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THE OCCURRENCE OF THE AIRBORNE BACTERIA GENUS *STAPHYLOCOCCUS* IN THE CITY OF OLSZTYN

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WYSTĘPOWANIE BAKTERII Z RODZAJU *STAPHYLOCOCCUS* W POWIETRZU ATMOSFERYCZNYM MIASTA OLSZTYNA

Praca przedstawia wyniki ilościowych i jakościowych badań bakterii potencjalnie chorobotwórczych z rodzaju *Staphylococcus* w powietrzu atmosferycznym w centrum i miejscach rekreacyjnych miasta Olsztyna. Ilościowe badania prowadzone były przez okres jednego roku, w odstępach jednomiesięcznych od października 2002 roku do września 2003 roku, na sześciu stanowiskach badawczych przy zastosowaniu sedymentacyjnej i zderzeniowej metody poboru prób. Przy poborze prób metodą sedymentacyjną średnie liczebności bakterii tego rodzaju na poszczególnych stanowiskach były zawsze wyższe niż przy poborze prób metoda zderzeniową. Wyizolowane kolonie bakterii z rodzaju *Staphylococcus*, z prób powietrza pobieranego czterokrotnie w ciągu okresu badawczego w miesiącach październik 2002 roku, styczeń, kwiecień i lipiec 2003 roku poddawano dalszej identyfikacji do gatunku, na podstawie badań biochemicznych. Większą różnorodność gatunkową bakterii tego rodzaju stwierdzono w powietrzu pobieranym metodą sedymentacyjną (8 gatunków) niż metodą zderzeniową (3 gatunki). Wśród wyizolowanych z powietrza bakterii z rodzaju *Staphylococcus* dominowały gatunki: *S. xylosus* 61% i *S. lentus* 15% przy poborze prób metodą sedymentacyjną i odpowiednio 31% i 62% przy metodzie zderzeniowej. Przy zastosowaniu metody sedymentacyjnej wśród wszystkich wyizolowanych bakterii tego rodzaju (627 jtk/m^3), stwierdzono 8% udział bakterii *Staphylococcus aureus* i 2% *Staphylococcus epidermidis*. Gatunków tych nie wykryto przy poborze prób metodą zderzeniową.

Summary

This paper presents the qualitative and quantitative results of the research into potential pathogens of genus *Staphylococcus* in the air in the centre and in the recreational areas of Olsztyn. The qualitative examinations were conducted for one year, from October 2002 to September 2003, with one-month intervals between measurements, which were done at testing sites with the use of sedimentation and impaction methods of sampling. When the samples were taken by the sedimentation method, the average count of this type of bacteria at various sites was higher than in those taken by the impaction method. The colonies of *Staphylococcus* bacteria, isolated from air samples taken four times during the test period in October 2002, January, April and July 2003, were further identified down to the species, based on biochemical tests. A wider species diversity of this type of bacteria was found in the samples taken by the sedimentation method (8 species) than by the impaction method (3 species). Among the *Staphylococcus* bacteria isolated from air the following species dominated: *S. xylosus* 61% and *S. lentus* 15% when the samples were taken by the

sedimentation method and 31% and 62%, respectively, with the impaction method. When the samples were taken by the sedimentation method, 8% of *Staphylococcus aureus* and 2% *Staphylococcus epidermidis* were found among all the isolated bacteria (627 cfu/m^3). These species were not found in the samples taken by the impaction method.

