Much lower average concentrations of heterotrophic bacteria—i.e.,

87 CFU m^{-3}—were recorded by Kallawicha et al. [34] in laboratories in Bangkok, Thailand, which can be associated with different climate, type of ventilation, number of users and their activities. Brągoszewska and Biedroń [11] emphasize the importance of adequate ventilation for microbiological air quality. The researchers recorded the highest average concentrations of heterotrophic bacteria in school offices in Gliwice, Poland in April (821 CFU m^{-3}), when the rooms were not aired regularly and the lowest, in June (424 CFU m^{-3}), when the rooms were well-aired.

Furthermore, as stated by Dumała and Dzudzińska [35] and Al Mijalli [36], microbiological air contamination in school rooms depends on many factors, the most important being the number of children and their physical activity.

According to Fang et al. [37], bacterial concentration in the air is also influenced by meteorological and environmental conditions. Brągoszewska and Pastuszka [22] report that it depends on the geographical location of the investigated facility and seasonal variations in the bacterial community structure. Mentese et al. [38] emphasize that in Ankara, Turkey, the highest microbial concentrations were observed in nursery schools—i.e., 649 CFU m^{-3} in winter and 1462 CFU m^{-3} in summer. Wolny-Koładka et al. [1] noted that in November, the critical values for bacteria were exceeded in an university office, toilet and corridor, and in June and September, in the biomass analysis laboratory.

4.2. Concentrations of Fungal Bioaerosol

Mold level in indoor air is associated with many factors: outdoor air pollutants, room ventilation, building materials, building maintenance, number of occupants in the room and mold infestation of the building [29,39,40]. Poor air quality may lead to lung diseases and allergies and cause non-specific symptoms known as sick building syndrome [41].

In the air of the studied schools, the concentration of molds was much lower than that of heterotrophic bacteria. Similar results were obtained by Kalwasińska et al. [42] examining air quality in the university library in Toruń, Poland. Comparable ranges were also noted by Brągoszewska et al. [3] in rural nursery schools in the Upper Silesia region of Poland (78–788 CFU m^{-3}). In Portuguese primary schools, a higher concentration range—i.e., 16–1792 CFU m^{-3}—was reported by Madureira et al. [31]. However, in nursery schools in Ankara, Turkey, lower levels of fungal aerosols—i.e., 27–53 CFU m^{-3}—were observed by Mentese et al. [43]. In our study, the average concentrations of molds in all the investigated rooms in School B were higher and amounted to approximately 300 CFU m^{-3}. Many researchers have recorded similar average values—i.e., 332 [31] and 368 CFU m^{-3} in school rooms [3] and 294.9 CFU m^{-3} in laboratories [34]. Mold level was lower at all sampling sites in winter, which is consistent with literature reports. Wolny-Koładka et al. [1] also recorded lower concentrations of molds at the University of Agriculture in Cracow in winter compared to other seasons.

4.3. Predominant Genera of Airborne Fungi

The presence of mold spores in indoor air is very common because they can survive on furniture, equipment, in ventilation systems, etc. for a long time [44]. Many mold species are associated with allergic reactions including respiratory and skin symptoms, mainly in people with immunodeficiency [45]. The most clinically important allergens are produced by fungi belonging to the following genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Mucor*, *Penicilium* and *Fusarium* [46,47].

Cladosporium species, ubiquitous worldwide, represent the most frequently isolated airborne fungi, especially in the temperate zone [48–50]. This observation is consistent with our results.

In studies by Madureira et al. [51] fungal bioaerosol in primary schools and childcare centers was dominated by *Penicillium* and *Cladosporium* species. Viegas et al. [52] recorded a variety of molds in gyms with swimming pools, with the highest shares of *Cladosporium* sp. (36.6%), *Penicillium* (19%), *Aspergillus* sp. (10.2%) and *Mucor* (7%).

Kallawich et al. [34] observed that *Aspergillus/Penicillium* were the most abundant fungal taxa in the laboratories (40.6%), followed by *Cladosporium* (30%) and ascospores (17%). Similarly, Verde et al. [53] confirmed the dominance of *Penicillium* (41%) and *Aspergillus* (24%) in the air of hospital rooms.

