Pol. J. Environ. Stud. Vol. 21, No. 2 (2012), 307-312

Original Research

Airborne Mesophilic Bacteria at the Ciechocinek Health Resort

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Received: 27 April 2011
Accepted: 27 September 2011

Abstract

The aim of the present study was to determine microbiological air quality in the Ciechocinek health resort and to identify the most common mesophilic bacteria present in the air. Sampling sites were located in the surroundings of inhalators (graduation towers and the "Grzyb" fountain), in recreation areas (promenade, parks), and urban areas. Samples were obtained by the impaction method in a Merck MAS-100 microbial air-sampler. Enumeration of mesophilic bacteria was conducted according to the Polish Standard (PN86/Z-04111/02). Bacterial strains were identified with appropriate API tests (bioMérieux). Due to the specific microclimate and low levels of microbiological air pollution, Ciechocinek can be considered an excellent upper respiratory tract spa. The lowest numbers of the investigated types of bacteria were found around the open inhalators. In the Ciechocinek area bacteria commonly occurring in air prevailed. Gram-positive bacilli (mainly *Bacillus* genus) were the most common among the isolated bacteria (53.8% of all strains), and Gram-positive cocci (*Staphylococcus*, *Micrococcus*) also were abundant (28%). The least abundant were Gram-negative rods (*Pseudomonas*, *Serratia*). Potentially pathogenic species (*Pseudomonas aeruginosa*, *Enterococcus faecium*, *Aeromonas hydrofila*) were found sporadically.

Keywords: air microbiology, air pollution, health resort, bacteria

Introduction

Due to rapid industrialization and the development of highly urbanized areas, atmospheric air has become contaminated with an increasing variety of pollutants that pose threats to human health. Large quantities of dust, organic compounds, and non-organic compounds of nitrogen, sulfur, and carbon are emitted to the atmosphere.

In addition to industrial pollution, atmospheric air contains microbiological pollutants, which include viruses, bacteria, hyphae fungus, and spores occurring in the air in the form of aerosols [1]. Apart from saprotrophs, other health-threatening substances such as pathogenic, allergenic, and toxinogenic microorganisms may also be present in the air. People become infected, *inter alia*, through respiratory tracts, oral, pharyngeal, and nasal cavities; therefore, air contaminated by germs attached to dust particles or bioaerosols (droplet infection) may also constitute a source of infection [2]. Furthermore, in favorable conditions numerous pathogenic streptococcus, staphylococcus, *Corynebacterium diphtheriae*, and tubercle bacilli may preserve their vitality in a dry state and remain potentially dangerous for human health [3].

It is also noteworthy that many saprotrophs become pathogenic after penetrating a weakened organism; therefore, it is quite justified to determine the microbiological state of air in areas frequented by individuals suffering from chronic respiratory diseases.

Ciechocinek is the largest Polish lowland health resort, and is visited by some 70,000 patients per year. Due to the

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presence of open-air inhalation facilities (graduation towers and Grzyb fountain), the health resort is also oriented towards treatment of respiratory system diseases. For that reason, it is necessary to precisely determine the microbiological purity of air in the resort area. Despite the fact that, according to the air quality classification of the Voivodeship Inspectorate of Environmental Protection where (WIOŚ), resort areas are subject to a special assessment where only dustiness and chemical parameters of air are evaluated. Microbiological analyses of the air in health resort areas is neither conducted nor taken into consideration when evaluating air quality [4, 5].

The purpose of this study was to determine microbiological air quality in the Ciechocinek area and to identify the most common mesophilic bacteria present.

Materials and Methods

Object of the Study

The town of Ciechocinek is located in Kujawy, on the left bank of the Vistula river, 25 kilometers from Toruń (52º52' N, 18º47' E). The Ciechocinek area is characterized by an exceptionally high annual mean temperature (ca. +8ºC) and is among the warmest regions in the lowland belt. The region also is characterized by low air humidity and unusually low precipitation; its annual total rarely exceeds 500 mm [6].