INTRODUCTION

Atmospheric air and indoor air are those of the most natural environments surrounding a human being that have a significant importance for his normal functioning. Advancing progress in industry, transportation as well as the creation of larger urban areas has resulted in a greater and greater concentration of sources of air pollutant emission [4]. A large amount of harmful substances are emitted to the atmosphere including: dust, organic compounds, inorganic compounds of N, S, C and also microorganisms [2, 8, 10, 13, 20]. Microorganisms existing in the air can have the form of so-called aerosols, i.e. fine drops of liquid or fine molecules of solid matter which contain bacteria, viruses, fungal spores, plant pollens and small animal organisms. Carried over significant distances with the air movement, they may cause infections and diseases of various types, and particularly of the respiratory system particulary of humans and animals [7, 14, 16, 25]. The atmospheric air mostly contains saprophytic microorganisms, but pathogenic microorganisms can be also found here [15]. They occur mostly in human surroundings and close to sewage treatment plants. The range of their propagation depends on weather conditions [5]. The qualitative and quantitative composition of the air microflora depends to a large extent on the survival of aerosol. The survival of aerosols in the air depends on many factors, such as: temperature, air pressure, relative humidity of the air, intensity of insolation, time of the day and of the year, location of the examined area [2, 4, 28] and on the size and composition of aerosols [6, 20, 22]. The drop in air pressure and temperature and rise in relative humidity of the air causes condensation of water vapor on the surface of aerosol particles, and in consequence their weight rises and they start to fall faster [8]. The bacteria included in the *Staphylococcus* genus, which are commonly found in the natural environment, are an indicator of contamination with pathogenic microorganisms originating in humans and in animals [17]. They can be isolated from various environments: from soil, air and water. They comprise the natural microflora in humans and animals [15] and their greatest numbers are found on the skin, in skin glands and in mucosa [27]. Being saprophytic as a rule, these bacteria do not pose any threat to human health; however, some species show pathogenic properties. The most important among these are: *Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus saprophyticus* [27]. With limited abilities of air for self-purification, atmospheric air has to be checked for its microbiological purity, with a simultaneous determination of the degree of dustiness and characterizing the weather conditions; additionally, measures must be taken in order to protect the air in towns from excessive pollution.

The objective of the study was to determine the degree of contamination of atmospheric air with *Staphylococcus* bacteria based on their count and species diversity, in the centre and in recreational areas of the city of Olsztyn, with the samples taken by two methods.

MATERIAL AND SAMPLING

Samples for quantitative determination of *Staphylococcus* bacteria were taken at selected sampling sites for one year, with one-month intervals, from October 2002 to

September 2003, each time in three series, by two methods:

- sedimentation sampling, according to the guidelines provided by Polish Standards [17, 18, 19];
- and impaction sampling, with the use of a microbiological air sampler MAS 100 Eco™ produced by MERCK.

Four times during the test period, in October 2002, January, April and July 2003, the species of the isolated *Staphylococcus* bacteria were determined.

In the sedimentation method, Petri dishes with Chapman's medium [3] were placed 1.5 m above the ground at each selected sampling site. The lids were opened and the medium exposed for 30 minutes. The lids were then closed and the dishes with the inoculated material (air) were taken to the laboratory.

The impaction method used an MAS 100 Eco™ apparatus, manufactured by MERCK. The operation of this high performance impaction – type apparatus is based on the air examination principles described by Andersen [1].

Each sample examined 80 dm^3 of air from the centre of the city and 100 dm^3 of air from recreational sites in relevant time intervals of 48 and 60 seconds. A stream of examined air with microorganisms was directed onto the surface of a Chapman's medium [3] on a standard Petri dish placed in an apparatus (the capacity of the air intake was 100 dm^3/min). The dishes with the inoculated material were then taken to the laboratory.

The air samples, taken by the two methods, were incubated at 37°C for 48 hrs. After the incubation, the characteristic, yellow colonies grown on the Chapman's medium were counted. The results were calculated by determining the cell shapes and staining by Gram's Method. This was followed by calculating the average numbers for three repetitions when the samples were taken by the sedimentation and impaction methods. In the impaction method, the numbers of grown colonies were adjusted with Feller's statistical calculating table [1]. The results were calculated for colonies forming units in 1 cubic meter (cfu/m^3) of air with Omeliański's formula, modified by Gogoberidze for the sedimentation method [18] and Krzysztofik's formula for impaction method [8].

The colonies isolated from the Chapman medium were sieved onto agar slants for identification. The ability to form catalase was determined (with 3% hydrogen peroxide), to form cytochrome oxidase (with 1% solution of tetramethyl-p-phenylodiamine) as well as the ability to ferment glucose on Hugh-Leifsona's medium [9]. The haemolytic abilities of the isolated bacterial strains were determined on the bouillon-agar with 2% glucose and 5% sheep's blood [3] when it was incubated at 37°C for 24 hrs. The bacterial strains were finally identified with Api STAPH tests, manufactured by bioMérieux. When the air samples for microbiological analyses were being taken, the weather conditions data were recorded: temperature, pressure, relative humidity, wind direction and speed, and insolation.

SAMPLING SITES

Microbiological examination of air was carried out at six sampling sites. Three test stands were situated in the centre of Olsztyn (Fig. 1):

station I – at the building of regional Sanitary-Epidemiological Station "Sanepid";
station II – at the fire station building;
station III – in the city park.

