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THE VARIABILITY OF BACTERIAL AEROSOL IN POULTRY HOUSES DEPENDING ON SELECTED FACTORS

KAROLINA BRÓDKA, ANNA KOZAJDA, ALINA BUCZYŃSKA, and IRENA SZADKOWSKA-STAŃCZYK

Nofer Institute of Occupational Medicine, Łódź, Poland
Department of Environmental Health Hazards

Abstract
Objectives: This study is aimed at evaluation of bacterial air contamination in intensive poultry breeding. The evaluation was based on the determined levels of bacterial concentrations and qualitative identification of isolated microorganisms. **Materials and Methods:** The study covered 5 poultry houses: two hatcheries and three hen houses with the litter bed system. The air was sampled in three measurement series in the central part of the investigated workplace at the height of about 1.5 m over the ground, using portable measuring sets consisting of a GilAir 5 (Sensidyne, USA) pump and a measuring head filled with a glass microfibre filter (Whatman, UK). For the quantitative and qualitative analysis of microorganisms were used appropriate microbiological media. **Results:** The total concentrations of airborne mesophilic bacteria inside the poultry breeding houses ranged from 4.74×10^4 cfu/m^3 to 1.89×10^8 cfu/m^3. For Gram-negative bacteria, the range comprised the values from 4.33×10^2 cfu/m^3 to 4.29×10^6 cfu/m^3. The concentrations of the cocci of *Enterococcus* genus ranged from 1.53×10^4 cfu/m^3 to 1.09×10^7 cfu/m^3, whereas those of other Gram-positive bacteria from 3.78×10^4 cfu/m^3 to 6.65×10^7 cfu/m^3. The lowest concentrations of each group of the examined microorganisms were noted in the second measurement series when the air exchange in the breeding houses was over twice higher than in first and third measurement series because the mechanical ventilation was supported by natural ventilation (opened gates in the buildings). The lowest concentrations of total bacteria were obtained in those buildings where one-day old chickens were kept. Gram-positive bacteria of the genera: *Staphylococcus*, *Enterococcus*, *Corynebacterium, Brevibacterium*, *Micrococcus*, *Cellulomonas*, *Bacillus*, *Aerococcus*, and Gram-negative bacteria of the genera: *Pseudomonas*, *Moraxella*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Pasteurella*, *Pantoea* were isolated. It was shown that for most of the investigated livestock premises the total bacteria concentrations exceeded the reference value of 1.0×10^5 cfu/m^3. Furthermore, pathogenic microorganisms which are a potential threat to human health (*Escherichia coli*, *Enterobacter cloacae, Klebsiella pneumoniae* ssp. *ozaenae*, *Enterococcus faecalis*, *Enterococcus faecium*) were found among the identified bacteria. **Conclusions:** The results indicate that the hygienic conditions of the working environment connected with litter bed system production of poultry are affected by changes of the efficiency of ventilation and create a direct health risk to employees. They should use personal protective measures to protect their respiratory tract, especially when the gates in the hen houses are closed.

Key words:
Bacterial aerosol, Poultry breeding, Occupational exposure, Health risks

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Address reprint request to K. Bródka, Department of Environmental Health Hazards, Nofer Institute of Occupational Medicine, św. Teresy 8, 91-348 Łódź, Poland (e-mail: kbrodka@imp.lodz.pl).

INTRODUCTION

The results of most of the existing studies demonstrate that the air inside the facilities where the intensive poultry breeding is conducted is contaminated by high concentrations of microorganisms, especially bacteria [1–6]. The bacterial aerosol consists of staphylococci, streptococci, numerous Gram-positive and Gram-negative rod-shaped bacteria, including *Bacillus* genus [4,5,7–13]. Both live cells and their components (endotoxins in case of Gram-negative bacteria) may induce in poultry breeding employees a number of symptoms and diseases, mainly within the respiratory tract, as well as the toxic syndrome induced by endotoxins found in the organic dust [14–21]. The degree of hazardousness of airborne bioaerosol particles in breeding facilities depends not only on the pathogenicity of microorganisms or toxicity of their products but also on the particle size which determines their ability to penetrate the human respiratory tract. The particles above 5 μm aerodynamic diameter settle mainly in the naso-pharyngeal area (inhalable fraction). On the other hand, the particles smaller than 5 μm (respirable fraction) may reach the alveoli through the trachea and bronchi [22].

