Staphylococci and Fecal Bacteria as Bioaerosol Components in Animal Housing Facilities in the Zoological Garden in Chorzów

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Abstract

Zoos are places open for a large number of visitors, adults and children, who can admire exotic as well as indigenous animal species. The premises for animals may contains pathogenic microbes, including those exhibiting antibiotic resistance. It poses a serious threat to people remaining within the zoo premises, both for animal keepers who meet animals on a daily basis and visitors who infrequently have contact with animals.

Unfortunately, there are almost no studies concerning on the presense, on the concentration of airborne bacteria, especially staphylococci and faecal bacteria in animal shelters in the zoo. No data about antibiotic resistance of staphylococci in these places. The results will enable to determine the scale of the threat that indicator bacteria from the bioaerosol pose to human health within zoo premises.

This study conducted in rooms for 5 animals group (giraffes, camels, elephants, kangaroos and colobinae) in the Silesian Zoological Garden in Chorzów (Poland). The bioaerosol samples were collected using a six-stage Andersen cascade impactor to assess the concentrations and size distribution of airborne bacteria. Staphylococci were isolated from bioaerosol and tested for antibiotic resistance.

In our study the highest concentration of staphylococci and fecal bacteria was recorded in rooms for camels and elephants, the lowest in rooms for colobinae. At least 2/3 of bacteria in bioaerosol constituted respirable fraction, that migrates into the lower respiratory tract of the animals and the people. In investigated animal rooms the greatest bacteria contribution was recorded for bioaerosol fraction sized 1.1–3.3μm. Bacterial intoxication was particularly strong in spring and autumn, what is related to shedding fur by animals.

Among the isolated staphylococci most often occurred *Staphylococcus succinus, S. sciuri* and *S. vitulinus*. The highest antibiotic resistance was noted in the case of *Staphylococcus epidermidis*, while the lowest for *S. xylosus*.

As the animals constitute a significant source of staphylococci and fecal bacteria, attention should be paid to thorough cleaning of their shelters.

Introduction

Zoological gardens in their present form are known for almost 250 years. During that period, especially within the last 100 years, a lot has changed, including significantly increased life expectancy of captive animals, development of exotic animal medicine, improving living conditions as well as intensifying breeding efforts (Tombarkiewicz et al. 2008).

Zoos are the centers responsible for preserving endangered animal species, what encourages maintaining high biodiversity and conserving genetic resources. Moreover, these places are open for a large number of visitors, adults and children, who can admire exotic as well as indigenous animal species. We must be aware that animals themselves and their surroundings generate bioaerosol. The problem becomes even more serious in the case of animals kept in closed facilities. The environment inside the premises intended

for animals contains pathogenic microbes, including those exhibiting antibiotic resistance (Górny and Dutkiewicz 2002). It poses a serious threat to people remaining within the zoo premises, both for animal keepers who meet animals on a daily basis and visitors who infrequently have contact with animals. Bioaerosols are especially dangerous to little children, what is connected with distinct structure of their respiratory tracks as compared with adults, inhaling greater quantity of air in relation to their body weight, increased mobility and not fully developed immune system (Choo and Jalaludin 2015).

Animals represent the largest source of microbiological contamination inside the facilities intended for them, specially their fur that is capable of transferring pathogens (Jo and Kang 2006). When it comes to that matter particular attention should be directed to staphylococci that are found on skin, fur, epithelium and mucous membranes, notably in moist areas (e.g. nose) (Irving et al. 2008).

The other sources of germs that may constitute bioaerosol include: feed scraps, feces (remaining within the facilities for some part of the day) and litter. The last two may contain fecal bacteria. The bacteria in faces and those constituting bioaerosol can cause health problems (de Rooij et al. 2019).

Numerous studies demonstrated that contamination of the internal environment is correlated with the occurrence of both acute and chronic health problems (Samadi et al. 2013). The most frequently reported health issues regarded the respiratory system (rhinitis, bronchitis, sinusitis, asthma) and involved gastrointestinal disorders (Farthing et al. 2009, Borlée et al. 2015, Walser et al. 2015, Douglas et al. 2018, Robertson et al. 2019).

Taking the above into consideration, it is critically important to provide people remaining in close proximity to animals with safe conditions in terms of microbial components. Unfortunately, there are no guidelines that would directly determine the microbiological quality of air for specific environments such as animal facilities in the zoo. Zoos are obliged to ensure proper welfare conditions (Kruszewicz 2011), as set forth in the Council Directive 1999/22/EC and the ordinance of the Minister of Environment of 2004 (J.L. of 2004, No. 5, item 32).

The purpose of the study conducted in selected animal facilities in the Silesian Zoological Garden in Chorzów was to:

- estimate the contamination with indicator bacteria – staphylococci and fecal bacteria,

- determine whether the concentration of total (TC) and repairable fraction (RF) of the bioaerosol differs depending on the group of studied animals,

- determine the distribution of aerodynamic diameters of the bioaerosol containing indicator bacteria,

- assess whether detected bioaerosol concentrations pose a threat to zoo employees and visitors,

- isolate staphylococci strains and determine its species,

- assess resistance to antibiotics of isolated staphylococci strains.