Fig. 1. Sampling sites situated in the city centre of Olsztyn

Three more test stands were situated in Kortowo district (Fig. 2):
station IV – in the university park at Kortowskie Lake;
station V – at the marina on Kortowskie Lake;
station VI – in the area of Kortowo housing estate.

RESULTS

The results showed that the number of *Staphylococcus* bacteria in the air of the city of Olsztyn in the years 2002/2003, when determined by the sedimentation method, ranged from 0 on all sampling days at various sites, to about 370 cfu/m^3 in January at site II. The

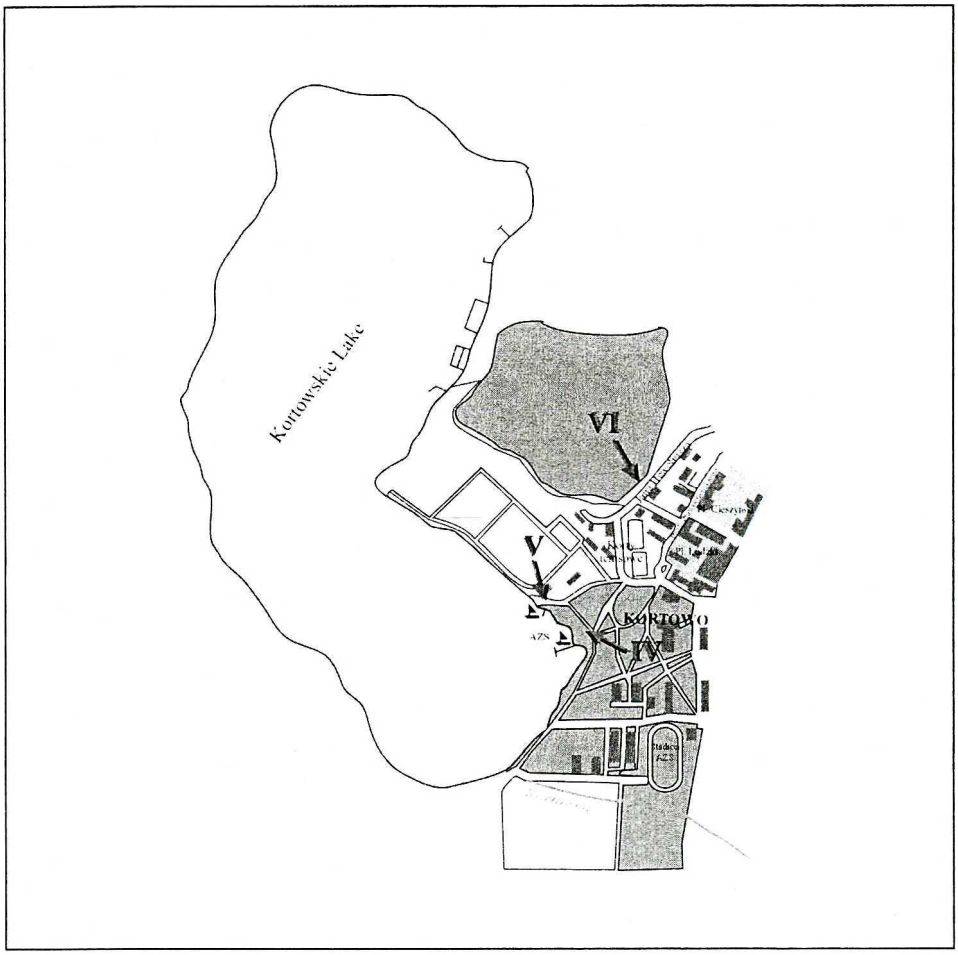

Fig. 2. Sampling sites situated in recreational areas in Kortowo district

highest *Staphylococcus* count in the air during the whole of the testing period was found at site II, which was situated in the city centre, whereas the lowest was found at site VI, situated at a recreational area (Tab. 1).

The count of bacteria in the air taken by the impaction method ranged from 0, in all months at various sites, to about 80 cfu/m^3 in August at site I. The highest level of contamination with the bacteria of this group during the whole testing period was measured at site I, situated at the city centre, whereas the lowest was found at site V, situated in a recreational area (Tab. 1).

The average *Staphylococcus* bacteria count was always higher in the measurements done with the sedimentation method than in those done with the impaction method. A significant difference was found at site II (Tab. 1).

