

Article

# Microbiological Air Quality in a Highschool Gym Located in an Urban Area of Southern Poland—Preliminary Research

Ewa Bragoszewska <sup>1,\*</sup> , Izabela Biedroń <sup>2</sup>  and Anna Mainka <sup>3</sup> 

<sup>1</sup> Faculty of Energy and Environmental Engineering, Department of Technologies and Installations for Waste Management, Silesian University of Technology, 18 Konarskiego St., 44-100 Gliwice, Poland

<sup>2</sup> Faculty of Science, Universität Bern, Hochschulstrasse 6, 3012 Bern, Switzerland; izabiedron@gmail.com

<sup>3</sup> Faculty of Energy and Environmental Engineering, Department of Air Protection, Silesian University of Technology, 22B Konarskiego Str., 44-100 Gliwice, Poland; Anna.Mainka@polsl.pl

\* Correspondence: Ewa.Bragoszewska@polsl.pl; Tel.: +48-322-372-762

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**Abstract:** The benefits of regular exercise include improved physical and mental health. The school gym is a particular micro-environment where students perform intensive physical training. The question is if there is an increased risk of microbiological contamination. This preliminary work studied the exposure of students to bacterial aerosol (BA) in a highschool gym located in an urban area of Southern Poland. A sampling of BA was undertaken with an Andersen six-stage impactor (ANDI). BA was identified using API (analytical profile index) tests. The BA concentrations were expressed as Colony Forming Units (CFU) per cubic metre of air. The results showed that before gym classes (BGC), the concentration of BA was  $4.20 \times 10^2 \pm 49.19$  CFU/m<sup>3</sup>, while during gym classes (DGC), the level of BA more than doubled ( $8.75 \times 10^2 \pm 121.39$  CFU/m<sup>3</sup>). There was also an increase in the respirable fraction of BA (particles less than 3.3 µm). Before the start of the sports activities, respirable fraction accounted for 30% of the BA, while during physical education classes, this share increased to over 80%. Identification of BA species showed that the dominant group of bacteria in the indoor air of the gym BGC was Gram-positive rods (61%) and for DGC it was Gram-positive cocci (81%). We detected that one bacteria strain (*Corynebacterium striatum*) was classified into risk group 2 (RG2) according to Directive 2000/54/EC. Additionally, multi-antibiotic resistance (MAR) showed that among the isolated airborne bacteria, the highest antibiotic resistance was demonstrated by *Staphylococcus epidermis* (isolated DGC) and *Pseudomonas* sp. (isolated BGC). The quantitative and qualitative information on microbiological air quality (MIAQ) in the school gym indicates that the actions to improve indoor physical activity spaces are recommended.

**Keywords:** microbiological indoor air quality (MIAQ); bacterial aerosol (BA); size distribution; gymnastic hall; multi-antibiotic resistance (MAR)

## 1. Introduction

According to available studies on indoor air quality (IAQ), it was found that both air pollution and physical conditions (including temperature, humidity and inefficient ventilation) have a negative impact on the health of building residents [1–9].

Previous studies conducted in educational buildings showed that poor microbiological indoor air quality (MIAQ) in classrooms exerts a negative effect on students' learning performance [10]. Poor air quality has been shown to increase absenteeism and increase the risk of asthma and other health-related issues [11,12]. In general, about 1% of school-age children in Poland suffer from chronic respiratory diseases [13]. There are 8000 secondary schools in Poland, with 13,360 students. The most popular

schools are post-gymnasium highschoools, which enable students to obtain a certificate of maturity and continue to education in universities. These schools are attended by 86.8% of all post-gymnasium students. Among the educational facilities, 3818 include highschoools, 424 of which are located in the cities of an urban area of Southern Poland (Silesia Province) [14].

Regulation from the Minister of National Education and Sport on safety and hygiene in public and non-public schools and institutions, and Framework Directive 89/391/EEC [15], oblige school principals to ensure safe and hygienic conditions for students and teachers [16,17]. This requirement is supported by the framework of school statutes as well as other general health and safety regulations.

Compared to other indoor spaces in educational buildings, it is interesting to note that school gyms offer a unique place to explore microbial diversity. Sports facilities exhibit exceptional conditions for bacterial aerosol (BA) proliferation, and the conditions in which students exercise are seldom examined. One of the parameters prevalent in this type of building is moisture emitted due to perspiration and water condensation as a result of the physical activity of occupants [18]. Although regular exercise improves overall well-being, decreases the prevalence of diseases and improves physical health, the public's passion for exercise has been discouraged by severe air pollution [19]. Increased physical activity in polluted areas consequently leads to elevated exposure of some pollutants producing adverse health effects [20–22]. During exercise, the air is generally inhaled through the mouth and at a higher-than-normal rate, so the intake of airborne contaminants increases, with increased penetration to the lower parts of the lungs [12].