Ten sampling sites were established in the Ciechocinek area (Fig. 1). Three sampling sites were located within the zone affected by the natural inhalators: 1 m (I1) from Grzyb Fountain (brine source No. 11), 1 m from graduation tower No. I on the side of the brine flow (I2), and 1 m from graduation tower No. I, on the side without the brine flow (I3). Sampling sites located within the recreation area were situated in Zdrojowy Park in front of Pijalnia Wód (Pump Room) (R1), by the Oaza Restaurant (R2), and on the promenade by Tężniowa Street (R3). Sampling sites located in the town were situated at the entrance to Ciechocinek from the Toruń side, next to the intersection of Kopernik and Bem streets (U1), on Kopernik Street, across from the railway station (U2), and on Widok Street (U3).

Sample Collection

The air samples were collected monthly according to Polish Standard PN-89/Z-04008/08 [7] 1.3 m above the ground level between May, 2008 and April, 2009. The samples were obtained by the impaction method in a Merck MAS-100 microbial air sampler. The air flow velocity equaled 11 m/s and coincides with level 5 of Andersen sampler [8]. This enabled detection of particles larger than 1 μm, which was important for translocation of microorganisms. The following meteorological parameters were measured during sample collection: air temperature, relative humidity, and wind velocity. These measurements were carried out using a Nielsen-Kellerman anemometer, Kestrel 3500 (Table 1).

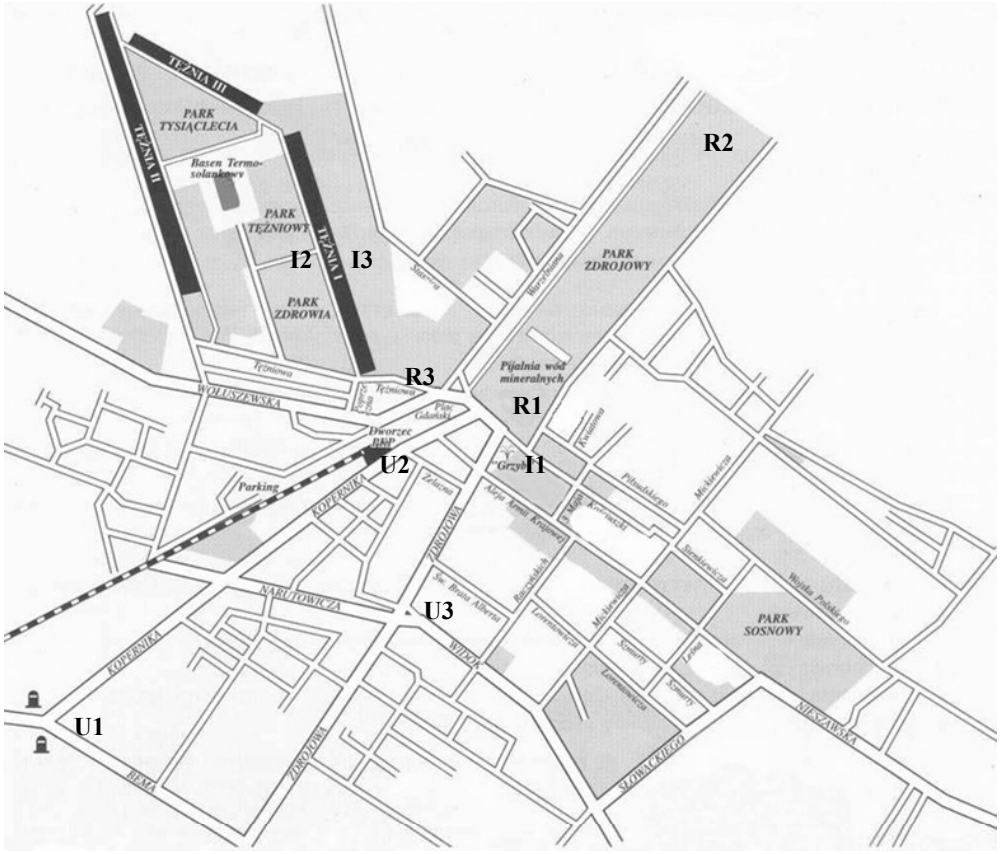

Fig. 1. The map of measurement sites in Ciechocinek health resort.
I1, I2, I3 – surroundings of inhalators, R1, R2, R3 – recreation areas, U1, U2, U3 – urban areas

Table 1. Meteorological parameter during sample collection.