Among the isolated strains of genus *staphylococcus*, 8 species were identified in the sedimentation method measurements (627 cfu/m^3) and 3 species in the impaction method measurements (87 cfu/m^3). The dominant species for the sedimentation method were:

Table 1. Number of individual *Staphylococcus* bacteria, determined on Chapman's medium by the sedimentation and impaction methods at 37°C in 1 cubic metre of air at sites situated in the city centre and in the recreational areas of Olsztyn in the year 2002/2003

Method of sampling												
	sedimentation method						impaction method					
sampling site / date of sampling	I	II	III	IV	V	VI	I	II	III	IV	V	VI
18.10.2002	13	65	26	39	13	0	19	6	6	0	12	0
18.11.2002	13	13	0	13	0	0	8	0	8	0	0	0
13.12.2002	0	0	0	0	26	13	5	0	0	0	0	0
17.01.2003	13	367	0	0	13	0	6	0	0	5	0	5
18.02.2003	0	0	0	0	0	0	5	0	0	5	0	0
18.03.2003	0	13	0	0	0	13	5	0	0	0	0	0
14.04.2003	13	0	0	0	13	0	6	0	0	0	0	0
26.05.2003	13	39	39	0	0	0	0	37	12	0	0	0
17.06.2003	0	0	13	13	13	13	x	x	x	x	x	x
04.07.2003	26	0	0	26	0	0	0	12	0	10	0	0
19.08.2003	52	13	13	39	65	0	75	17	0	12	0	12
25.09.2003	133	0	44	0	29	14	6	18	12	0	0	0
Average	23	42,5	11,2	10,8	14,3	4,4	11,3	7,5	3,1	2,6	1	1,4

x- do not examine

S. xylosus 61% and *S. lentus* 15% (Fig. 3a), and 31% and 62%, respectively, in the impaction method (Fig. 3b).

Among the strains under study, the species *S. aureus* accounted for 8% and *S. epidermidis* for 2%. These species were not detected by the impaction method (Fig. 3a).