Regardless of the existence of many reports on MIAQ in educational buildings [2,3,11,23–26], studies on potential risks of exposure to BA in gyms situated in academic environments during physical education or exercise lessons are very limited [18,27–29]. Additionally, there is still a shortage of information on the transmission of antimicrobial resistance through the air [30]. Multi-antibiotic resistance (MAR) is considered to be a rapidly progressing global public health issue with the potential of extensive environmental transmission. It is a widespread and alarming issue in global health, causing more than 700,000 deaths every year [31]. Including information about MIAQ in indoor spaces is recommended for health and well-being [32].

The objectives of this study are: (a) to assess bacterial aerosol (BA) air quality in the gymnastic hall of a highschoool building located in the urban area of Southern Poland, (b) to determine the concentration and BA particle size distribution in the gymnastic hall before gym classes (BGC), during gym classes (DGC) and in the outdoor air (OUT), (c) to identify isolated strains of bacteria BGC and DGC and (d) to determine the antibiotic resistance of isolated strains of BA.

## 2. Experiments

### 2.1. Sampling Sites

The study was carried out in a highschoool gym located in an urban area of Southern Poland (50°15'44.121" N 19°0'57.16" E). The research was conducted during the spring season. The spring season was selected for this study because recent research of MIAQ conducted in Southern Poland indicates that the highest BA concentration is consistently found in the spring [9,11,33]. Every measurement was conducted between 7:00 and 9:00 a.m. (BGC), and 10:00–12:00 a.m. (DGC, attended by 15–17 students).

In the analyzed school gym, MIAQ is primarily ensured by means of stack ventilation and airing through open and unsealed windows. The gym is aired before classes and during breaks when students leave the hall. Following Polish legislation, the ventilation system in each educational building is checked each year and should ensure three to five air changes per hour [12]. In the gymnastic hall, deep wet cleaning was carried out once a day after the occupancy period. Table 1 presents a description of the educational building. The device used for air temperature and humidity measurement was an portable Weather Station WMR 200 (Oregon Scientific, Portland, OR, USA).

**Table 1.** Environmental parameters and description of the analyzed gymnastic hall.

| School Localization           | In the City Center,<br>Near a Busy Street |
|-------------------------------|---|
| Building, built in            | 1900's                                    |
| Ventilation system            | natural                                   |
| Volume, m <sup>3</sup>        | 320                                       |
| Number of children            | 15–17                                     |
| Age of students               | 16–18                                     |
| Floor covered with            | PVC (polyvinyl chloride)                  |
| Indoor temperature (°C)       | 19.1 ± 4.6                                |
| Outdoor temperature (°C)      | 16.2 ± 3.1                                |
| Indoor relative humidity (%)  | 28.1 ± 5.4                                |
| Outdoor relative humidity (%) | 36.2 ± 7.1                                |

## 2.2. Sampling and Analytical Methods

BA was collected using an Andersen six-stage impactor ANDI (Thermo Fisher Scientific, Waltham, MA, USA) with cut-off diameters of 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 µm, with constant flow rate (28.3 L/min). The sampling time was 10 min, following Nevalainen et al. [34]. The device was disinfected using 70% ethanol-immersed cotton balls between each sampling. Samples were collected on nutrient media. Tryptic soy agar (TSA, BioMaxima) was used for BA, with cycloheximide (500 mg/L, 95%, ACROS Organics™, USA) added to inhibit fungal growth.

All Petri dishes were incubated for 48 h at 36 ± 1 °C. The samples of BA were collected in the center of the gymnastic hall and taken at the height of the student's breathing zone, about 1.5 m from the ground. In total, we quantitatively analyzed 324 Petri dishes (18 measurement series for OUT, BGC and DGC) and qualitatively, we analyzed 216 Petri dishes (18 measurement series both for BGC and DGC) with biological material.

The testing of blank plates was performed per batch of prepared medium at the temperature used during the performed procedure. The blanks were not contaminated. The sampling equipment (ANDI) and laboratory equipment (laminar flow cabinet, autoclave, incubators and microscope) were regularly checked and had current certificates.