The results will enable to determine the scale of the threat that indicator bacteria from the bioaerosol pose to human health within zoo premises.

This study is a continuation of previous experiments carried out in the zoos in Kraków and Chorzów.

Materials And Methods

The study was conducted in the Silesian Zoological Garden in Chorzów (Poland). The zoo is located 272 meters above the sea level and its area amounts to 47.62ha. The object is situated on almost flat area.

The measurements were taken throughout the whole calendar year. The study involved rooms for the following animals: elephants (*Elephas maximus*), giraffes (*Giraffa camelopardalis reticulata*), kangaroos (*Macropus rufus*), camels (*Camelus bactrianus*) and colobinae (*Colobus guereza*). The animals remained within their premises during cold months, and could walk into enclosures during warm months. The research facilities were selected based on the following criteria: the size of animals (large – giraffes, elephants, camels vs. small – colobinae and kangaroos) and the age of the animal facilities (new – for giraffes and colobinae vs. older – for elephants, kangaroos and camels). The control area was located on the parking lot, 5 m from the front of the office building.

Location of the sampling sites is shown in Fig. 1 and their characteristics in Table 1.

Table 1
Characteristics of the studied premises

parameter	**Group of animals**				
	elephants *(Elephas maximus)*	**camels** *(Camelus bactrianus)*	**colobinae** *(Colobus guereza)*	**giraffes** *(Giraffa camelopardalis reticulata)*	**kangaroos** (*Macropus rufus)*
Total area (m^2)	200	50	38	343	25
Year of construction	1960s	1960s	2010	2013	1960s
Number of animals	2	2	10	5	5
Area per 1 animal [m^2]	100	25	3.8	68.6	5
Type of ventilation	lack	lack	mechanical	mechanical	lack
Mean animal weight [kg]	3,500	480	14	1,500	47
Ratio – kg of animal weight per 1 m^2 of area	35	19.2	3.7	21.9	9.4
Type of litter	lack	sawdust beech	lack	sawdust beech	sawdust beech

Air samples were taken using a 6-stage cascade impactor WES-710 model Andersen-Graseby (Westech Instrument, Great Britain). This instrument enables to determine bioaerosol fractions based on the aerodynamic particle size: fraction 1: above 7µm; fraction 2: 4.7–7µm; fraction 3: 3.3–4.7µm; fraction 4: 2.1–3.3µm; fraction 5: 1.1–2.1µm and fraction 6: 0.65–1.1µm. Fractions 3–6 (below 4.7µm) are classified as respirable.

The samples were taken about the same time (between 10.00am and 1.00pm). At this time animals have already been fed and boxes cleaned. Air samples were collected 1.5m above the ground, what is tantamount to the location of the human breathing zone. Six Petri dishes were used to collect the samples – one for each impactor stage. The time necessary to collect the samples depended on anticipated concentration of bacteria in a given location. The flow rate through the impactor was constant and amounted to 28.3l/min. The samples were collected within 20 to 180 seconds, and the volume of sucked air ranged between 9.4 and 84.9l. The impactor was disinfected using gauze pads moisten in 70% isopropanol before taking each sample.

The following media were used in the study: Mannitol Salt Lab-Agar called Chapman medium (Biomaxima) – for isolating staphylococci, and EMB (Eosin Methylene Blue Agar; Biomaxima) – for growing fecal bacteria. After sampling, the Petri plates were immediately transported to the laboratory, where they were incubated at 36 ± 1°C for 48h. After the incubation, the colonies were counted and the results were expressed as colony forming units per 1m^3 of air (CFU/m^3). The tests were performed in triplicate and the results were presented as the means.

As the guidelines on the acceptable concentrations of bioaerosol inside facilities intended for animals have not been developed yet, the results obtained in the course of this study were evaluated against the values proposed by the Team of Experts in Biological Factors (Polish.: ZECB) (Augustyńska and Pośniak 2016), with animals rooms being classified as working premises contaminated with organic dust (Table 2).

Table 2
Proposals for acceptable concentrations of airborne microorganisms in the working environment according to the Team of Experts in Biological Factors (ZECB) – the values applicable in Poland

Microbiological agent	Acceptable concentration [CFU/m^3]
Gram-negative bacteria (total count - TC)	20,000
Gram-negative bacteria (respirable fraction - RF)	10,000
Staphylococci	No data

Staphylococci strains were identified according to the methodology devised by Lenart-Boroń et al. (2016). Pure staphylococci strains were obtained from the colonies growing on Chapman medium by means of streak plating. Pure strains were subjected to microscopic examination and furazolidone susceptibility testing. Micrococci were not included in the study due to their resistance to furazolidone, while sensitive strains were classified as staphylococci. Staphylococcal species were identified using a MALDI-TOF method.