Besides the basic assessment of quantitative and qualitative bacterial aerosol present in the air of poultry houses, the authors also studied the influence of several aspects of hen breeding on concentrations of these microorganisms in the air of poultry premises. Several authors determined the levels of airborne bacteria depending on age of hens or broilers and housing density of birds [5,11,12,23,29,30]. Furthermore, Nimmermark et al. [2], Saleh et al. [6] and Kirychuk et al. [17] showed that poultry production system (floor housing system with litter, house with cages) was also important.

Number of microorganisms occurring in the air of breeding buildings also depends on many factors such as temperature, humidity, airflow velocity inside the building, efficiency and type of ventilation. This article presents the concentrations of bacteria depending on the measurement series expressed by microclimate parameters and the type of ventilation in poultry houses. This seems to be an important aspect of science which was not discussed previously in studies on the working environment of intensive poultry production. The publication will also expand the knowledge on identified bacterial aerosol contamination in poultry houses.

The presented study is aimed at evaluation of the degree of bacterial aerosol contamination inside the intensive poultry production premises, based on the results of the quantitative and qualitative analysis of isolated microorganisms. Differences in bacterial aerosol concentrations related to selected microclimate characteristics and type of ventilation were analysed.

MATERIALS AND METHODS

Farm buildings and measurement series characteristics

The study covered 5 facilities located in central Poland, where intensive production of poultry was conducted. Two buildings of those facilities were used as hatcheries and the other three buildings – for hen fattening and for industrial production of eggs, using the litter bed system (straw). The investigations were carried out from April to November 2009 in three measurement series. In April (I series) the mean monthly temperature in the region under study was 11°C and on sampling day 14.6°C and consecutively in August (II series) with 18°C and 23.4°C and in November (III series) with 6.5°C and 7.4°C. In the first series, the measurements were carried out in all of the five buildings and at one point outside (background), in two consecutive series – only in three buildings (hen houses) and at one point outside. Hatchery buildings were characterized by: small room (approximately 30 m^2), without litter bed system, mean temperature 23.2°C, in non summer season additionally central heating. In the hatchery chickens stayed 2 days, whereas in the other buildings – from 3 days to 64 weeks. The hen houses covered by the study were comparable in

respect of construction and building materials, as well as ventilation capacity. The rooms were equipped with an automatic drinking and feeding system. The floorage of each buildings was approximately 1000 m^2 for 6.5 thousand hens. The buildings microclimate parameters (mainly temperature) were maintained by mechanical ventilation. The ventilator efficiency in each of the buildings was 4000 m^3/h. In summer, the mechanical ventilation was supported by natural ventilation (opened gates in the buildings). The hen buildings were not additionally heated. The building characteristics are presented in Table 1. Each consecutive breeding cycle was preceded by removal of the bedding and cleaning and disinfection of the building.

Air Sampling and Measuring Microclimate Parameters

The sampling strategy was based on Polish Standard [23]. In view of the expected high concentrations of bacterial microflora in breeding facilities, a filtration method was used in this study. Indoor and outdoor air samples for determining concentrations of bacteria were collected using the measuring sets consisting of GilAir 5 pump (Sensidyne, Clearwater, Florida, USA) and the open faced aerosol sampler (Two-Met, Zgierz, Poland), with a GF/A glass microfibre filter (Whatman International Ltd, Maidstone, Kent, UK) with a pore size of 1.6 μm. The sets were operated at a flow rate of 2 l/min. The measuring sets were calibrated before each sampling procedure, using Gillibrator 2 calibrator (Sensidyne, Clearwater, Florida, USA). The equipment was placed at a height of 1.5 m above the floor. The sampling took 4–6 h. Duplicate samples were collected at each measuring point. After the sampling, the filters with collected biological material were put, using sterile tweezers, into tightly closed containers with Stuart-Ringertz medium (Sigma-Aldrich Chemie GmbH, Munich, Germany) and transported to the laboratory. Then the filters in the containers with the transport medium were covered with 10 ml of the phosphate buffer solution (BTL, Łódź, Poland) and by shaking on a shaker (shaking time: 50 min, shaking rate: 420 revolutions per minute) the biological material on filters was eluted. A series