## 2.3. Bacteria Identification

BA strains obtained from each intake were isolated. In the first stage, colony morphology was macroscopically determined (shape, pigmentation, edges, etc.). The next stage was microscopic analysis (bacterial morphology, motility and reaction to Gram staining, etc.). The next steps focused on selecting a group of microorganisms present in each of the tested samples. For this purpose, a comparative analysis of the created feature matrix was used. A series of screening analyses led to the acquisition of strains of bacteria, present during each collection. The selected microorganisms were then identified, and their antibiotic resistance was determined.

Next, we gathered information about the main groups of bacteria present in the indoor air in the gymnastic hall. Isolated strains were cultivated on the agar medium with the addition of blood (trypticase-soy agar with 5% sheep blood). Selected strains were characterized in terms of their metabolic characteristics by using the biochemical test API (analytical profile index), which is supported by APIweb (bioMérieux, Marcy-l'Étoile, France). The following API systems were used: API 20E, API 20NE, API 50CH, API CORYNE, API STAPH and API STREP.

## 2.4. Multi-Antibiotic Resistance Test (MAR)

For antimicrobial susceptibility testing, the disc diffusion method was carried out according to the Kirby–Bauer Disk Diffusion Susceptibility Test Protocol [35]. Isolated strains of the BA were determined. During night cultures, the bacterial isolates were diluted to 1 McFarland (3 × 10<sup>8</sup> colony

forming units/mL). 100 µL of bacterial inoculum was spread over the surface of a Mueller–Hinton agar plate (Oxoid, Columbia, MD, USA). Next, antimicrobial susceptibility testing discs (Oxoid, Columbia, MD, USA) were placed on inoculated Mueller–Hinton agar plates. 20 different antibiotics and their concentrations were chosen to take into consideration the common antibiotic resistance referred to in the literature on bacterial species. Three repetitions of each antibiotic were performed. Specific doses of the antibiotics are presented in Table 2.

**Table 2.** Antibiotics and their doses used in multi-antibiotic resistance test (MAR).

| Group of Antibiotics | Antibiotic      | Symbol/Dose (µg) |
|----------------------|-----------------|------------------|
| Penicillins          | Amoxicillin     | AML (25)         |
|                      | Ampicillin      | AMP (25)         |
| Cephalosporines      | Ceftazidime     | CAZ (30)         |
|                      | Cephalothin     | KF (30)          |
|                      | Cefuroxime      | CXM (30)         |
| Quinolones           | Nalidic acid    | NA (30)          |
| Aminoglycosides      | Amikacin        | AK (30)          |
|                      | Doxycycline     | DO (30)          |
|                      | Erythromycin    | E (30)           |
|                      | Gentamicin      | CN (30)          |
|                      | Kanamycin       | K (30)           |
|                      | Neomycin        | N (30)           |
|                      | Streptomycin    | S (25)           |
| Tobramycin           | TOB (10)        |                  |
| Tetracyclines        | Tetracycline    | TE (30)          |
| Sulfonamides         | Trimethoprim    | W (5)            |
| Rifampicins          | Rifampicin      | RD (30)          |
| Other                | Chloramphenicol | C (30)           |
|                      | Nitrofurantoin  | F (200)          |
|                      | Novobiocin      | NV (30)          |

Petri dishes with bacteria were incubated at 37 °C for 24 h. After incubation, the areas of inhibition growth were measured. A three-stage scale was used in order to assess the bacteria resistance to antibiotics: diameter of growth inhibition < 15 mm—bacterial resistance to an antibiotic, diameter of growth inhibition between 16 and 25 mm—intermediate bacterial resistance to an antibiotic and diameter of growth inhibition > 25 mm—bacteria sensitive to the antibiotic.

### 2.5. Statistical Analysis

The R Studio 1.2.5042 was used to perform all statistical analyses, and the ggplot2 package was used to generate all plots [36]. The presence of significant differences was determined using one-way analysis of variance (ANOVA). Pairwise *t*-test with Bonferroni correction for multiple comparisons was performed if significant results were obtained in the ANOVA test ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1. Quantity of Bacterial Aerosol (BA) in a Highschool Gym: Before Gym Classes (BGC), during Gym