The sensitivity of staphylococci to antibiotics was evaluated using filer paper disks immersed in selected antibiotic solutions. The antibiotics listed hereafter were used in the study: cefoxitin (FOX 30 µg), chloramphenicol (C 30 µg), ciprofloxacin (CIP 5 µg), fusidic acid (FA 10 µg), gentamycin (CN 10 µg), tigecycline (TGC 15 µg), erythromycin (E 15 µg), clindamycin (DA 2 µg), tetracycline (TE 30 µg) and rifampicin (RA 5 µg). Paper disks were transferred to Mueller-Hinton medium (Biomaxima Poland). Antibiotic resistance was eventuated according to the guidelines provided by the National Reference Center for Antimicrobial Susceptibility (Polish: KORLD), as set forth in the document titled “Guidelines for selecting tests to assess bacterial susceptibility to antibiotics and chemotherapeutics. Evaluation of susceptibility of Gram-positive cocci from the genus *Staphylococcus* spp.” (Żabicka and Hryniewicz 2010).

Statistical analysis was performed using the Statistica 13.1 (StatSoft, USA). The values obtained for the concentration of bacterial bioaerosol were expressed as means with standard deviations and range. Normal distribution of data was examined applying the Shapiro-Wilk test.

The distribution of total (TC) and respirable (RF) fraction of bioaerosol values was close to normal and other data were not normally distributed, therefore both parametric (a one-way ANOVA followed by the post-hoc Tukey’s test) and non-parametric (the Kruskal-Wallis test) tests were applied to assess the significance of differences between the concentrations of bioaerosols in rooms for different animals.

Results

The normative values were determined only for fecal bacteria. The concentrations calculated for this group (Table 3) fall within the range of 0 to 1,060CFU/m^3 and do not exceed values recommended by ZECB (see Table 2). As compared with acceptable concentration, maximum concentration for fecal bacteria amounted to approx. 5% for TC and 10% for RF.

Table 3
Average, standard deviation and range of bioaerosol of indicator bacteria in animal premises in Chorzów zoological garden [CFU/m3]

Group of animals	Fractions	Mannitol-positive staphylococci (ST-pos)	Mannitol-negative staphylococci (ST-neg)	Fecal bacteria (FB)
giraffes	TC	3,499 ± 3,100 (636-7,635)	3,128 ± 1,040 (1,908-4,028)	70 ± 99 (0-212)
	RF	2,933 ± 2,680 (636-6,504)	2,438 ± 966 (1,767-3,816)	70 ± 99 (0-212)
elephants	TC	1,899 ± 1,373 (283-3,286)	18,402 ± 34,244 (472 – 69,748)	435 ± 506 (0–1,060)
	RF	1,431 ± 1,231 (94 – 2,968)	16,132 ± 30,594 (212 – 62,010)	418 ± 498 (0–1,060)
camels	TC	6,251 ± 3,135 (3,304-9,827)	6,539 ± 6,452 (1,416 – 15,978)	52 ± 105 (0-211)
	RF	4,041 ± 1,543 (2,312-5,726)	5,332 ± 6,057 (896 – 14,210)	52 ± 105 (0-211)
colobinae	TC	1,009 ± 1,083 (105-2,332)	736 ± 1,023 (70 – 2,261)	47 ± 66 (0-141)
	RF	630 ± 811 (0–1,766)	400 ± 643 (70 – 1,366)	11 ± 23 (0–47)
kangaroos	TC	5,902 ± 6,630 (0–12,225)	1,837 ± 2,597 (282-5,724)	123 ± 105 (0-212)
	RF	3,834 ± 4,458 (0–8,978)	1,254 ± 1,757 (282-3,886)	88 ± 88 (0-212)
control	TC	133 ± 114 (0-275)	93 ± 59 (23–162)	0 ± 1 (0–2)
	RF	93 ± 112 (0-254)	78 ± 56 (11–148)	0 ± 0 (0–0)

Table 3 shows mean bioaerosol concentrations with standard deviations and concentration ranges. The highest bioaerosol concentration was obtained for mannitolo-negative staphylococci (ST-POS), the lowest for mannitolo-positive staphylococci (ST-NEG) and the lowest for fecal bacteria (FB). The mean concentrations for ST-POS and ST-NEG differ by up to one order of magnitude, while for ST-NEG and FB by two orders of magnitude. The highest mean concentration for ST-POS, both for TC and RF, was detected in shelters for camels – it amounted to 6,251 ± 3,135CFU/m^3 and 4,041 ± 1,543CFU/m^3, respectively. The largest mean concentration for ST-NEG (TC: 18,402 ± 34,244CFU/m^3, RF: 16,132 ± 30,594CFU/m^3) and FB (TC: 435 ± 506CFU/m^3, RF: 418 ± 498CFU/m^3) was recorded in rooms for elephants. The lowest mean bioaerosol concentrations for 3 studied bacterial groups were measured in housing facilities for colobinae (ST-POS TC: 1,009 ± 1,083CFU/m^3, RF: 630 ± 811CFU/m^3; ST-NEG TC: 736 ± 1,023CFU/m^3, RF: 400 ± 643CFU/m^3; FB TC: 47 ± 66CFU/m^3, RF: 11 ± 23CFU/m^3).