Table 1. Farm buildings and measurement series characteristics

Measurement series	Type of building	Measurements (n)	Microclimate parameters (mean)				Ventilation	Farm buildings characteristics
			temperature (°C)	relative humidity (%)	concentration of CO_2 (ppm)	airflow velocity (m/s)		
I	hatchery	2	23.2	57.0	1 410	0.03	data not available	the floorage of a room approximately 30 m^2, smooth floors without litter bed system, in non summer season additionally central heating
	hen building	3	22.2	47.0	1 263	0.50	mechanical (ventilators)	the floorage of each building approximately 1000 m^2; about 6,5 thousand hens in each building; ventilators' efficiency amounted to 4000 m^3/h; buildings without central heating
II	hen building	3	23.7	84.1	1 128	0.87	mechanical (ventilators), natural (open gates)	
III	hen building	3	19.1	67.5	1 473	0.35	mechanical (ventilators)	

of 10-fold dilutions was prepared from the resultant eluates. Plates with nutrient agar (BTL, Łódź, Poland) containing nystatin, Columbia Agar with 5% sheep blood (GRASO, Starogard Gdański, Poland), MacConkey agar (BTL, Łódź, Poland) and agar with esculin, bile, sodium azide, and citrate (BTL, Łódź, Poland) were inoculated with specific volumes of eluates and their dilutions by spreading them onto the plates. In order to determine the total mesophilic bacteria, plates with nutrient agar were incubated for 48 h at 30°C, followed by 24 h at 37°C. In order to determine the total number of Gram-positive bacteria with the exception of the cocci of *Enterococcus* genus, plates with Columbia agar were incubated for 48 h at 37°C. The same conditions and times of incubation were used for the plates in order to determine the total number of Gram-negative bacteria (MacConkey agar medium) and the total number of the cocci of *Enterococcus* genus (agar with esculin, bile, sodium azide and citrate). After incubation, grown colonies were counted on the plates and after taking into account the dilution of the sample and the volume of aspired air the resultant bacteria concentration was expressed in terms of the number of colony forming units on the microbiological medium per 1 m^3 of the examined air (cfu/m^3). Qualitative identification of isolated microorganisms was carried out using standard microbiological procedures with microscopic analysis, diagnostic nutrient media (Nutrient agar; MacConkey agar; agar with esculin, bile, sodium azide and citrate; Columbia agar with 5% sheep blood; Mueller-Hinton medium, Hugh-Leifson medium, agar with esculin; API M Medium) and biochemical API tests (API Staph, API 20NE, API 20E) (BioMerieux, France).

During indoor bioaerosol sampling at the same point, simultaneously the basic parameters of microclimate, such as: temperature, relative humidity, CO_2 concentration and airflow velocity were measured. The measurements were carried out using the microclimate multifunction meter Testo 435-2 (Testo AG, Germany) at a heigh of 1.5 m above the ground for 10 min. The values of each parameter were read out every minute, after which the results were averaged for the measurement point.

Statistical Analysis

Concentrations of airborne bacteria in breeding facilities depending on measurement series (selected microclimate characteristics and type of ventilation) were characterized by using arithmetic means (AM), standard deviations (SD), minimal values (Min.) and maximal values (Max). Univariate analysis was used to determine effects of microclimate characteristics and type of ventilation on the level of bacterial aerosol. Thus obtained dimensionless data were statistically analyzed using one-way ANOVA. Comparisons of mean concentrations of bacterial microflora to different measurement series defined by the parameters of microclimate (temperature, relative humidity, CO_2 concentration and airflow velocity) and type of ventilation (only mechanical ventilation or mechanical ventilation supported by natural ventilation – opened gates) were made using the Fisher's least significant difference test. To analyze differences, data of the buildings from 3 to 5 were used. Also statistically significant differences between the concentrations of bacteria in hatcheries and other buildings in first measurement series were assessed. The influence of the age of hens on the level of bacterial aerosol was not analyzed in detail (only hatcheries versus other buildings). Significant differences between the groups were evaluated using post hoc analysis by means of the Tukey's test, at $p < 0.05$ selected for statistical significance [25]. Statistical calculations were made with the software package Statistica version 8.

RESULTS

The mean temperature inside the hen buildings in consecutive measurement series was 22.2°C, 23.7°C and 19.1°C, respectively. The values of relative humidity and

airflow velocity were 47.0% and 0.50 m/s (I series), 84.1% and 0.87 m/s (II series) and 67.5% and 0.35 m/s (III series), respectively. The mean concentration of CO_2 was 1263 ppm, 1128 ppm and 1473 ppm in first, second and third measurement series, respectively. In hatcheries, mean values of temperature, relative humidity, airflow velocity and concentration of CO_2 were 23.2°C, 57%, 0.03 m/s, 1410 ppm, respectively. The mean values of temperature, relative humidity, CO_2 concentration and airflow velocity determined inside the intensive poultry breeding houses are shown in Table 1.

The mean bacteria concentrations inside poultry houses are presented in Table 2.