The BA concentration of the highschool gym before gym classes (BGC), during gym classes (DGC) and the outdoor air (OUT) was measured. The highest mean value of the concentration of BA was DGC ( $8.75 \times 10^2 \pm 121.39$  CFU/m<sup>3</sup>), whilst the mean concentration of bacteria BGC was  $4.20 \times 10^2 \pm 49.19$  CFU/m<sup>3</sup>. The OUT concentration of BA was  $2.34 \times 10^2 \pm 36.33$  CFU/m<sup>3</sup>. The maximum value of BA concentration was obtained DGC ( $3.78 \times 10^2$  CFU/m<sup>3</sup>), and the minimum

was obtained OUT ( $4.0 \text{ CFU/m}^3$ ). The BA concentration indoors was nearly four times the concentration outdoors. These results indicate that the source of the increased concentration DGC is simply due to the presence of students, because humans are the main source of bacterial emission indoors, and are confirmed with the data available in the literature [8,29,37]. BA contamination levels obtained in our study were lower than the threshold values of occupational exposure specified by the Expert Group on Biological Agents at the Polish Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment for public service buildings ( $5.0 \times 10^3 \text{ CFU/m}^3$  for mesophilic bacteria) [38,39]. Comparing the values observed in our study with the limits suggested by the Commission of the European Communities (CEC) [40] indicates that the range of values obtained for DGC should be considered as moderate contamination ( $<2000 \text{ CFU/m}^3$ ), and also, for BGC and OUT, should be considered as average contamination ( $<500 \text{ CFU/m}^3$ ).

The ANOVA test was significant with F-value equal to 13.81. Based on these results, t-tests were used to determine for which variants there are significant differences. A significant difference was observed between BGC and DGC ( $p = 0.0015$ ) and between DGC and OUT ( $p = 0.00086$ ). No significant difference was found between OUT and BGC ( $p = 0.4577$ ).

The Environmental Protection Agency (EPA) do not give any specific guidelines for bioaerosol concentration levels, so they are only as proposed by different governmental and private organizations [41]. Nevalainen, in 1989 [42], suggested an upper limit for bacterial aerosols ranging from 4500 to 10,000  $\text{CFU/m}^3$ . However, research conducted by the WHO expert group on the assessment of the health risk of biological agents in the indoor environment suggests that the total concentration of microorganisms should not exceed  $1000 \text{ CFU/m}^3$  [43].

Similar studies carried out in Poland 16 years ago showed that the level of BA before gym classes was  $5.50 \times 10^2 \text{ CFU/m}^3$ . During the first lesson in the gym, the concentration of BA was 10 times higher ( $5.50 \times 10^3 \text{ CFU/m}^3$ ) [44]. As it can be seen, the concentration of BA before gym classes is on the comparable level, but during gym classes, the study of Pastuszka et al. [44] reported significantly higher levels of BA. The probable reason was the higher occupancy in the gym.

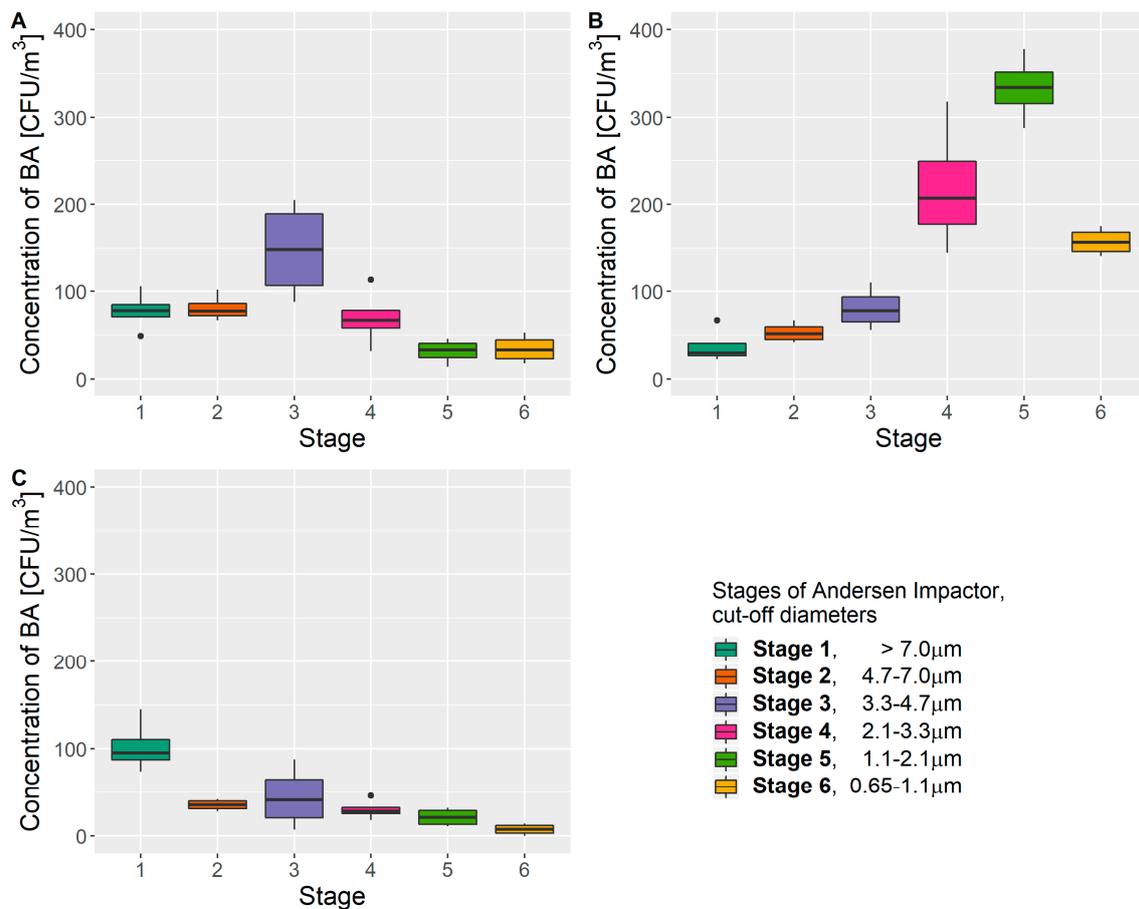
Studies of MIAQ in fitness centers in Portugal reported that the average BA concentration was  $5.56 \times 10^2 \text{ CFU/m}^3$  and  $8.24 \times 10^2 \text{ CFU/m}^3$  in the studio and bodybuilding room, respectively. As it can be seen, the average level of BA during gym classes is in agreement with results from the bodybuilding room, while the BA concentration before gym classes is comparable to the levels in the gym studio [18]. The results of our measures BGC also agree with the studies of Dacarro et al. [27] and Grisoli et al. [28], who reported the levels of mesophilic bacteria in Italian gyms, which were  $4.93 \times 10^2 \text{ CFU/m}^3$  and  $3.93 \times 10^2 \text{ CFU/m}^3$ , respectively.