Regarding bioaerosol concentration broken down by fractions established based on aerodynamic particle size (Table 4), the highest bioaerosol concentration for ST-POS was detected in rooms for camels for the faction 2.1–1.1µm (1,909CFU/m^3), for ST-NEG for the same fraction but in the facilities for elephants (5,765CFU/m^3). The highest concentration for FB was also detected in elephant facilities but concerned larger particle size – 3.3–2.1µm (5,686CFU/m^3). After summing up all generated results for all studied bacterial groups, the share of individual bioaerosol factions can be arranged in the descending order: 2.1–1.1µm > 3.3–2.1µm > 4.7–3.3µm > 1.1–0.65µm > 11–7µm > 4.7–7µm. The highest bacteria concentrations were detected in factions classified as respirable, as confirmed by the data shown in Table 5. Depending on the bacterial group, season and facilities intended for a given animal species the share of respirable fraction ranged from 0 to 100%. 100% RF was achieved most frequently for fecal bacteria – e.g. in spring in rooms for giraffes, elephants, colobinae and kangaroos. That relationship results from the fact that fecal bacteria form large consortia, consisting of bacteria alone or bacteria attached to dust particles, less frequently than staphylococci. The average share of FR in TC, taking into account all studied animal facilities and 3 bacterial groups, was the largest in winter (74.28%), while the lowest in summer (65.93%). The analysis of the results for particular season indicated that the above-mentioned pattern can be directly connected with time spent by animals inside their rooms, that is undoubtedly the longest in winter. As far as the animal groups are concerned, the greatest RF share amounting to 85.35% was recorded in shelters for giraffes, while the lowest in rooms for kangaroos. It seems that it is related to the animal size, weight as well as the presence or absence of litter.

Table 4
Fraction average concentations of bioaerosol of indicator bacteria in animal premises in Chorzów zoological garden [CFU/m3]

Fraction of bioaerosol [µm]	Group of bacteria	Group of animals					
		giraffes	elephants	camels	colobinae	kangaroos	control
11 – 7	St-pos	247	183	1,161	280	866	19
	St-neg	424	1,063	648	91	424	8
	FB	0	0	0	24	35	0
7-4.7	St-pos	318	286	1,049	100	1,202	21
	St-neg	265	1,207	560	244	159	8
	FB	0	18	0	12	0	1
4.7–3.3	St-pos	636	256	1,102	135	1,060	18
	St-neg	424	2,541	790	138	495	18
	FB	0	88	0	0	0	0
3.3–2.1	St-pos	636	439	666	253	1,396	29
	St-neg	548	5,686	943	132	389	31
	FB	0	247	35	0	0	0
2.1–1.1	St-pos	866	286	1,909	171	1,149	24
	St-neg	760	5,765	1,868	47	177	19
	FB	53	82	0	0	0	0
1.1–0.65	St-pos	795	451	365	71	230	21
	St-neg	707	2,141	1,732	82	194	10
	FB	18	0	18	12	88	0

Table 5
Percentage share of respirable fraction (RF) depends on season [%]

Season	Group of bacteria	Group of animals						Average share for season
		giraffes	elephants	camels	colobinae	kangaroos	control	
spring	ST-POS	66.7%	90.3%	58.3%	66.7%	60.0%	23.1%	71.11%
	ST-NEG	60.7%	88.9%	88.9%	28.6%	55.6%	50.0%	
	FB	100.0%	100.0%	NA	100.0%	100.0%	NA	
summer	ST-POS	86.0%	64.1%	61.6%	75.8%	50.3%	77.8%	65.93%
	ST-NEG	94.7%	30.0%	54.4%	100.0%	67.9%	70.0%	
	FB	NA	88.9%	100.0%	NA	33.3%	0.0%	
autumn	ST-POS	85.2%	33.3%	70.0%	0.0%	81.4%	NA	69.70%
	ST-NEG	67.6%	80.0%	63.3%	25.0%	70.0%	100.0%	
	FB	100.0%	100.0%	NA	NA	100.0%	NA	
winter	ST-POS	100.0%	70.4%	82.1%	45.2%	NA	92.3%	74.28%
	ST-NEG	92.6%	71.9%	85.1%	60.4%	100.0%	91.3%	
	FB	NA	NA	NA	0.0%	NA	NA	
Average share RF for group of animals		85,35%	74.35%	73.74%	50.17%	71.85%	63.06%	

Legend: ST-POS – Mannitol-positive Staphylococci; ST-NEG – Mannitol-negative Staphylococci; FB – fecal bacteria; NA- not applicable

The results of cluster analysis for bioaerosol concentrations in rooms for different animals are shown in Fig. 2. As presented in Fig. 2, the parameters obtained for colobinae facilities show the greatest similarity to those recorded in the control area. It means that parameters in rooms for colobinae are comparable to the ones recorded in the external environment. The environment in the facilities for elephants differed considerably from the control site and remaining animal rooms.