According to Cabral [54], high humidity in sick buildings promotes fungal growth (mainly of *Penicillium* and *Aspergillus* species) with the release of conidia and cell fragments into the atmosphere.

4.4. Concentrations of Mannitol-Positive Staphylococci and Their Identification

Staphylococci are abundant bacteria of the human skin microbiome [55] that often do not cause infections. However, in certain circumstances, they may pose a threat to both immunocompetent people and people whose immune system is weakened or impaired [1].

Manniotol-positive staphylococci were identified in the air of the studied schools in Bydgoszcz, but their concentrations were lower than those reported by Wolny-Koładka et al. [1] at the university—i.e., from 0 to 65 CFU m^{-3}. According to Boada et al. [56], one fifth of the population (mainly children) are persistent carriers of *S. aureus* This seems to have been confirmed by our results, which indicated that this particular species dominated among the identified staphylococci. The species composition of airborne staphylococci in Bydgoszcz schools was similar to that in the University of Ibadan library in Nigeria [57] where *S. aureus*, *S. arlattae*, *S. cohnii*, *S. haemolyticus* and *S. muscae* were identified. Different results were obtained by Wolny-Koładka et al. [1] at the University of Agricultural in Cracow, where three dominant species—i.e., *S. xylosus* (18%), *S. sciuri* (17%) and *S. hominis* (15%)—were distinguished. At the same time, Brągoszewska et al. [13] identified four staphylococcal species in three educational buildings in the industrial region of Upper Silesia, Poland, namely *S. lentus*, *S. epidermidis*, *S. sciuri* and *S. chromogenes*, while Brągoszewska et al. [58] confirmed the dominance of *S. lentus*, *S. epidermidis* and *S. chromogens* in the high school gym located in an urban area of Southern Poland.

4.5. Antimicrobial Resistance of Staphylococci

The presence of airborne *Staphylococcus* spp. indicates the possible presence of pathogenic microorganisms, in which antibiotic resistance has been observed with increasing frequency over the past several decades. According to Peterson et al. [59], the growth of antibiotic-resistant bacteria poses a serious threat to public health, food security and development today. The importance of this problem on a global scale is emphasized by the World Health Organization [60], which warns that without urgent action, the world is heading for a post-antibiotic era. Moreover, WHO indicates that when new resistance mechanisms are emerging and spreading globally, there is increased demand for new drugs, while the possibilities of obtaining them are currently very limited.

Antibiotic resistance is associated mainly with overuse and misuse of these medications [61,62]. Bacterial strains acquire resistance which spreads rapidly even to modern drugs [63]. Among the factors contributing to the emergence of drug resistance is bacterial ability to adapt to environmental conditions through biofilm formation, which facilitates the spread of their resistance genes [64]. In order to survive stress triggered by antibiotics, bacteria alter their gene expression and activate latent defense mechanisms [65]. A shortage of new drugs and the availability of non-prescription antibiotics combined with the rapid growth of multidrug-resistance in bacteria necessitate an interdisciplinary approach to complementary and alternative treatments [66,67].

The analysis conducted in two Bydgoszcz schools indicated both resistance and sensitivity to selected antibiotics in the isolated Staphyloccocal strains. Levofloxacin and gentamicin turned out to be the most effective medicines. Similar results were obtained by Małecka-Adamowicz et al. [68] investigating the antibiotic resistance of staphylococci isolated from the air in several sports facilities in Bydgoszcz. Sensitivity to levofloxacin most likely results from its increased activity against Gram-positive microorganisms [69]. In the present study the isolated staphylococci had the highest resistance to erythromycin (62.5%). Comparable results were obtained by Frías-De León et al. [70] and Lenart-Boroń et al. [62].