Besides, temperature and relative humidity are closely associated with bacterial growth and have significant effects on microbial diffusion in the air [28,45–47]. The ASHRAE's standard recommends that indoor temperatures in the winter and summer are between 20 and 23.8 °C and 22.7 and 26.1 °C, respectively [48]. The EPA recommends that relative humidity levels indoors should be between 30% and 50% [49]. Controlling these parameters, especially relative humidity, may be an effective strategy to prevent asthma and allergy symptoms among students and teachers [50].

### 3.2. Particle Size Distribution of Bacterial Aerosol (BA) in a Highschool Gym: Before Gym Classes (BGC), during Gym Classes (DGC) and Outdoor Air (OUT)

Figure 1 presents the analysis of the average concentration of BA collected from the different stages of the Andersen six-stage impactor (ANDI) in the indoor and outdoor air of the school building areas.

The highest average concentration of BA in the OUT was observed on the stage with the aerodynamic diameter ranging  $> 7.0 \mu\text{m}$  (Figure 1C). For BGC, the highest concentration of BA was observed for particles with an aerodynamic diameter ranging from 3.3 to 4.7  $\mu\text{m}$  (Figure 1A). Stages with the aerodynamic diameter ranging from 0.65 to 3.3  $\mu\text{m}$  had the highest concentration of BA from indoor samples DGC (Figure 1B).



**Figure 1.** Average concentration of bacterial colony-forming units (CFU) per cubic meter collected from the different stages of the Andersen six-stage impactor (ANDI): (A) before gym classes (BGC), (B) during gym classes (DGC) and (C) in the outdoor air (OUT).

As it can be seen, the size distribution of bacteria OUT and BGC were similar. Both distributions were characterized with a large share of coarse particles of BA. However, indoor bioaerosol collected BGC indicated a little higher contribution of smaller particles and lower contribution of particles, >7.0 μm, compared to outdoor BA. The sports activity significantly changed the patterns of this size distribution, shifting the peak into the smaller BA particles—in the range from 1.1 to 3.3 μm. The size distributions of BA collected DGC might indicate that the particles of BA were relatively fresh, and generally originating from exercising students. Some studies have found that the activities of humans increase the concentrations of aerosol diameter > 1 μm [51,52] in indoor environments. We hypothesize that human activity and human presence may affect the concentration of biological particles, as they do for ordinary aerosol particles.

The results suggest that during gym classes, students are at risk of being exposed to respirable particles (less than 3.3 μm) that can reach the trachea, bronchi and alveoli, and contribute to adverse respiratory symptoms. It can be seen that the concentration levels of BA obtained in our study are below these proposed standards. However, their long-term inhalation may cause adverse health effects, especially in students sensitive to this type of air pollution.