The indoor/outdoor ratio (I/O ratio), that is the ratio between bioaerosol concentration indoors and outside, is an important indicator of microbial air pollution. When I/O ratio is higher than 1 it means that the room has been intoxicated. Table 6 shows I/O ratios arranged from the lowest to the highest values, separately for 3 studied bacterial groups, including TC and RF. The greatest intoxication occurred in spring and autumn, especially in the case of ST-POS, what can be easily explained as staphylococci constitute natural skin, fur and mucous membrane microflora. The lowest indoor intoxication with staphylococci and fecal bacteria was recorded in winter, what might be related to low animal activity and infrequent stay within the enclosures. In the case of animals shedding fur, winter is a stable period. However, the situation changes in

spring and autumn, when fur is shed: patches of shed fur facilitate bacterial growth, including staphylococci (predominantly) and fecal bacteria (when the animal lies in feces that has not been cleaned).

Table 6
Dependence ratio I/O on group of animals/season for total concentration of bacterial bioaerosol (TC) and respirable fraction of bacterial bioaerosol (RF)

Group of bacteria					
ST-pos		ST-neg		FB	
I/O for TC	**I/O for RF**	**I/O for TC**	**I/O for RF**	**I/O for TC**	**I/O for RF**
0 K/win	0 Cl/aut	1 Cl/sum	1 Cl/sum	0 G/sum	0 G/sum
1 Cl/spr	0 K/win	2 K/win	1 Cl/aut	0 G/win	0 G/win
2 G/win	3 G/win	4 Cl/aut	2 K/win	0 E/win	0 E/win
2 K/spr	3 Cl/spr	6 E/sum	3 E/sum	0 Cm/spr	0 Cm/spr
5 Cl/win	3 Cl/win	7 E/aut	5 E/aut	0 Cm/aut	0 Cm/aut
5 E/win	4 E/win	10 K/aut	7 K/aut	0 Cm/win	0 Cm/win
11 G/spr	6 K/spr	12 G/win	8 Cl/spr	0 Cl/sum	0 Cl/sum
14 Cm/win	13 Cm/win	14 Cl/spr	9 Cl/win	0 Cl/aut	0 Cl/aut
21 E/spr	21 E/sum	14 Cl/win	12 G/win	0 K/win	0 Cl/win
22 Cl/sum	21 Cl/sum	17 E/win	13 E/win	47 E/aut	0 K/win
26 E/sum	32 G/spr	20 Cm/aut	13 Cm/aut	47 Cl/spr	47 E/aut
38 G/sum	42 G/sum	27 K/spr	25 G/aut	71 G/aut	47 Cl/spr
64 Cm/spr	59 Cm/sum	29 Cm/win	27 Cm/sum	71 K/spr	71 G/aut
75 Cm/sum	75 K/sum	34 G/sum	27 Cm/win	106 Cm/sum	71 K/spr
106 Cl/aut	84 E/spr	34 Cm/sum	30 K/spr	106 K/sum	71 K/sum
115 K/sum	94 E/aut	37 G/aut	46 G/sum	141 Cl/win	212 G/spr
283 E/aut	162 Cm/spr	49 K/sum	47 K/sum	212 G/spr	212 Cm/sum
3,304 Cm/aut	2,313 Cm/aut	168 G/spr	204 G/spr	212 K/aut	212 K/aut
7,636 G/aut	6,504 G/aut	677 Cm/spr	1,204 Cm/spr	318 E/sum	565 E/sum
11,029 K/aut	8,979 K/aut	2,955 E/spr	5,255 E/spr	1,060 E/spr	1,060 E/sp
Legend: G-giraffes, E-elephants, Cm-camels, Cl-colobinae, K-kangaroos, spr-spring, sum-summer, aut-autumn, win-winter					

This study also involved identification of staphylococci species occurring in rooms intended for animals in the zoo (Table 7). *Staphylococcus succinus* was the most frequently found bacteria. It constituted almost

1/3 of all isolated staphylococci strains. *S. sciuri* was the second most frequently occurring bacteria (19.1%) and *S. vitulinus* the third *(*12.6%). *S. haemolyticus* was the least frequently detected among isolated staphylococci species.