The total concentrations of airborne mesophilic bacteria inside the poultry houses ranged from 4.74×10^4 to 1.89×10^8 cfu/m^3, with the arithmetic mean value 5.69×10^7 cfu/m^3. For Gram-positive bacteria, the range of the values was from 3.78×10^4 to 6.65×10^7 cfu/m^3 with the arithmetic mean value of 1.87×10^7 cfu/m^3. The concentrations of Gram-negative bacteria ranged from 4.33×10^2 to 4.29×10^6 cfu/m^3 with the arithmetic mean value 9.38×10^5 cfu/m^3 whereas those of the cocci of *Enterococcus* genus – from 1.53×10^4 to 1.09×10^7 cfu/m^3 with the arithmetic mean value 2.78×10^6 cfu/m^3. Table 3 shows bacteria concentrations found in poultry houses depending on the type of building and measurement series (microclimate parameters and type of ventilation) and outside the building.

The lowest mean concentrations of all types of bacteria were noted in the hatcheries. The consecutive measurement series showed that the highest arithmetic mean concentration of total mesophilic bacteria in breeding houses (without hatcheries) was in III measurement series, whereas the lowest in II series. These values were: 4.21×10^7, 9.03×10^6 and 1.57×10^8 cfu/m^3 for first, second and third measurement series, respectively. In case of the Gram-positive bacteria, the highest mean concentration was obtained in III series – 3.60×10^7 cfu/m^3, a slightly lower was in I series – 2.50×10^7 cfu/m^3 and by almost one order of magnitude lower in II series – 7.30×10^6 cfu/m^3. The mean concentration of Gram-negative bacteria in third measurement series was 3.32×10^6 cfu/m^3 and was over 10-fold higher than the concentration obtained in the first series (1.10×10^5 cfu/m^3) and almost 100-fold higher than the bacteria concentration in the second measurement series (9.91×10^3 cfu/m^3). The values of the mean concentrations of cocci isolated on agar with esculin reached the same order of magnitude in each measurement series and were 3.98×10^6, 1.79×10^6 and 4.38×10^6 cfu/m^3 in first, second and third series, respectively.

Results of analysis of air samples collected outside breeding houses are shown in Table 3. The mean concentrations of total bacteria obtained in the first and second measurement series were very similar and amounted to 9.50×10^3 and 9.25×10^3 cfu/m^3, while the concentration

Table 2. Mean concentrations of airborne culturable bacteria in the poultry houses

Microorganisms	Measurements* (n)	Concentrations (cfu/m^3)		
		AM	SD	min–max
Total bacteria	11	5.69×10^7	7.25×10^7	4.74×10^4–1.89×10^8
Gram-positive bacteria**	11	1.87×10^7	2.18×10^7	3.78×10^4–6.65×10^7
Gram-negative bacteria	11	9.38×10^5	1.58×10^6	4.33×10^2–4.29×10^6
Bacteria of *Enterococcus* genus	11	2.78×10^6	3.20×10^6	1.53×10^4–1.09×10^7

* Each in 2 repetitions.
** Gram-positive bacteria without the cocci of *Enterococcus* genus.
AM – arithmetic mean; SD – standard deviation; min – minimal value; max – maximal value.

Table 3. Concentrations of airborne culturable bacteria in the poultry houses depending on the type of building and measurement series (microclimate parameters and type of ventilation) and outdoor

Measurement series**	Type of building	N	Total bacteria				Gram-positive bacteria*			Gram-negative bacteria			Bacteria of *Enterococcus* genus			
			M (cfu/m^3)	SD	p^1	p^2	M (cfu/m^3)	SD	p^1	p^2	M (cfu/m^3)	SD	p^1	p^2	M (cfu/m^3)	SD	p^1	p^2
I	hatchery	2	3.03×10^5	3.61×10^5	0.32	0.006	1.62×10^5	1.76×10^5	0.46	0.31	6.01×10^3	2.13×10^3	0.52	<0.001	4.13×10^4	3.67×10^4	0.48	0.64
	hen building	3	4.21×10^7	4.30×10^7			2.50×10^7	3.59×10^7			1.10×10^5	1.76×10^5			3.98×10^6	5.98×10^6		
	outdoor	1	9.50×10^3				3.55×10^2				0.00				0.00			
II	hen building	3	9.03×10^6	7.46×10^5			7.30×10^6	1.15×10^6			9.91×10^3	1.01×10^4			1.79×10^6	6.62×10^5		
	outdoor	1	9.25×10^3				9.25×10^3				0.00				5.09×10^2			
III	hen building	3	1.57×10^8	4.82×10^7			3.60×10^7	6.03×10^6			3.32×10^6	8.79×10^5			4.38×10^6	4.34×10^5		
	outdoor	1	3.56×10^6				2.83×10^6				3.96×10^5				0.00			