In the study by Wolny-Koładka et al. [1], more than 50% of staphylococci isolated from the air on the premises of the University of Agricultural in Cracow were resistant to erythromycin and about 33%, to tetracycline. A much higher resistance to tetracycline (75% of strains) was recorded by Giwa et al. [57] in the air of university libraries in Ibadan, Nigeria. According to Lenart-Boroń et al. [71], high resistance of the studied strains to erythromycin and tetracycline may stem from the fact that these antibiotics have been in use for a long time, which may have contributed to the increased resistance of bacteria to these drugs.

As recommended by the European Committee for Susceptibility Testing [19], the analysis of susceptibility to penicillin was performed only for *S. aureus* due to fact that no currently available method can reliably detect penicillinase production in coagulase-negative staphylococci. All isolated *S. aureus* strains were penicillin resistant, which is connected with their ability to produce penicillinase. More than 90% of all staphylococci are capable of producing this enzyme, resulting in their resistance to most penicillins [19].

The rapid emergence of resistant bacteria, which occurs worldwide, endangers the efficacy of antibiotics and may lead to a serious crisis in healthcare. Therefore, a need for better understanding of antibiotic-resistant bacterial populations is obvious [23]. In view of that, the monitoring of air quality in schools in order to assess bacterial acquisition of antibiotic resistance seems a necessary measure.

Exposure to bioaerosols has become a serious health problem. However, no international standards specifying its acceptable maximum levels for indoor environments are available [72].

In addition, it should be emphasized that with the use of different equipment and sampling methods it is not possible to provide a reliable comparison of the results obtained by different researchers [73]. Therefore, relevant organizations should develop clear criteria for assessing indoor and outdoor air quality Wolny-Koładka et al. [1].

5. Conclusions

Our study was conducted in two schools in northern Poland, where air samples were collected not only from classrooms (standard approach) but also from libraries and cafeterias, which are frequently used by students. The results led to the following conclusions:

1. A statistically significant relationship between the size of the rooms and the concentration of heterotrophic bacteria was observed. In both schools, higher microbial concentrations were recorded in smaller rooms (the cafeterias). There were no statistically significant differences in the concentration of molds and staphylococci depending on the sampling site. The average concentrations of these groups of microorganisms was similar in the studied rooms.
2. In both schools, only mold concentration significantly depended on the sampling month ($p < 0.001$), which may be connected with relative humidity.
3. The air temperature was similar in all the investigated rooms; therefore, this factor did not significantly affect microbial concentration.
4. In the air of the investigated school rooms, *Cladosporium* and *Penicillium* species were dominant fungi, which may be related to the use of natural ventilation—i.e., opening the windows—allowing the inflow of these molds from the outside.
5. The taxonomic analysis indicated that *Staphylococcus aureus* dominated among mannitol-positive staphylococci in both schools, while *S. xylosus*, *S. haemolyticus* and *S. saprophyticus* were slightly less abundant. The predominance of the opportunistic *Staphylococcus aureus* in the air of the studied schools confirms the observation that there are carriers of this bacteria among students.
6. Antibiogram patterns of the isolated staphylococcal strains showed their high resistance to erythromycin, while levofloxacin and gentamicin were the most effective antibiotics. No spread of multidrug-resistant staphylococcal strains in the air of the studied schools was observed.

Our research may serve as a basis for future studies, which should be supplemented with measurements of particulate matter (PM) (with special emphasis on respirable PM) and of physicochemical parameters. The amount of aerosol inhaled by room users should also be determined. In addition, given the current lack of precise indoor air quality guidelines in Poland, the research may be a valuable contribution.