Table 7
The frequency of occurrence of staphylococci species in animal shelters in the zoo in Chorzów

Group of animals	Species of bacteria	Frequency of occurrence [%]
giraffes	*Staphylococcus succinus*	34.8
	Staphylococcus sciuri	23.2
	Staphylococcus xylosus	17.4
	Staphylococcus vitulinus	16.2
	Staphylococcus equorum	7
	Staphylococcus chromogenes	1.4
elephants	*Staphylococcus succinus*	34
	Staphylococcus xylosus	18.7
	Staphylococcus sciuri	17
	Staphylococcus vitulinus	15.3
	Staphylococcus equorum	9.9
	Staphylococcus chromogenes	1.7
	Staphylococcus cohnii	1.7
	Staphylococcus epidermidis	1.7
camels	*Staphylococcus succinus*	29.9
	Staphylococcus xylosus	20.4
	Staphylococcus sciuri	17.7
	Staphylococcus vitulinus	12.2
	Staphylococcus equorum	5.4
	Staphylococcus gallinarum	4.2
	Staphylococcus capitis	4.1
	Staphylococcus lentus	3.3
	Staphylococcus chromogenes	2.7
colobinae	*Staphylococcus succinus*	57
	Staphylococcus vitulinus	17.1
	Staphylococcus epidermidis	14.3
	Staphylococcus gallinarum	5.7

Group of animals	Species of bacteria	Frequency of occurrence [%]
	Staphylococcus capitis	3.1
	Staphylococcus haemolyticus	2.9
kangaroos	*Staphylococcus succinus*	40.3
	Staphylococcus vitulinus	15
	Staphylococcus xylosus	12.7
	Staphylococcus sciuri	11.5
	Staphylococcus chromogenes	6.8
	Staphylococcus capitis	4.6
	Staphylococcus gallinarum	4.6
	Staphylococcus equorum	3.5
	Staphylococcus epidermidis	1.2
control	*Staphylococcus sciuri*	45.4
	Staphylococcus cohnii	27.2
	Staphylococcus epidermidis	13.6
	Staphylococcus lentus	9.1
	Staphylococcus capitis	4.7

The frequency of occurrence of individual staphylococci species for different animal rooms is presented in Table 8. *S. succinus* occurred most frequently in all animal facilities and its share ranged from 29.9% in rooms for camels to 57% in facilities for colobinae. The smallest share in the case of individual animal facilities was recorded for: *S. chromogenes* (giraffes, camels), *S. epidermidis* (elephants, kangaroos) and *S. haemolyticus* (colobinae). As regards the number of identified staphylococci species in animal rooms, it ranged from 6 (giraffes and colobinae) to 9 (camels, colobinae). Five staphylococci species were detected within the control area (outside animal shelters).

Table 8
The total percentage of staphylococci species in animal shelters in the zoo in Chorzów

Species of bacteria	Frequency of occurrence [%]
Staphylococcus succinus	32.7
Staphylococcus sciuri	19.1
Staphylococcus vitulinus	12.6
Staphylococcus xylosus	11.5
Staphylococcus epidermidis	5.1
Staphylococcus cohnii	4.8
Staphylococcus equorum	4.3
Staphylococcus capitis	2.7
Staphylococcus gallinarum	2.4
Staphylococcus chromogenes	2.1
Staphylococcus lentus	2.1
Staphylococcus haemolyticus	0.5

Isolated staphylococci – 100 strains belonging to 12 species – were subjected to antibiotic resistance testing. Ten antibiotics were used in the study. Antibiotic names and doses used to immerse paper disks are shown in Table 9. It has been established that studied bacterial strains showed the highest resistance to fusidic acid and rifampicin, and overall susceptibility to 5 antibiotics: ciprofloxacin, chloramphenicol, gentamycin tigecycline and erythromycin. The highest average resistance to tested antibiotics (Table 10) was recorded for strains belonging to *S. epidermidis* (22.5%), similar (approx. 20%) for *S. sciuri* and *S. lentus*, while the lowest for *S. xylosus* (4%).

Table 9
The total resistance of staphylococci strains isolated from the air in animal shelters in the zoo in Chorzów

Antibiotic name	Dose [mg]	Code	Share of strains resistant [%]
fusidic acid	10	FA10	42
rifampicin	5	RA5	36
tetracycline	30	TE30	27
clindamycine	2	DA2	22
cefoxitin	30	FOX30	1
ciprofloxacin	5	CIP5	1
chloramphenicol	30	C30	0
gentamycin	10	CN10	0
tigecycline	15	TGC15	0
eryhromycin	15	E15	0

Table 10
Antibiotics resistance of staphylococci isolated from the air in animal shelters in the zoo in Chorzów

Species of staphylococci	Number of strains	Antibiotic code (see Table 9)						Average resistance of the species [%]
		DA2	FA10	FOX30	RA5	TE30	C30, CIP5, CN10, E15, TGC15	
Staphylococcus capitis	1	0	0	0	0	100	0	10
Staphylococcus cohnii	3	0	33	0	0	33	0	6.6
Staphylococcus chromogenes	2	0	50	0	50	50	0	15
Staphylococcus epidermidis	4	25	100	25	50	25	0	22.5
Staphylococcus equorum	6	0	0	0	0	17	0	1.7
Staphylococcus gallinarum	6	50	100	0	0	0	0	15
Staphylococcus haemolylitus	1	0	100	0	0	0	0	10
Staphylococcus lentus	3	0	100	0	33	67	0	20
Staphylococcus sciuri	16	28	62	0	56	61	0	20.7
Staphylococcus succinus	30	29	29	0	42	32	0	13.2
Staphylococcus vitulinus	14	0	0	0	58	14	0	7.2
Staphylococcus xylosus	14	0	40	0	0	0	0	4