N – number of measurements (each in 2 repetitions); M – mean; SD – standard deviation.
p – level of statistical significance: p^1 – p-value for hatcheries versus hen buildings in first measurement series, p^2 – p-value for differences in measurement series.
* Gram-positive bacteria without the cocci of *Enterococcus* genus.
** Measurement series characterized by values of selected microclimate parameters and type of ventilation (Table 1).

in the third series was almost three orders of magnitude higher (3.56×10^6 cfu/m^3). For Gram-positive bacteria, the lowest level of mean concentration was noted in I measurement series (3.55×10^2 cfu/m^3), the highest in III series (2.83×10^6 cfu/m^3). Gram-negative bacteria were isolated only in the third measurement series at concentration equal to 3.96×10^5 cfu/m^3, Gram-positive bacteria of the genus *Enterococcus* were isolated only in the second series at concentration equal to 5.09×10^2 cfu/m^3.

The variance analysis indicated a significant impact of the measurement series (selected microclimate characteristics and type of ventilation) on the concentrations of total bacteria ($F = 13$; $p = 0.006$) and Gram-negative bacteria ($F = 39$; $p < 0.001$) in the breeding facilities. The variance analysis did not indicate any significant impact of the measurement series on the level of Gram-positive bacteria ($F = 1.4$; $p = 0.312$) and bacteria of *Enterococcus* genus ($F = 0.5$; $p = 0.641$) found in bioaerosol of breeding facilities. The first measurement series showed no statistically significant differences between the concentrations of bacteria in hatcheries and the other buildings for all types of bacteria ($p > 0.05$) – Table 3.

A comparison of the mean bacterial concentrations for different measurement series, carried out by the Fisher test and then Tukey test, indicated that the bacterial aerosol was characterized by a significantly higher concentration in third series, as compared to first or second series. The p-values for significance differences in concentrations of bacteria depending on the measurement series defined by selected microclimate characteristics and type of ventilation are shown in Table 4.

The results of qualitative analysis of airborne bacterial aerosol in the buildings and outside are presented in Table 5. The bacterial microflora identified in each of tested buildings was differentiated and characterized by the presence of representatives of 21 genera/species.

Cocci and Gram-positive rod-shaped bacteria, *Bacillus* genus and Gram-negative rod-shaped bacteria were found

Table 4. Assessment of significance differences (p-value) in concentrations of bacteria depending on the measurement series ($p < 0.05$)

Microorganisms	Measurement series**	Significance differences in concentrations p		
		I	II	III
Total bacteria	I	–	0.55	0.020
	II	0.55	–	0.006
Gram-positive bacteria*	I	–	0.59	0.800
	II	0.59	–	0.290
Gram-negative bacteria	I	–	0.97	< 0.001
	II	0.97	–	< 0.001
Bacteria of *Enterococcus* genus	I	–	0.73	0.990
	II	0.73	–	0.650

* Gram-positive bacteria without the cocci of *Enterococcus* genus.
** Measurement series in hen buildings characterized by values of selected microclimate parameters and type of ventilation (Table 1).

among the mesophilic bacterial microflora. Most prevalent in the breeding houses were Gram-positive cocci, mainly coagulase-negative species of the genus of *Staphylococcus* (*Staphylococcus lentus*, *Staphylococcus xylosus*, *Staphylococcus sciuri*, *Staphylococcus chromogenes*), which constituted almost 42% of all microorganisms isolated from Columbia agar, and faecal cocci, i.e. bacteria of the genus of *Enterococcus* (*Enterococcus faecalis* – 89.88%, *Enterococcus faecium* – 10.12%) isolated from agar with esculin. Cocci belonging to the genus of *Micrococcus* and species *Aerococcus viridans* were less abundant. Another numerously represented group of Gram-positive microorganisms isolated from Columbia agar were rods of the genera: *Brevibacterium* (19.23%), *Corynebacterium* (15.95%) and *Cellulomonas* (7.83%). Bacteria of the *Bacillus* genus constituted over 3% of Gram-positive bacteria found in the poultry houses.