Author Contributions: Conceptualization, M.M.-A.; Data curation, M.M.-A.; Methodology, M.M.-A. and B.K.-P.; Supervision, M.M.-A.; Visualization, M.M.-A.; Writing—original draft, M.M.-A., B.K.-P. and E.A.D.; Writing—review and editing, M.M.-A., B.K.-P. and E.A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This paper was funded by the Polish Minister of Science and Higher Education under the programme "Regional Initiative of Excellence" in 2019–2022 (Grant No. 008/RID/2018/19).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wolny-Koładka, K.; Malinowski, M.; Pieklik, A.; Kurpaska, S. Microbiological air contamination in university premises and the evaluation of drug resistance of staphylococci occurring in the form of a bioaerosol. *Indoor Built Environ.* **2017**, *28*, 235–246. [CrossRef]
2. Meadow, J.F.; Altrichter, A.E.; Kembel, S.W.; Kline, J.; Mhuireach, G.; Moriyama, M.; Northcutt, D.; O'Connor, T.K.; Womack, A.M.; Brown, G.Z.; et al. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* **2013**, *24*, 41–48. [CrossRef]
3. Brągoszewska, E.; Mainka, A.; Pastuszka, J.S. Bacterial and Fungal Aerosols in Rural Nursery Schools in Southern Poland. *Atmosphere* **2016**, *7*, 142. [CrossRef]
4. Ramos, C.; Wolterbeek, H.; Almeida, S. Exposure to indoor air pollutants during physical activity in fitness centers. *Build. Environ.* **2014**, *82*, 349–360. [CrossRef]
5. Andualem, Z.; Gizaw, Z.; Bogale, L.; Dagne, H. Indoor bacterial load and its correlation to physical indoor air quality parameters in public primary schools. *Multidiscip. Respir. Med.* **2019**, *14*, 2. [CrossRef]
6. Naruka, K.; Gaur, J. Microbial air contamination in a school. *Int. J. Curr. Microbiol. App. Sci.* **2013**, *2*, 404–405.
7. Canha, N.; Almeida, S.; Freitas, M.C.; Täubel, M.; Hänninen, O. Winter Ventilation Rates at Primary Schools: Comparison between Portugal and Finland. *J. Toxicol. Environ. Health Part A* **2013**, *76*, 400–408. [CrossRef]
8. Patelarou, E.; Tzanakis, N.; Kelly, F.J. Exposure to Indoor Pollutants and Wheeze and Asthma Development during Early Childhood. *Int. J. Environ. Res. Public Health* **2015**, *12*, 3993–4017. [CrossRef]
9. Mainka, A.; Zajusz-Zubek, E. Indoor Air Quality in Urban and Rural Preschools in Upper Silesia, Poland: Particulate Matter and Carbon Dioxide. *Int. J. Environ. Res. Public Health* **2015**, *12*, 7697–7711. [CrossRef] [PubMed]
10. Du, P.; Du, R.; Lu, Z.; Ren, W.; Fu, P. Variation of Bacterial and Fungal Community Structures in PM2.5 Collected during the 2014 APEC Summit Periods. *Aerosol Air Qual. Res.* **2018**, *18*, 444–455. [CrossRef]
11. Brągoszewska, E.; Biedroń, I.; Kozielska, B.; Pastuszka, J.S. Microbiological indoor air quality in an office building in Gliwice, Poland: Analysis of the case study. *Air Qual. Atmos. Health* **2018**, *11*, 729–740. [CrossRef]
12. Fsadni, P.; Frank, B.; Fsadni, C.; Montefort, S. The Impact of Microbiological Pollutants on School Indoor Air Quality. *J. Geosci. Environ. Prot.* **2017**, *5*, 54–65. [CrossRef]
13. Brągoszewska, E.; Mainka, A.; Pastuszka, J.S.; Lizończyk, K.; Desta, Y.G. Assessment of Bacterial Aerosol in a Preschool, Primary School and High School in Poland. *Atmosphere* **2018**, *9*, 87. [CrossRef]
14. Demirel, G.; Özden, Ö; Döğeroğlu, T.; Gaga, E.O. Personal exposure of primary school children to BTEX, NO_2 and ozone in Eskişehir, Turkey: Relationship with indoor/outdoor concentrations and risk assessment. *Sci. Total Environ.* **2014**, *473*, 537–548. [CrossRef]
15. Yoon, C.; Lee, K.; Park, N. Indoor air quality differences between urban and rural preschools in Korea. *Environ. Sci. Pollut. Res.* **2010**, *18*, 333–345. [CrossRef]
16. Labi, A.-K.; Obeng-Nkrumah, N.; Bjerrum, S.; Aryee, N.A.A.; Ofori-Adjei, Y.A.; Yawson, A.E.; Newman, M.J. Physicians' knowledge, attitudes, and perceptions concerning antibiotic resistance: A survey in a Ghanaian tertiary care hospital. *BMC Health Serv. Res.* **2018**, *18*, 126. [CrossRef]
17. Blaga, A.B. The importance of indoor air quality study in schools. *AMT* **2016**, *21*, 19.