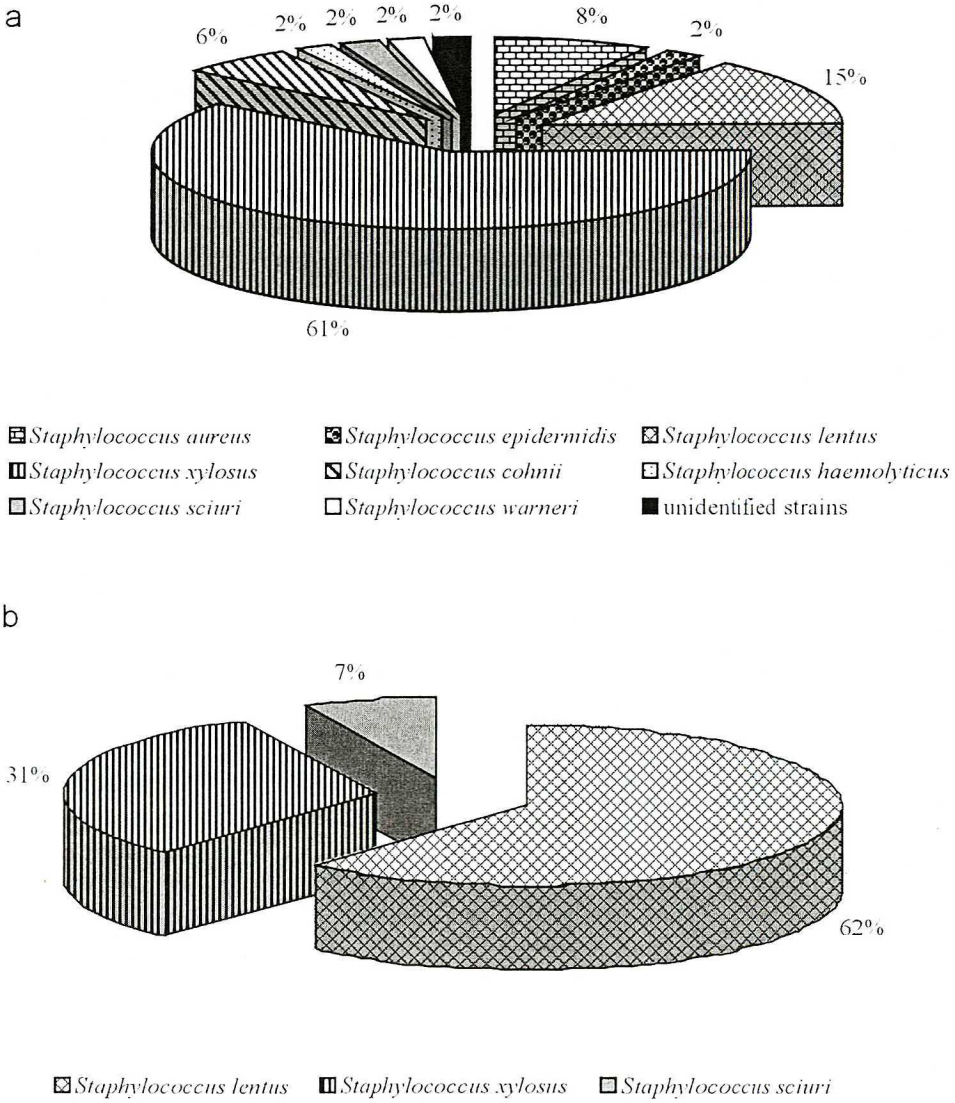

Fig. 3. The percentage of *Staphylococcus* bacteria, identified in the atmospheric air at sites situated in the city centre and in recreational areas of Olsztyn (I-VI) in the months of: October 2002, January, April and July 2003, determined by the
a) sedimentation, **b)** impaction method

Throughout the study period, the highest diversity of species was found in October in the samples of air taken by the sedimentation method, (Fig. 4a), whereas in January and July for the impaction method (Fig. 4b).