Bacteria of *Pseudomonas* genus dominated among Gram-negative microorganisms; they were most numerously represented by the species of *Pseudomonas fluorescens* (55.93%), less numerously by species *Pseudomonas alcaligenes* and *Pseudomonas stutzeri* and bacteria of the genus of *Moraxella* (38.36%). The bacteria of the genera of *Pasteurella* and *Pantoea* as well as *Enterobacteriaceae* bacteria (*Escherichia coli*, *Klebsiella pneumoniae* ssp. *ozaenae* and *Enterobacter cloacae*) were also isolated; however, their share among all isolated Gram-negative rod-shaped bacteria was low, slightly over 1%.

The results of qualitative analysis of bacterial microflora in outdoor air showed that over half of the Gram-positive bacteria isolated from the Columbia agar were the genera of *Corynebacterium* (39.90%) and *Brevibacterium* (26.58%), the remaining part were Gram-positive cocci of the genera *Micrococcus* (20.02%) and *Staphylococcus* (0.22%), and bacilli of the genus *Bacillus* (13.29%). Microorganisms of the genus *Enterococcus faecalis*, and Gram-negative bacteria of the species *Pseudomonas fluorescens* were also isolated.

The pool of the determined bacteria contained microorganisms which were qualified to the second exposure group according to the criteria specified in the ordinance issued by the Polish Ministry of Health on 22 April 2005 on biological hazards in the working environment in its recent version harmonized to the EU Directive 2000/54/EC. These are: *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* ssp. *ozaenae*, *Enterococcus faecalis*,

Table 5. Genera / species of bacteria isolated from indoor and outdoor air

Type of bacteria	Location	Isolated genus / species name	%*	Risk group**
Gram-positive	indoor	*Staphylococcus lentus*	22.99	–
		Brevibacterium spp.	19.23	–
		Corynebacterium spp.***	15.95	2
		Staphylococcus xylosus	13.25	–
		Micrococcus spp.	9.85	–
		Cellulomonas spp.	7.83	–
		Staphylococcus sciuri	5.29	–
		Bacillus spp.	3.34	–
		Aerococcus viridans	2.21	–
		Staphylococcus chromogenes	0.05	–
	outdoor	*Corynebacterium* spp.	39.90	2
		Brevibacterium spp.	26.58	–
		Micrococcus spp.	20.02	–
		Bacillus spp.	13.29	–
		Staphylococcus lentus	0.21	–
		Staphylococcus xylosus	0.01	–
Gram-negative	indoor	*Pseudomonas fluorescens*	55.93	–
		Moraxella spp.	38.36	–
		Pseudomonas alcaligenes	4.15	–
		Escherichia coli	0.96	2
		Klebsiella pneumoniae ssp. *ozaenae*	0.30	2
		Pseudomonas stutzeri	0.16	–
		Pasteurella spp.	0.06	2
		Enterobacter cloacae	0.06	2
		Pantoea sp.	0.01	–
	outdoor	*Pseudomonas fluorescens*	100.00	–
Gram-positive faecal cocci	indoor	*Enterococcus faecalis*	89.88	2
		Enterococcus faecium	10.12	2
	outdoor	*Enterococcus faecalis*	100.00	2

* Percentage of individual microorganisms in the pool of all diagnosed bacteria in each group.

** Based on the ordinance of the Ministry of Health on 22 April 2005 regarding harmful biological agents in the working environment and health protection of employees occupationally exposed to these agents (Off J L No 81, sec. 716) [26].

*** Among genus of *Corynebacterium* may occur pathogenic species which are qualified to the second exposure group according to the Polish ordinance and EU Directive.

Enterococcus faecium and two genera specified in the ordinance, *Corynebacterium* spp. and *Pasteurella* spp., among which pathogenic species may occur [26,27].

DISCUSSION

Our studies have shown that the air inside the buildings where industrial breeding of poultry with the litter bed system is conducted contains high concentrations of total bacteria, within the wide range of 10^4–10^8 cfu/m^3. These values were comparable with the levels of concentrations of this type of microorganisms presented in the literature [1,2,4,6,13,28]. Lee et al. [1], Romanowska-Słomka et al. [13] and Bakutis et al. [28] found airborne total bacteria in poultry houses at mean level equal to 10^5 cfu/m^3, while Awad et al. [4] and Saleh et al. [6] reported respective concentrations of 10^5–10^6 cfu/m^3. In research conducted in Norway, Nimmermark et al. [2] reported higher concentrations of bacteria (10^7 cfu/m^3). Higher than our mean concentrations of airborne bacteria inside the poultry houses equal to 4.7×10^9 cfu/m^3 were found by Radon et al. [3]: from 2.7×10^7 to 4.2×10^{10} cfu/m^3