18. Feller, W. *An Introduction to the Probability Theory and Its Application*; John Wiley & Sons Inc.: New York, NY, USA, 1950.
19. European Committee on Antimicrobial Susceptibility Testing—EUCAST. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 8.1 2018. 2018. Available online: http://www.eucast.org (accessed on 15 May 2018).
20. Samson, R.A.; Hoekstra, E.S.; Frisvad, J.C. *Introduction to Food and Airborne Fungi*, 7th ed.; Centraalbureau voor Schimmelcultures: Utrecht, The Netherlands, 2004.
21. Tolabi, Z.; Alimohammadi, M.; Hassanvand, M.S.; Nabizadeh, R.; Soleimani, H.; Zarei, A. The investigation of type and concentration of bio-aerosols in the air of surgical rooms: A case study in Shariati hospital, Karaj. *MethodsX* **2019**, *6*, 641–650. [CrossRef] [PubMed]
22. Brągoszewska, E.; Pastuszka, J.S. Influence of meteorological factors on the level and characteristics of culturable bacteria in the air in Gliwice, Upper Silesia (Poland). *Aerobiology* **2018**, *34*, 241–255. [CrossRef] [PubMed]
23. Brągoszewska, E.; Biedroń, I.; Hryb, W. Microbiological Air Quality and Drug Resistance in Airborne Bacteria Isolated from a Waste Sorting Plant Located in Poland—A Case Study. *Microorganism* **2020**, *8*, 202. [CrossRef]
24. Jiayu, C.; Qiaoqiao, R.; Feilong, C.; Chen, L.; Jiguo, W.; Zhendong, W.; Lingyun, C.; Liu, R.; Guoxia, Z. Microbiology Community Structure in Bioaerosols and the Respiratory Diseases. *J. Environ. Sci. Public Health* **2019**, *3*, 347–357. [CrossRef]
25. Brągoszewska, E.; Bogacka, M.; Pikoń, K. Efficiency and Eco-Costs of Air Purifiers in Terms of Improving Microbiological Indoor Air Quality in Dwellings—A Case Study. *Atmosphere* **2019**, *10*, 742. [CrossRef]
26. Moore, M.N. Do airborne biogenic chemicals interact with the PI3K/Akt/mTOR cell signalling pathway to benefit human health and wellbeing in rural and coastal environments? *Environ. Res.* **2015**, *140*, 65–75. [CrossRef]
27. Cincinelli, A.; Martellini, T. Indoor Air Quality and Health. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1286. [CrossRef] [PubMed]
28. Gizaw, Z.; Gebrehiwot, M.; Yenew, C. High bacterial load of indoor air in hospital wards: The case of University of Gondar teaching hospital, Northwest Ethiopia. *Multidiscip. Respir. Med.* **2016**, *11*, 24. [CrossRef] [PubMed]
29. Madureira, J.; Aguiar, L.; Pereira, C.C.; Mendes, A.; Querido, M.; Neves, P.; Teixeira, J.P. Indoor exposure to bioaerosol particles: Levels and implications for inhalation dose rates in schoolchildren. *Air Qual. Atmos. Health* **2018**, *11*, 955–964. [CrossRef]
30. Mainka, A.; Brągoszewska, E.; Kozielska, B.; Pastuszka, J.S.; Zajusz-Zubek, E. Indoor air quality in urban nursery schools in Gliwice, Poland: Analysis of the case study. *Atmos. Pollut. Res.* **2015**, *6*, 1098–1104. [CrossRef]
31. Madureira, J.; Paciência, I.; Fernandes, E.D.O. Levels and Indoor–Outdoor Relationships of Size-Specific Particulate Matter in Naturally Ventilated Portuguese Schools. *J. Toxicol. Environ. Health Part A* **2012**, *75*, 1423–1436. [CrossRef]
32. Yang, J.; Nam, I.; Yun, H.; Kim, J.; Oh, H.-J.; Lee, D.; Jeon, S.-M.; Yoo, S.-H.; Sohn, J.-R. Characteristics of indoor air quality at urban elementary schools in Seoul, Korea: Assessment of effect of surrounding environments. *Atmos. Pollut. Res.* **2015**, *6*, 1113–1122. [CrossRef]
33. Sheik, G.B.; Ismail, A.; Abd, A.; Rheam, A.; Saad, Z.; Shehri, A.; Bin, O.; Al, M. Assessment of Bacteria and Fungi in air from College of Applied Medical Sciences (Male) at AD-Dawadmi, Saudi Arabia. *Int. J. Sci. Technol. Res.* **2015**, *4*, 48–53.
34. Kallawicha, K.; Chao, H.J.; Kotchasatan, N. Bioaerosol levels and the indoor air quality of laboratories in Bangkok metropolis. *Aerobiologia* **2018**, *35*, 1–14. [CrossRef]
35. Dumała, S.M.; Dudzińska, M.R. Microbiological indoor air quality in Polish schools. *Annu. Set Environ. Prot.* **2013**, *15*, 231–244.
36. Al-Mijalli, S.H.S. Bacterial Contamination of Indoor Air in Schools of Riyadh, Saudi Arabia. *Air Water Borne Dis.* **2017**, *6*, 1–8. [CrossRef]
37. Fang, Z.; Guo, W.; Zhang, J.; Lou, X. Influence of Heat Events on the Composition of Airborne Bacterial Communities in Urban Ecosystems. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2295. [CrossRef]
38. Mentese, S.; Rad, A.Y.; Arısoy, M.; Güllü, G. Seasonal and Spatial Variations of Bioaerosols in Indoor Urban Environments, Ankara, Turkey. *Indoor Built Environ.* **2011**, *21*, 797–810. [CrossRef]