	Temperature [ºC]	Humidity [%]	Wind velocity [m/s]	Atmospheric precipitation
10.04.2008	10.2	54.8	0.9	-
11.05.2008	21.8	49.1	1.2	-
19.06.2008	25.5	51.5	0.5	-
13.07.2008	32.0	48.1	1.1	-
24.08.2008	20.3	72.1	1.4	-
14.09.2008	23.2	65.0	1.0	-
17.10.2008	14.0	60.7	0.8	-
16.11.2008	14.1	71.1	0.8	-
18.12.2008	5.3	79.3	1.0	shower
11.01.2009	3.0	99.7	3.1	-
08.02.2009	-0.3	92.8	1.3	-
23.03.2009	10.3	52.7	2.1	-

Concentration of Bacteria

Enumeration of mesophilic bacteria was conducted according to the Polish Standard [9] on nutrient agar for 48 hours at 37ºC. Results were expressed in the number of CFU (colony-forming units) in 1 m^3 of air and analyzed in STATISTICA 6.0.

Identification of Bacteria

In order to identify bacteria, pure bacterial cultures were isolated from surfaces of nutrient agar plates. Each month, 100 randomly selected colonies were inoculated into slants containing the same nutrient. Bacterial morphology was determined using the Gram staining procedure performed on 2- and 5-day bacterial cultures grown in nutrient broth. Bacterial strains were identified with appropriate API tests (bioMérieux). Gram-negative rods were identified based on API 20 E test, cocci-based on API Staph test, and API 20 Strep test, and Gram-positive bacilli, with API 50 CH test. After test results were recorded and numerical profile obtained, the identification was performed in apiweb™.

Results

The concentration of mesophilic bacteria in Ciechocinek air ranged from 23 CFU/m^3 in February in the graduation tower area to 566 CFU/m^3 in June in urbanized areas. In all testing stations, the highest concentration of mesophilic bacteria were observed in August with abundances of mesophiles remaining at high levels in urbanized areas until October (Fig. 2). Based on the obtained concentration levels of mesophilic bacteria, the air in Ciechocinek was classified in accordance with Polish Standard PN89/Z-04111/02 as not polluted (93.7% of all collected samples) or moderately polluted (6.3% of samples) (Table 2).

Table 2. The evaluation of microbiological pollution of air in Ciechocinek according to PN-89 Z-04111/02.

Air pollution levels (Polish Standard PN89/Z-04111/02)	Permissible concentration of mesophilic bacteria	Percentage of samples in category
Not polluted	<1000 CFU/m^3	93.7%
Moderately polluted	>1000 and <3000 CFU/m^3	6.3%
Highly polluted	>3000 CFU/m^3	0%

The highest numbers of bacteria were recorded in sampling sites located in urban areas of Ciechocinek, and the lowest around open-air inhalation facilities. The difference in concentration of mesophiles between spa areas and open-air inhalation areas was not significant (Fig. 3).

A strong positive correlation between bacterial concentration level and air temperature was observed in the surroundings of the open-air inhalation facilities and spa areas with correlation coefficients r equaling 0.79 and 0.74, respectively. However, the correlation between concentrations of mesophilic bacteria and other recorded meteorological parameters (i.e. relative humidity and wind velocity) was not significant.

Gram-positive bacilli, whose vast majority belonged to *Bacillus* genus (53.8% of all strains), were the most common among the isolated bacteria. Gram-positive cocci were also abundant (28%) and were primarily represented by genera *Staphylococcus* and *Micrococcus.* The least abundant were Gram-negative rods, represented mainly by genera *Pseudomonas* and *Serratia* (Fig. 4, Table 3).

Discussion

In addition to chemical pollution, the atmosphere is contaminated with organic substances in the form of bioaerosols, including potentially pathogenic microorganisms (viruses, bacteria, fungus hyphae, and spores).

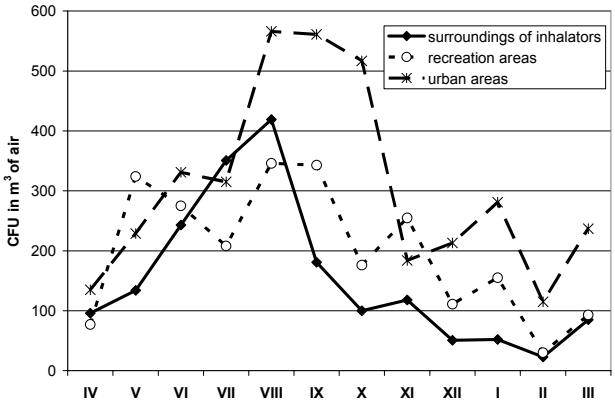

Fig. 2. Number of mesophilic bacteria in Ciechocinek air.