The lowest concentrations of total bacteria and Gram-positive bacteria were found in hatcheries (buildings with the youngest chickens). In the facilities with older flocks (other buildings), the level of microorganisms was higher by several orders of magnitude. The same tendency was reported by other authors [11,29,30]. The mean concentration of Gram-negative bacteria obtained in our study (9.38×10^5 cfu/m^3) exceeded the levels of this group of bacteria presented by several authors. Zucker et al. [9] and Clark et al. [31] carried out research in buildings with different-than-ours poultry maintenance system (without litter) and this could be reason of lower values of concentration of Gram-negative bacteria. Also Bakutis et al. [28] found lower concentration of Gram-negative bacteria than we showed in our study, but those authors did not report what type of housing system was studied.

The changes we obtained in concentrations of bacteria in the three measurement series are best explained by the intensity of air exchange in hen houses depending on outside temperature. The lowest concentrations of each of the groups of the examined microorganisms were noted in second measurement series and the highest in third. In the warmest months (II measurement series) the air exchange in the breeding houses was enhanced owing to use of different ventilation methods. In summer, the mechanical air exchange with ventilators was supported by natural ventilation through open gates; in that case the airflow values were the highest. In non-summer months the premises were made tighter, the mechanical ventilation system was not augmented and the airflow values were lower.

Unlike with most of physical and chemical factors, there are no worldwide acceptable criteria of evaluation of occupational exposure to biological agents. In Poland in 2004, the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment accepted the recommended maximum values of concentrations of biological agents [32]. For the total number of mesophillic bacteria, this value (proposed by Górny [33]) amounts to 1.0×10^5 cfu/m^3. In our study, concentrations of total bacteria exceeded this value in all cases, except for the concentration in the building with one-day old chickens (hatcheries), which was by one order of magnitude lower (4.74×10^4 cfu/m^3) than the recommended maximum concentration. The concentrations of the total number of mesophillic bacteria only obtained during the second measurement series were similar to the 1.0×10^7 cfu/m^3 reference value proposed by Clark, while in first and third series this value was significantly exceeded [34]. Also occupational exposure threshold value for the total number of bacteria reported by Malmros et al. (1.0×10^4 cfu/m^3) was exceeded in all cases [35]. The concentrations of Gram-negative bacteria were compared with the reference value proposed by Górny [33] for the highest admissible concentration of this type of bacteria present in the working environment polluted by organic dust. It amounts to 2.0×10^4 cfu/m^3. This value was exceeded 10-fold and 100-fold in the first and third measurement series, respectively. Concentrations of Gram-negative bacteria in our study in all cases exceeded the reference value (1.0×10^3 cfu/m^3) proposed for his group of bacteria by Clark [34] and Malmros et al. [35].

The qualitative analysis made it possible to isolate 21 genera/species of Gram-positive and Gram-negative bacteria present inside the breeding facilities. The Gram-positive bacteria group was found to contain microorganisms belonging to 7 genera. The Gram-negative bacteria group also contained 7 genera. The predominant microflora included bacteria of the following genera: *Staphylococcus*, *Brevibacterium*, *Corynebacterium*, *Enterococcus*, *Pseudomonas* and *Moraxella*, mostly specified as commonly found microorganisms. Furthermore, bacteria of the genera of *Micrococcus*, *Cellulomonas*, *Bacillus*, *Aerococcus*, *Pasteurella*, *Pantoea* and intestinal rod-shaped bacteria were found. The latter constituted a bit over one percent of all determined Gram-negative bacteria. The presence of microorganisms belonging to the mentioned genera in the poultry breeding facilities is confirmed by the results obtained by other authors [5,7,8–11,36]. Among the Gram-positive bacteria, *Staphylococcus*, *Enterococcus*, *Micrococcus* and *Bacillus* were the genera most commonly identified in air of hen buildings [4,5,7,10,12].

Furthermore, Budzińska et al. [10] isolated also cocci of the genus *Aerococcus*, while Dutkiewicz et al. [7] detected rod-shaped microbes of the genus *Corynebacterium*. In research of Awad et al. [4], Vucemilo et al. [5], Zucker et al. [9], Budzińska et al. [10] and Sauter et al. [12], microorganisms of genus *Pseudomonas* and *Enterobacteriaceae* predominated among Gram-negative bacteria isolated from the air of poultry buildings. In addition, bacteria of genus *Acinetobacter* constituted high percentage of Gram-negative bacteria isolated by Awad et al. [4] and Dutkiewicz et al. [7].