39. Womack, A.M.; Bohannan, B.J.M.; Green, J.L. Biodiversity and biogeography of the atmosphere. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 3645–3653. [CrossRef]
40. Bowers, R.M.; McCubbin, I.B.; Hallar, A.G.; Fierer, N. Seasonal variability in airborne bacterial communities at a high-elevation site. *Atmos. Environ.* **2012**, *50*, 41–49. [CrossRef]
41. Pallabi, P. Review on Common Microbiological Contamination Found in Hospital Air. *J. Microbiol. Pathol.* **2018**, *2*, 103.
42. Kalwasińska, A.; Burkowska, A.; Wilk, I. Microbial air contamination in indoor environment of a university library. *Ann. Agric. Environ. Med.* **2012**, *19*, 25–29.
43. Mentese, S.; Arısoy, M.; Rad, A.Y.; Güllü, G.; Arisoy, M. Bacteria and Fungi Levels in Various Indoor and Outdoor Environments in Ankara, Turkey. *Clean Soil Air Water* **2009**, *37*, 487–493. [CrossRef]
44. Kobza, J.; Pastuszka, J.S.; Brągoszewska, E. Do exposures to aerosols pose a risk to dental professionals? *Occup. Med.* **2018**, *68*, 454–458. [CrossRef]
45. Twaroch, T.E.; Curin, M.; Valenta, R.; Swoboda, I. Mold Allergens in Respiratory Allergy: From Structure to Therapy. *Allergy Asthma Immunol. Res.* **2015**, *7*, 205–220. [CrossRef] [PubMed]
46. Crameri, R.; Garbani, M.; Rhyner, C.; Huitema, C. Fungi: The neglected allergenic sources. *Allergy* **2013**, *69*, 176–185. [CrossRef] [PubMed]
47. Pusz, W.; Kita, W.; Weber, R. Microhabitat Influences the Occurrence of Airborne Fungi in Copper Mine in Poland. *J. Cave Karst Stud.* **2014**, *76*, 14–19. [CrossRef]
48. Almaguer, M.; Aira, M.J.; Rodríguez-Rajo, F.J.; Fernández-González, M.; Rojas-Flores, T.I. Thirty-four identifiable airborne fungal spores in Havana, Cuba. *Ann. Agric. Environ. Med.* **2015**, *22*, 215–220. [CrossRef]
49. Khan, M.; Perveen, A.; Qaiser, M. Seasonal and diurnal variation of atmospheric fungal spore concentrations in Hyderabad, Tandojam-Sindh and the effects of climatic conditions. *Pak. J. Bot.* **2016**, *48*, 1657–1663.
50. Antón, S.F.; De La Cruz, D.R.; Sánchez, J.S.; Reyes, E.S. Analysis of the airborne fungal spores present in the atmosphere of Salamanca (MW Spain): A preliminary survey. *Aerobiologia* **2019**, *35*, 447–462. [CrossRef]