The ratio of respirable BA during gym classes was 80% compared to the reports [53,54] of a ratio of respirable BA inside public buildings ranging between 30% and 60%. Our result is likely to be an accurate level and may be evidence for the appearance of adverse respiratory symptoms, especially in students sensitive to this type of air pollution.

On the grounds of the statistical analysis, significant differences were found between differences between BA concentration in the OUT–BGC–DGC on some stages of the Andersen six-stage impactor

(ANDI), with  $p$ -values  $< 0.05$  (Table 3). The similar size distribution of BA we found at stage 1: between OUT and DGC, at stage 2: between OUT and BGC and BGC and DGC, at stage 3: between OUT and BGC and at stage 4, between OUT and DGC and BGC and DGC.

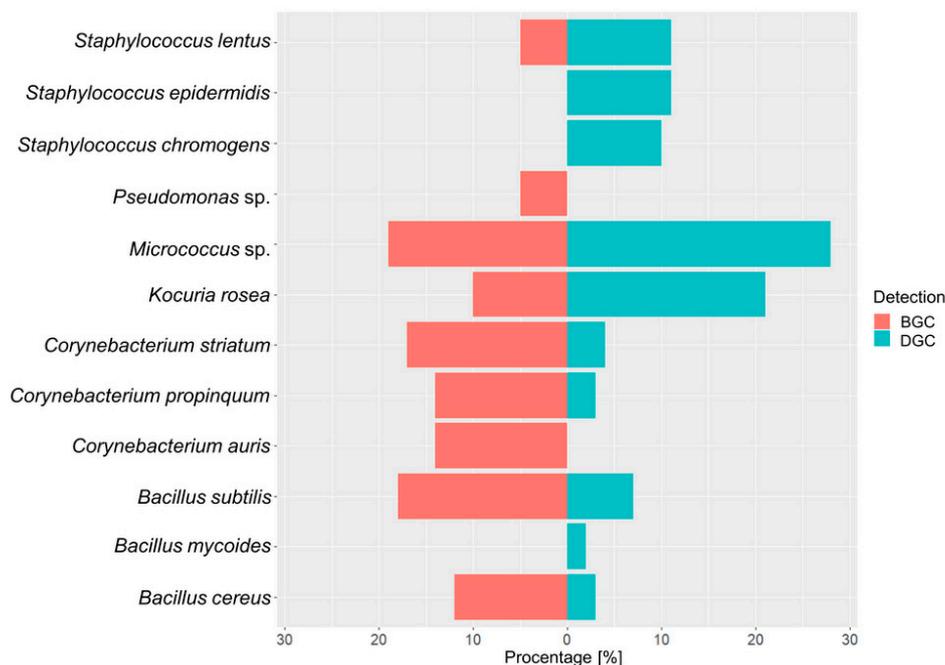
**Table 3.** Pairwise comparisons between bacterial aerosol (BA) concentrations on different stages of ANDI.

|     | Stage 1 |       | Stage 2 |                      | Stage 3              |     |                      |                      |
|-----|---------|-------|---------|----------------------|----------------------|-----|----------------------|----------------------|
|     | OUT     | BGC   | OUT     | BGC                  | OUT                  | BGC |                      |                      |
| BGC | 0.579   | -     | BGC     | 0.001                | -                    | BGC | 0.018                | -                    |
| DGC | 0.015   | 0.144 | DGC     | 0.177                | 0.022                | DGC | 0.718                | 0.134                |
|     | Stage 4 |       | Stage 5 |                      | Stage 6              |     |                      |                      |
|     | OUT     | BGC   | OUT     | BGC                  | OUT                  | BGC |                      |                      |
| BGC | 0.007   | -     | BGC     | 1                    | -                    | BGC | 0.056                | -                    |
| DGC | 0.001   | 0.001 | DGC     | $4.3 \times 10^{-8}$ | $6.9 \times 10^{-8}$ | DGC | $2.1 \times 10^{-7}$ | $1.2 \times 10^{-6}$ |

Italic entries indicate that the correlation is significant at the  $p$ -value less than 0.05; outdoor air (OUT), before gym classes (BGC) and during gym classes (DGC).

### 3.3. Bacterial Diversity and Antibiotic Resistance of Bacterial Aerosol (BA) in a Highschool Gym before Gym Classes (BGC) and during Gym Classes (DGC)

Detailed analysis of BA quality included 10 bacterial species (Figure 2). The statistical analysis of BA qualitative composition points to significant differences for all series between BGC and DGC for four analyzed groups of bacteria: Gram-positive cocci, non-sporing Gram-positive rods, sporing Gram-positive rods, family *Bacillaceae* and Gram-negative rods ( $p$ -values  $< 0.05$ ).