A number of bacteria isolated in the hen house air also were found in outside air (but at lover concentrations). This suggest that microorganisms in the air of the neighbourhood of the hen house come from the indoor air.

Among determined microorganisms, some bacteria were qualified to the second risk group (based on the Polish ordinance No 81 sec. 716 and EU Directive No 2000/54/EC), i.e. they were the factors which may induce diseases in people but their spread in the human population is hardly probable; usually there are some relevant effective methods to prevent or treat them. *Enterococcus faecalis* and *Enterococcus faecium* cocci constitute a natural flora of gastrointestinal tract of humans and animals. They may, however, induce opportunistic inflammations of urinary tract, gallbladder and endocardium, particularly in people with immune deficiency, as well as cause hospital infections [8,37].

The other rod-shaped bacteria, qualified to the second risk group, are defined as intestine rod-shaped bacteria which inhabit intestines of healthy people and animals. Bacteria of *Escherichia coli* species, numbered among this exposure group, may become an opportunistic pathogen colonizing the organism outside the alimentary tract. They then usually become a cause of urinary tract infections, but also nephritis and otitis media. Rod-shaped bacteria of *Enterobacter cloacae* species are opportunistic pathogens which quite often induce diseases in people with deficient immunity. They may attack the upper respiratory tract, urinary tract, and may induce general infections, now and then causing also hospital infections. *Klebsiella pneumoniae* ssp. *ozaenae* is a subspecies of *K. pneumoniae*; the microorganisms which belong to it are opportunistic pathogens causing the upper respiratory tract diseases with a characteristic clinical picture [8,38].

Among the isolated microflora were also two genera of bacteria *Corynebacterium* and *Pasteurella*, which were listed in the aforementioned ordinance as potentially pathogenic for humans. Both saprophytic and pathogenic species may be found within these genera, but in this study we failed to identify them. Gram-positive rod-shaped bacteria of *Corynebacterium* genus may induce opportunistic infections which occur with the organism's deficient immunity. The infection may enter through wounds and respiratory tract. Gram-negative rod-shaped bacteria belonging to *Pasteurella* genus are common microorganisms found in respiratory tract and gastrointestinal tract of pets and wild animals. The bacteria of this genus may induce in humans infections of wounds resulting from animal scratches and bites. These infections may cause inflammations of the connective tissue and lymphatic nodes, and in complications – arthritis and osteomyelitis. The rod-shaped bacteria may also induce chronic infections of lung and intestines [8,39].

Other airborne bacteria in breeding facilities may be defined as environmental bacteria; they have not been mentioned in the quoted regulation of the Minister of Health, and it is hardly probable that they could induce diseases. However, we should remember that Gram-negative bacteria even from environmental group may be a source of endotoxins inducing immunotoxic reactions in people [8,14–16,18].

The number of samples collected in our study was limited; therefore, it would not be reasonable to generalize our conclusions about differences in bioaerosol concentrations in poultry houses. However, after careful analysis we have decided to present our conclusions, which are valid, although limited in their scope.

CONCLUSIONS

1. Workers employed at the intensive poultry production with litter bed system are exposed to high concentrations of bacterial microorganisms. The determined mean concentration of total bacteria (5.7×10^7 cfu/m^3) as well as Gram-negative bacteria exceeds the reference limit value for working facilities.
2. In hatcheries, the concentrations of bacteria were lower than in hen houses.

3. A significant variability of mean concentrations of bacteria was detected. The lowest concentrations of each group of the examined microorganisms were noted in the second measurement series, when the air exchange in the breeding houses was over twice higher than in first and third measurement series and the mechanical ventilation was supported by natural ventilation (opened gates in the buildings). The higher concentration of bacteria was found when the efficiency of ventilation was lower.
4. Within the pool of identified predominant microorganisms, 5 microorganisms (*Escherichia coli, Enterobacter cloacae*, *Klebsiella pneumoniae* ssp. *ozaenae*, *Enterococcus faecalis*, *Enterococcus faecium*) were qualified to the second exposure group according to the Polish ordinance and EU Directive and two genera of bacteria, *Corynebacterium* and *Pasteurella*, were classified as those among which pathogenic species may occur.
5. Due to the high levels of bacterial aerosol in the working environment associated with intensive poultry production and their potential adverse effects on the respiratory system of exposed workers, special attention should be paid to improving the effectiveness of the ventilation system and ensuring that individual workers are protecting against harmful biological agents by using FFP2 masks.

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