51. Madureira, J.; Paciência, I.; Rufo, J.C.; Pereira, C.; Teixeira, J.P.; Fernandes, E.D.O. Assessment and determinants of airborne bacterial and fungal concentrations in different indoor environments: Homes, child day-care centres, primary schools and elderly care centres. *Atmos. Environ.* **2015**, *109*, 139–146. [CrossRef]
52. Viegas, C.; Alves, C.; Carolino, E.; Rosado, L.; Santos, C.S. Prevalence of Fungi in Indoor Air with Reference to Gymnasiums with Swimming Pools. *Indoor Built Environ.* **2010**, *19*, 555–561. [CrossRef]
53. Verde, S.C.; Almeida, S.; Matos, J.; Guerreiro, D.; Meneses, M.; Faria, T.; Botelho, D.; Santos, M.; Viegas, C. Microbiological assessment of indoor air quality at different hospital sites. *Res. Microbiol.* **2015**, *166*, 557–563. [CrossRef]
54. Cabral, J.P.S. Can we use indoor fungi as bioindicators of indoor air quality? Historical perspectives and open questions. *Sci. Total Environ.* **2010**, *408*, 4285–4295. [CrossRef]
55. Tršan, M.; Seme, K.; Srčič, S. The environmental monitoring in hospital pharmacy cleanroom and microbiota catalogue preparation. *Saudi Pharm. J.* **2019**, *27*, 455–462. [CrossRef] [PubMed]
56. Boada, A.; Pons-Vigués, M.; Real, J.; Grezner, E.; Bolíbar, B.; Llor, C. Previous antibiotic exposure and antibiotic resistance of commensal *Staphylococcus aureus* in Spanish primary care. *Eur. J. Gen. Pract.* **2018**, *24*, 125–130. [CrossRef]
57. Giwa, H.J.; Ogunjobi, A.A. Prevalence of multiple antibiotic resistant bacteria in selected libraries of University of Ibadan, Nigeria. *J. Am. Sci.* **2017**, *13*, 18–25. [CrossRef]
58. Brągoszewska, E.; Biedroń, I.; Mainka, A. Microbiological Air Quality in a Highschool Gym Located in an Urban Area of Southern Poland—Preliminary Research. *Atmosphere* **2020**, *11*, 797. [CrossRef]
59. Peterson, E.; Kaur, P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front. Microbiol.* **2018**, *9*, 2928. [CrossRef]
60. WHO—World Health Organization. *Antimicrobial Resistance Global Report on Surveillance*; World Health Organizations: Geneva, Switzerland, 2014.
61. Alhomoud, F.; Almahasnah, R.; Alhomoud, F.K. "You could lose when you misuse"—Factors affecting over-the-counter sale of antibiotics in community pharmacies in Saudi Arabia: A qualitative study. *BMC Health Serv. Res.* **2018**, *18*, 915. [CrossRef]