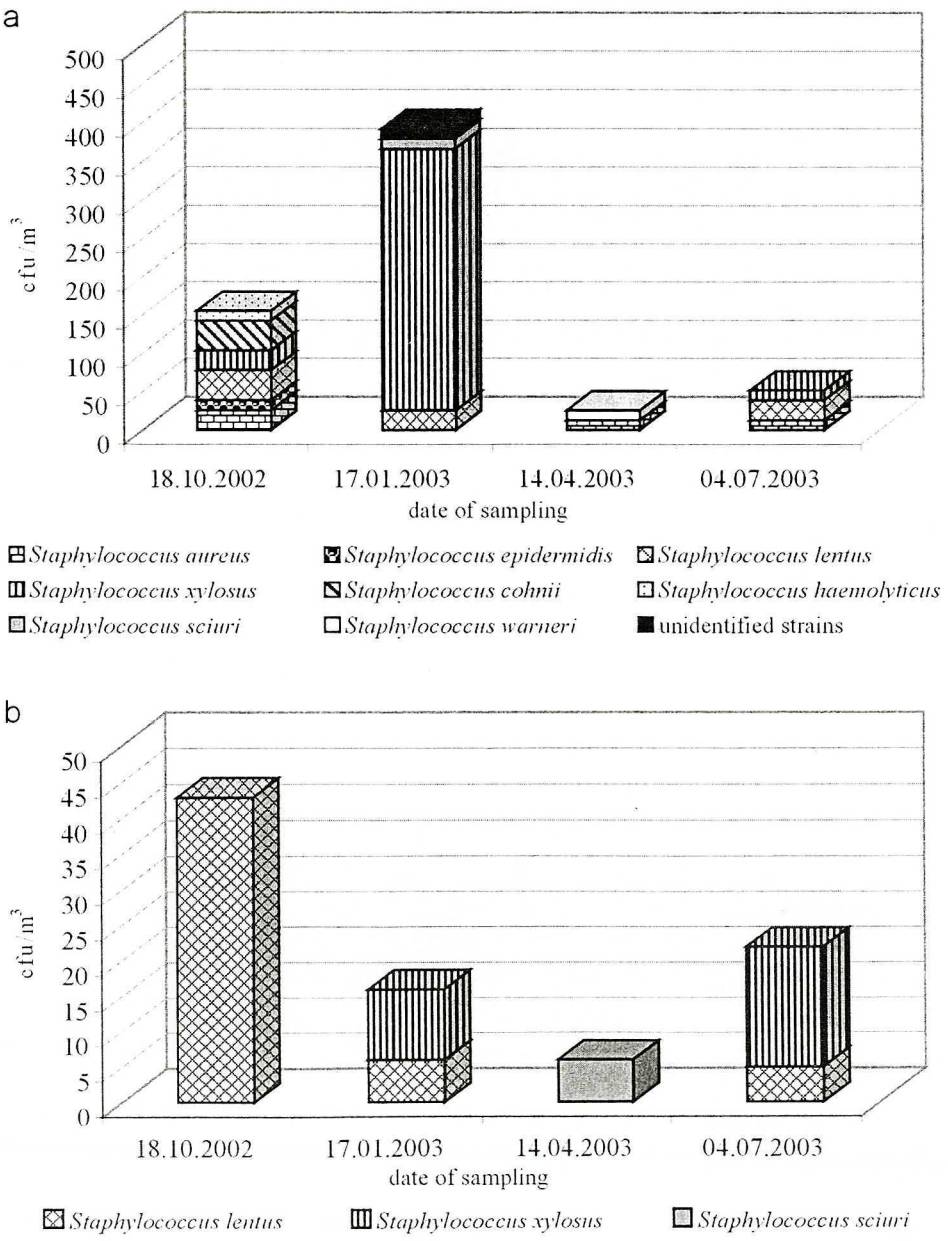

Fig. 4. The species composition of *Staphylococcus* bacteria, identified in the atmospheric air at sites situated in the city centre and in recreational areas of Olsztyn (I-VI) in the months of: October 2002, January, April and July 2003, determined by the
a) sedimentation, b) impaction method

The results obtained with both methods are incomparable. One of the reasons is the fact that on the exposed Petri dishes fat particles are more numerous than on the impactor dish in an aspirator and the concentrations received by the sedimentation method additionally undergo a comparison with the results.

The results of the meteorological observations were shown in Table 2.

Table 2. The results of the meteorological observations

Date of sampling	Sampling site	Temperature °C	Atmospherical pressure hPa	Wind direction and speed m/s	Insolation
18.10.2002	I	10	985	SW 7,5	cloudy, without atmospherical fall
	II	11		SW 7,5	
	III	10		SW 7,5	
	IV	12		SW 7,5	
	V	12		SW 7,5	
	VI	11		SW 7,5	
17.01.2003	I	4	999	SW 4,2	cloudy, without atmospherical fall
	II	5,5		SW 6,3	
	III	4		SW 1,6	
	IV	7,5		SW 3,1	
	V	5		SW 4,8	
	VI	5		SW 2,4	
14.04.2003	I	12	1014	NW 0,9	sunny
	II	14		NW 1,1	
	III	16		NW 3,5	
	IV	20		NW 2,8	
	V	23		NE 2,1	
	VI	21		NE 2,4	
04.07.2003	I	24	987	SW 0,9	cloudy
	II	25		SW 2,4	
	III	26		SW 2,7	
	IV	23		SW 0,4	
	V	27		SW 4,4	
	VI	28		SW 5,2	

DISCUSSION OF RESULTS

Staphylococcus, mainly the coagulase-positive strains such as *Staphylococcus aureus*, are important pathogens of people and animals and are responsible, among others, for hospital infections, bacteriemias, pericarditis, cerebrospinal fluid infections and urinary tract infections [26]. Nevertheless, there has been an increase in infection incidents caused by commonly considered non-pathogenic coagulase-negative staphylococci – mainly *Staphylococcus epidermidis* as well as *Staphylococcus saprophyticus*, *Staphylococcus warneri* and *Staphylococcus cohnii* [24]. They were also identified in previous experiments. The majority of the *Staphylococcus* bacteria isolated from the atmospheric air of Olsztyn were coagulase-negative natural microflora of people and animals [11, 12, 15, 21]. Hospital infections caused by staphylococci resistant to many major antibiotics with the ability to easily acquire genes determining resistance to antibiotics have become an increasingly important problem. *Staphylococcus aureus* is considered to be particularly dangerous and

is resistant to all â-lactam antibiotics [11].

Due to the increasing resistance to antibiotics and the rapid spread of resistant strains, their monitoring in the studied environment is recommended [23]. The highest contamination of atmospheric air with *Staphylococcus* throughout the studied period was recorded in the centre of Olsztyn in January at site II (367 cfu/m^3) and in August at site I (75 cfu/m^3) when sampled with both sedimentation and impaction methods. This could have been due to the fact that the air examinations in the centre of the city were carried out in morning hours with intense pedestrian traffic, which could have been a source of these bacteria.

CONCLUSIONS

1. Higher counts of *Staphylococcus* bacteria were found at sites situated in the city centre as compared to those in recreational areas, for both sampling methods.
2. When samples were taken by the sedimentation method, the average counts of *Staphylococcus* bacteria were higher than for the impaction method.
3. A higher species diversity was found for the sedimentation than for the impaction method.
4. *Staphylococcus xylosus, Staphylococcus lentus* dominated in the species composition.

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