Discussion

Available literature fails to provide reports on the presence and concentration of staphylococci and fecal bacteria in very specific environments, such as animal facilities in the zoos. Another difficulty is concerned with the lack of normative values for staphylococci. When we compare the results against values established for mesophilic bacteria recommend by ZECB – $1x10^5$CFU/m^3 (TC) and $5x10^4$CFU/m^3 (RF) – the concentrations for TC were not exceeded, while the concentration for RF was exceeded by 24% only once. As regards fecal bacteria, existing normative values were not exceeded. Recorded concentrations, as compared with normative values, amounted to approx. 5% for TC and 10% for RF.

The staphylococci concentrations recorded in the course of the study conducted in the zoo in Chorzów ranged from 0 to $6.9x10^4$CFU/m^3. The lowest concentrations were noted in winter, while the highest in spring. Staphylococci count obtained by Masclaux et al. (2013) carrying out research on a pig farm in Switzerland fell within the following range: $1.9x10^3$–$4x10^8$CFU/m^3. However, in summer the concentration ranged from $1.9x10^3$ to $4.7x10^7$CFU/m^3, while in winter it increased by one order of magnitude ($5.9x10^4$–$4x10^8$CFU/m^3). In our study the concentrations for staphylococci were higher by at least three orders of magnitude, as compared with the results obtained by Masclaux et al.

Popescu et al. (2011) conducted research in stables in Romania (Brasov County) to evaluate the changes in staphylococci concentrations depending on the time of the day. The values measured in the evening were slightly higher: $5.91x\ 10^4$CFU/m^3 vs. $5.34x10^4$CFU/m^3 in the morning – the difference in mean values amounted to approx. 10%. The concentrations for fecal bacteria were also higher in the evening – in that case the difference for mean concentrations reached 44%. The concentration of fecal bacteria in the morning amounted to $1.54x10^4$ CFU/m^3, while in the evening to $2.75x10^4$CFU/m^3. In our study the daily mean concentration for fecal bacteria was significantly lower. It amounted to $2.4x10^1$CFU/m^3, what means that it was three orders of magnitude lower. It indicates that zoo keepers maintain proper cleanness standards in rooms intended for animals, what is surely associated with much lower stock as compared with large-scale farms.

The application of an Andersen cascade impactor in the experiments enabled us to estimate the potential level of bioaerosol penetration into the human respiratory system based on the bacteria aerodynamic size (Górny et al. 2016). Madsen et al. (2018) used an Andersen cascade impactor to determine which bioaerosol fraction contains staphylococci based on the aerodynamic size of particles/aggregates formed by these bacteria. According to Madsen 70% of bioaerosol contains staphylococci aggregates sized 7–11µm. It indicates that they are deposited in the upper respiratory tract – 22% of bioaerosol in the primary and secondary bronchi and 8% in the terminal bronchi and alveoli. Our study delivered contradictory results – fraction sized 7–11µm constituted only 11.2%, and the largest number of staphylococci was detected in fraction sized 2.1–1.1µm (27%). It means that this part of bioaerosol reaches terminal bronchioles. A significant part of bioaerosol (77.6%) was qualified as FR.

Clauβ (2015) in a review article addressed the topic of the distribution of bioaerosol fractions for fecal bacteria and staphylococci. The data for fecal bacteria presented in that article are consistent with the results generated in this study. However, the results obtained for staphylococci were varying. In the above-cited article the fraction above 4.7µm constituted more than 50%, while in our study as little as 22.4%.

As seen in various research, bioaerosol particles with the diameter lower than 2.5µm pose the most serious threat to the exposed people. This fraction is capable of penetrating into the lower pulmonary tract (to pulmonary alveoli), what often leads to health problems, such as low birth weight, heart and lung diseases, cancer and premature death [Morakinyo et al. 2016]. As claimed by Clauβ (2015), in the case of rooms intended for animals it may depend on many factors like room area, number of animals, animal size and weight, the presence or absence of litter.

The research conducted previously in the same object (Grzyb and Pawlak 2021) revealed that I/O ratio for bacteria reached maximum value of 344 for TC and 785 for RF. In the case of indicator bacteria, ST-POS in particular, I/O ratio reached the value of 11,029 for TC and 8,979 for RF. It means that the difference between staphylococci concentration indoors and in the outside air was high. Staphylococci intoxication inside facilities for zoo animals can be easily explained, as staphylococci constitute natural fur, skin and mucous membrane microflora. This assumption can be supported by data delivered by Chmiel et al. (2019) who reported instances of bacterial intoxication in churches and museums in Kraków. In that case worshipers and visitors were the source of contamination.