62. Lenart-Boron, A.; Wolny-Koładka, K.; Juraszek, K.; Kasprowicz, A. Phenotypic and molecular assessment of antimicrobial resistance profile of airborne *Staphylococcus* spp. isolated from flats in Kraków. *Aerobiologia* **2017**, *33*, 435–444. [CrossRef] [PubMed]
63. Nahaei, M.R.; Shahmohammadi, M.R.; Ebrahimi, S.; Milani, M. Detection of Methicillin-Resistant Coagulase-Negative Staphylococci and Surveillance of Antibacterial Resistance in a Multi-Center Study from Iran. *Jundishapur J. Microbiol.* **2015**, *8*, e19945. [CrossRef]
64. Brągoszewska, E.; Biedroń, I. Indoor Air Quality and Potential Health Risk Impacts of Exposure to Antibiotic Resistant Bacteria in an Office Rooms in Southern Poland. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2604. [CrossRef]
65. Palmer, A.; Chait, R.; Kishony, R. Nonoptimal Gene Expression Creates Latent Potential for Antibiotic Resistance. *Mol. Biol. Evol.* **2018**, *35*, 2669–2684. [CrossRef] [PubMed]
66. Aslam, B.; Wang, W.; Arshad, M.I.; Khurshid, M.; Muzammil, S.; Rasool, M.H.; Nisar, M.A.; Alvi, R.F.; Aslam, M.A.; Qamar, M.U.; et al. Antibiotic resistance: A rundown of a global crisis. *Infect. Drug Resist.* **2018**, *11*, 1645–1658. [CrossRef] [PubMed]
67. Horumpende, P.G.; Sonda, T.B.; Van Zwetselaar, M.; Antony, M.L.; Tenu, F.F.; Mwanziva, C.E.; Shao, E.R.; Mshana, S.E.; Mmbaga, B.T.; Chilongola, J.O. Prescription and non-prescription antibiotic dispensing practices in part I and part II pharmacies in Moshi Municipality, Kilimanjaro Region in Tanzania: A simulated clients approach. *PLoS ONE* **2018**, *13*, e0207465. [CrossRef]
68. Małecka-Adamowicz, M.; Kubera, Ł.; Jankowiak, E.; Dembowska, E. Microbial diversity of bioaerosol inside sports facilities and antibiotic resistance of isolated *Staphylococcus* spp. *Aerobiologia* **2019**, *35*, 731–742. [CrossRef]
69. May, L.; Klein, E.Y.; Rothman, R.E.; Laxminarayan, R. Trends in Antibiotic Resistance in Coagulase-Negative Staphylococci in the United States, 1999 to 2012. *Antimicrob. Agents Chemother.* **2013**, *58*, 1404–1409. [CrossRef]
70. León, M.G.F.-D.; Duarte-Escalante, E.; Calderón-Ezquerro, M.D.C.; Jiménez-Martínez, M.D.C.; Acosta-Altamirano, G.; Moreno-Eutimio, M.A.; Zúñiga, G.; García-González, R.; Ramírez-Pérez, M.; Reyes-Montes, M.D.R. Diversity and characterization of airborne bacteria at two health institutions. *Aerobiologia* **2015**, *32*, 187–198. [CrossRef]
71. Lenart-Boron, A.; Wolny-Koładka, K.; Stec, J.; Kasprowic, A. Phenotypic and Molecular Antibiotic Resistance Determination of Airborne Coagulase Negative *Staphylococcus* spp. Strains from Healthcare Facilities in Southern Poland. *Microb. Drug Resist.* **2016**, *22*, 515–522. [CrossRef] [PubMed]
72. Mirhoseini, S.H.; Nikaeen, M.; Satoh, K.; Makimura, K. Assessment of Airborne Particles in Indoor Environments: Applicability of Particle Counting for Prediction of Bioaerosol Concentrations. *Aerosol Air Qual. Res.* **2016**, *16*, 1903–1910. [CrossRef]
73. D'Arcy, N.; Canales, M.; Spratt, D.A.; Lai, K.M. Healthy schools: Standardisation of culturing methods for seeking airborne pathogens in bioaerosols emitted from human sources. *Aerobiologia* **2012**, *28*, 413–422. [CrossRef]