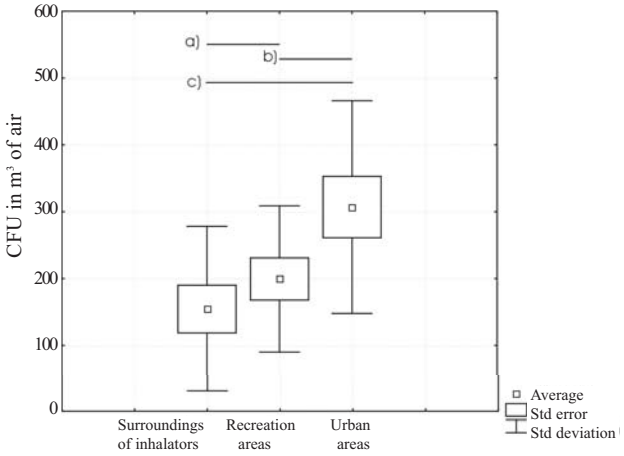

Fig. 3. The influence of open inhalators on the number of airborne mesophilic bacteria in Ciechocinek.
b, c – significant differences at $p<0.05$

Many authors believe that from the epidemiological and economical point of view, microbiological evaluation of air quality is fully justified [1, 2, 10, 11]. In spite of that, microbiological studies are not taken into consideration when accessing air quality even in spa areas.

The results presented above demonstrated that preserving the curative character of Ciechocinek (lack of industry or high-density building developments, and large green areas) had a positive impact on microbiological purity of atmospheric air. The air in Ciechocinek can be considered clean, even in urbanized areas, in comparison to air in large and densely populated urban centers. Filipiak et al [12] and Bugajny et al. [13] reported that during similar studies conducted in Poznań, obtained values were even 10 times higher; in summer, the concentration of mesophilic bacteria reached 13,000 CFU/m^3. Fang et al. [14], during a study conducted in Beijing in areas with high levels of traffic and human activity, observed even 22,000 CFU/m^3. Unfortunately, publications related to microbiological air pollution in spa areas are scarce. Due to the fact that the majority of published data is related to urban areas and the surroundings of municipal facilities such as sewage treatment plants and dumps [8, 15-18], the possibilities to discuss obtained results are limited.

Table 3. Identification of airborne mesophilic bacteria at Ciechocinek health resort.

Morphological forms	Genus	[%]	Dominant species
Bacilli	*Bacillus*	66.15	*Bacillus subtilis,*
			Bacillus cereus,
			Bacillus mycoides
	Brevibacillus	1.32	*Brevibacillus agri*
	others	0.72	
Cocci	*Staphylococcus*	1.09	*Staphylococcus lentus*
			Staphylococcus xylosus
	Micrococcus	8.73	*Micrococcus luteus*
	Enterococcus	3.45	*Enterococcus faecium*
	Aerococcus	2.14	*Aerococcus viridans*
	others	1.59	
Rods	*Pseudomonas*	1.65	*Pseudomonas luteola,*
			Pseudomonas aeruginosa
	Serratia	1.49	*Serratia liquefaciens*
	Aeromonas	0.42	*Aeromonas hydrophila*
	others	0.24	

Exceptionally low values of mesophilic bacterial abundances recorded in the air of the resort could have been a result of a method used to collect samples. Studies conducted by Michałkiewicz and Piskorek [19], Kruczalk et al. [20], and Zmysłowska and Jackowska [21], compared sedimentation and impaction methods of sampling and demonstrated that these methods produced different results in the same locations. Sample collection with the sedimentation method yielded higher results than the impaction method. Considering the specificity and techniques used in these methods, the impaction method seems to be more accurate. In the sedimentation method, due to the variable sedimenta-