**Figure 2.** The most frequent BA species identified in a highschool gym before gym classes (BGC) and during gym classes (DGC).

Gram-positive cocci of the genera *Micrococcus*, *Kocuria* and *Staphylococcus* were the main components of the bacteria DGC. When there were no students exercising at the gym, BGC Gram-positive cocci constituted 34% of the total BA. During the sporting activity, the percentage

of these bacteria increased and constituted 81%. This group of bacteria commonly occur in natural environments and colonize the surface of human skin.

DGC, three *Staphylococcus* species were identified, with a high percentage of *S. lentus*, *S. epidermidis* and *S. chromogens*. This species composition is comparable to that identified in the indoor air of preschools, primary schools and highschools during the spring in 2016 and 2017 in Poland [11]. *Staphylococcus* was also the dominant bacteria group in sports facilities at the Centre of Physical Culture and Sport at the University in Northern Poland [12]. This bacteria genus may particularly affect the health of students [55].

The most frequently isolated group bacteria BGC was Gram-positive rods. Bacteria in this group are common in samples of food, water, soil and outdoor air [45]. Many of the Gram-positive rods are found in normal skin and mucous membrane flora of humans and various animals and can cause human infections [56].

According to the classifications in Directive 2000/54/EC [57], biological agents from risk group 2 (RG2) were also detected among isolated microorganisms. It was only one strain—*Corynebacterium striatum*, for which BGC constituted 14%, while DGC constituted 4% of total microbiota. Bacteria from RG2 are associated with human disease, which is rarely serious or for which preventive and therapeutic interventions are often available.

### 3.4. Multi Antibiotic Resistance Test (MAR) of Bacterial Aerosol (BA) in a Highschool Gym before Gym Classes (BGC) and during Gym Classes (DGC)

Antibiotic-resistant genes are rapidly evolving into a significant environmental problem and have become a global threat to public health [58]. Results of MAR (Figure 3) can be presented as the inhibition rate of growth diameter around antimicrobial susceptibility testing disks, mean values for each antibiotic and tested strain (in millimeters). The results of MAR demonstrated that the air environment DGC was highly impacted by antibiotic resistance bacteria, while in BGC, a smaller proportion of resistant microorganisms was observed.

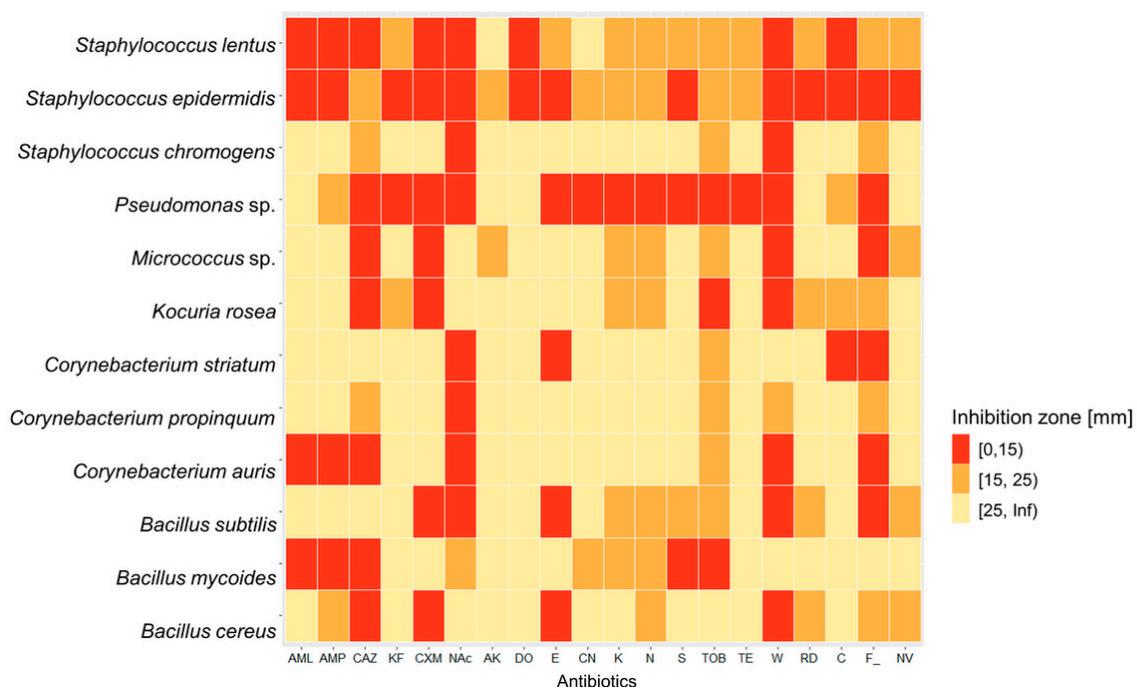


Figure 3. Results of antibiotic resistance testing. Expressed in the values of the growth inhibition zone (mm).