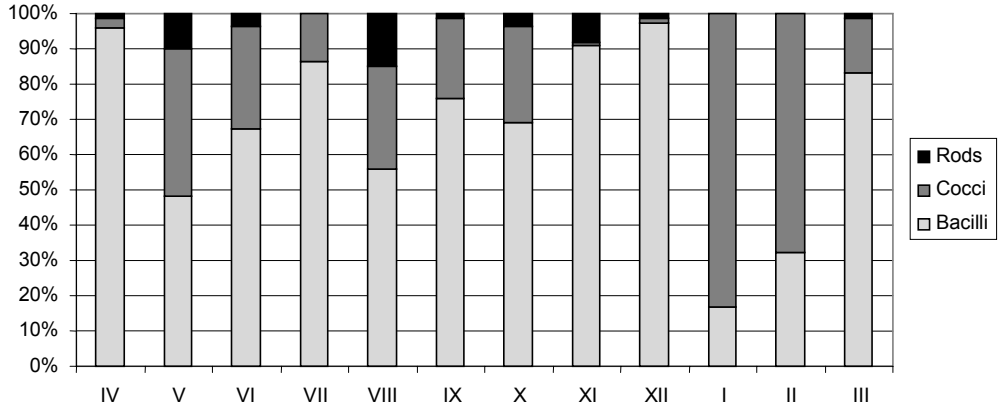

Fig. 4. Morphological forms of mesophilic bacteria in Ciechocinek air.

tion conditions caused by air movements, bacteria from outside of the examined air column may settle on a pan. Furthermore, the volume of air from which microorganisms settling on a pan originated can only be approximated.

In the urban area, concentrations of mesophilic bacteria were decidedly higher than in the surroundings of the open-air inhalation facilities. A similar relationship was also observed during earlier studies conducted in Ciechocinek [22, 23]. The highest abundances of mesophilic bacteria, molds, mannitol-positive staphylococci, and hemolytic bacteria were observed at stations located in the urban area of Ciechocinek, with the lowest around the open inhalators. Only in the case of actinomycetes was the highest annual mean abundance observed at the research stations located in the recreation area, that is primarily in the parks. Concentration levels of microorganism in the air were most probably affected by aerosol produced in the open-air inhalation area. The presence of brine aerosol particles accelerates sedimentation of pollutants, including cells of microorganisms that occur in the air. In addition to Cl^- and Na^+ ions, Ciechocinek brine sprayed by graduation towers contains F^+, Br^+, J^+, and B^+ ions, which undoubtedly improve antibacterial effects of graduation tower aerosol [24, 25].

The study also showed a strong positive correlation between concentration of bacteria and air temperature observed in open-air inhalation and in spa areas. It is possible that the high abundance of mesophilic bacteria recorded in summer season was associated not only with optimal temperature for these microorganisms, but also with high numbers of tourists visiting the town. According to the (oral) information provided by Ciechocinek Spa an increased number of tourists in the graduation tower area is observed between May and the end of September, with the highest numbers in July and August. As demonstrated in Fig. 2, these periods precisely coincide with fluctuations in mesophilic bacterial abundances in the surroundings of open-air inhalation facilities. Unfortunately, accurate data is not available (the spa does not provide precise information about ticket sales to the graduation tower areas); therefore, it is impossible to precisely determine the impact of tourism on microbiological air pollution.

The majority of bacteria isolated from Ciechocinek air belonged to the genus *Bacillus* as well as genera *Staphylococcus* and *Micrococcus*. These bacteria are commonly found in air and their occurrence has been confirmed by Filipiak et al. [12] and Fang et al. [14], among others. The majority of bacteria found in the spa air were saprotrophs; however, potentially pathogenic species, for example *Pseudomonas aeruginosa*, *Enterococcus faecium*, or *Aeromonas hydrofila,* also were sporadically found.

When considering the above results, it should be taken into account that the occurrence of airborne microflora has a temporary and accidental character, and is affected by physicochemical properties of air as well as topographic and microclimatic conditions of the examined area [26]. Therefore, the presented results are momentary approximated values that correspond to microbiological pollution, which occurs at a given moment in a specific area.

Conclusions

Due to the low levels of microbiological air pollution, Ciechocinek can be considered an excellent upper respiratory tract spa.

The preservation of the spa character of Ciechocinek has been beneficial for microbiological air quality.

Aerosol produced in open-air inhalation facilities has a positive effect on the microbiological condition of air; in areas within their reach, abundances of microorganisms were lower than in other sections of the resort.

Bacteria commonly occurring in air prevailed in the spa area; however, potentially pathogenic species such as *Pseudomonas aeruginosa*, *Enterococcus faecium*, or *Aeromonas hydrofila* also were sporadically found.

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