The highest antibiotic resistance bacteria isolated DGC was *Staphylococcus epidermis*. *S. epidermidis* is from a group of mannitol-fermenting coagulase-negative staphylococci characterized by multiple antibiotic resistance [59,60].

*S. epidermidis* was one of the isolates showing the highest antibiotic resistance. Although it is an opportunistic pathogen and the most common skin commensal [61], it plays an important role in balancing the skin microflora and serves as a source of resistance genes [62]. Despite its widespread presence, we must not forget that infections are mainly caused by endogenous microflora [63]. In the case of exercises in the gym, we can expect the appearance of abrasions or scratches among students that are the result of physical activity. It is known that this species exhibits resistance to many antibiotics, and in addition, in recent years, methicillin-resistant *Staphylococcus epidermidis* (MRSE) is the most common of the species in medical facilities [64]. Among strains isolated in hospitals, resistance to beta-lactam antibiotics—mainly penicillin and cephalosporins—was most commonly observed [63]. The isolated strain also had such a resistance pattern.

Our attention was drawn to the fact that in hospital cases, we often talk about postoperative infections (often associated with implant placement), where high antibiotic resistance is explained by the formation of biofilms by microorganisms [62,65]. In this case, a strain with a similar resistance pattern was isolated from the air sample.

The highest antibiotic resistance bacteria isolated BGC was *Pseudomonas* sp. *Pseudomonas* species are known to harbor multiple intrinsic and acquired resistance genes and host several mobile genetic elements [66]. *Pseudomonas* sp. includes species with both clinical and environmental implications. Important members of this genus include *P. aeruginosa*, *P. fluorescens* and *P. stutzeri*.

However, the presence of resistant *Pseudomonas* representatives, even if they do not belong to pathogenic species, confirms the results of Kittinger, in which it has been shown that these species can be a reservoir of antibiotic resistance and in favorable conditions pass it on to pathogenic species through horizontal gene transfer [66]. It was noted that *Pseudomonas* isolates from environmental samples (freshwater) show much lower antibiotic resistance than clinical isolates [67]. In our research, the bioaerosol isolate presents a resistance pattern more similar to clinical isolates. This confirms the assumption that the source of bioaerosol emissions in the gym is mainly human.

The most sensitive bacteria to antibiotics are *Corynebacterium striatum* and *Corynebacterium propinquum* (isolated in both BGC and DGC). *Corynebacterium* species are widely distributed in the environment and in the microbiota of humans and animals [68].

The most effective antibiotics were amikacin and doxycycline (aminoglycosides group), which are on the World Health Organization list of basic medicines (the safest and most effective medicines needed in the healthcare system). Aminoglycosides are natural or semisynthetic antibiotics derived from actinomycetes. They are potent, broad-spectrum antibiotics which act by inhibiting protein synthesis [69].

#### 4. Conclusions

Although the presented research is the result of preliminary studies, the data cover one season and a limited number of repetitions, they allow the following conclusions to be drawn:

1. Indoor level of bacterial aerosol (BA) is higher than outdoor.
2. During gym classes (DGC) concentration of BA is  $>500$  CFU/m<sup>3</sup>, pointing to moderate contamination.
3. The sports activity shifted the peak of this size distribution into the smaller particles (1.1 to 3.3  $\mu$ m), pointing to fresh human origin particles.
4. Dominating bacterial species is Gram-positive cocci, which commonly occur on human skin.
5. Among determined biological agents, only one strain, *Corynebacterium striatum*, belongs to RG2 (risk group 2), and it constituted 14% of BA before gym classes (BCG); however, this bacteria rarely causes serious diseases.

6. DGC, the proportion of antibiotic resistance bacteria was higher than BGC.
7. The highest antibiotic resistance revealed *Staphylococcus epidermis* (isolated DGC) and *Pseudomonas* sp. (isolated BGC).
8. The most sensitive bacteria to antibiotics are *Corynebacterium striatum* and *Corynebacterium propinquum* (isolated both BGC and DGC).

The results of this study indicate that the concentrations of bacterial aerosol (BA) in a highschool gym located in the naturally ventilated historic building are not particularly hazardous for the occupants, but the air in the gym is more contaminated than outdoor air, so we recommend to perform gym classes outside the building as often as possible.

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