As results from the research carried out by Schulz et al. (2004), staphylococci can be used as a reliable and useful indicator for determining safe distance between rooms intended for animals and residential buildings as well as the spread of bioaerosol in the air surrounding animal shelters.

The most burning issue relating to the antibiotic resistance is concerned with viewing staphylococci as a serious threat to humans and animals. The most frequent reports regarding the risks associated with staphylococci concern *Staphylococcus aureus*, that can show resistance to methicillin (methicillin-resistant SA – MRSA) or vankomycine (vankomycine-resistant SA – VRSA). The information that majority of its strains are saprophytic and occur on human skin, animal fur as well as mucous membranes of the two is less popular. *S. aureus* can be found in 20–40% of human population. It colonizes nostrils and does not cause any health problems. However, *S. aureus* can cause opportunistic infections of the skin and soft tissues as well as inflammation of the entire body (sepsis). The incidence rate for staphylococci in the air samples depends on the sampling spot. Messi et al. (2015) carried out experiments in public places in Italy and reported that staphylococci constituted 17% of all isolated bacteria, while *S. aureus* strains approx. 1.7%. Our study covered 200 staphylococci strains but *S. aureus* was not detected.

The studies undertaken by Ferguson et al. (2016) confirmed that disinfecting rooms for animals results in killing staphylococci, including MRSA. Thus, when the concentration of staphylococci is high, it is advised to schedule periodic disinfection of animal rooms as an efficient measure reducing the number of undesired germs. It must be remembered that indicator bacteria analyzed in this study are potentially pathogenic. They migrate by means of direct transmission through dirty hands or orally, and as bioaerosol, what poses threat to animal keepers and – to a lesser degree – to zoo visitors (Bos et al. 2016).

In this study *Staphylococcus succinus* was the most numerous staphylococci species (approx. 33%), followed by *S. sciuri* (19.1%) and *S. vitulinus* (12.6%). Schulz et al. (2004) detected *Staphylococcus saprophyticus, S. cohnii, S. arlettae* and *S. lentus* on a broiler farm. Similar results were obtained by Popescu et al. (2011), who conducted research in stables. They identified two staphylococci species with more than 20% share in the entire number of isolated strains: *S. sciuri* and *S. xylosus*. Comparable species composition with the highest *S. sciuri* and *S. lentus* share was shown in Italian stables by DeMartino et al. (2010). Contradictory results were delivered by Popescu et al. (2011), who established that *Staphylococcus epidermidis* is the most numerous bacteria species with the share amounting to approx. 25%. In our study it constituted 5% of all identified bacterial species. Haas et al. (2020) investigating pig barns also received

conflicting results. The greatest share among all isolated staphylococci species was recorded for *S. pasteuri* (47.9%), *S. cohnii* subsp. *cohnii* (24.5%), while the lowest for *S. chromogenes* (1.06%).

Antibiotic resistance represents key staphylococci characteristics. It results from activating few mechanisms in the staphylococci cells: enzymatic inactivation of antibiotic, active removal of antibiotic from the cell or changing drug affinity to target site in the bacterial cell (Lenart-Boroń et al. 2016). The results of resistance testing for selected antibiotics presented here are in line with the data released by Wolny-Koładka (2018).

Conclusions

In this study the highest concentration of mannitol-positive staphylococci was recorded in rooms for camels, while the greatest concentration of mannitol-negative staphylococci in housing facilities for elephants. The lowest concentrations for 3 analyzed bacterial groups were detected in rooms for colobinae. Acceptable microbial concentrations for fecal bacteria were not exceeded.

In investigated animal rooms the greatest bacteria contribution was recorded for bioaerosol fraction sized 1.1–3.3μm. At least 2/3 of bacteria in bioaerosol constituted respirable fraction, that migrates into the lower respiratory tract of the animals. The concentration of microbes inside animal rooms was higher as compared with the external environment, what means that rooms for animals were intoxicated. Bacterial intoxication inside animal facilities was particularly strong in spring and autumn, what is related to shedding fur by animals.

The analysis of isolated staphylococci revealed that *Staphylococcus succinus, S. sciuri* and *S. vitulinus* are the most frequently occurring bacteria. Antibiotic susceptibility testing revealed that studied bacteria strains displayed the highest resistance to fusidic acid and rifampicin. The highest antibiotic resistance was noted in the case of *Staphylococcus epidermidis*, while the lowest for *S. xylosus.*

Declarations

Author contributions

JG and KP conceived, designed, and conducted the study. KP conducted the literature search. JG was involved in the analysis interpretation of data and drafted the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests

The authors declare that they have no competing interests.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Figures

Figure 1

Location of the sampling sites Legend: shelters for: 1. giraffes. 2. elephants. 3. camels. 4. colobinae. 5. kangaroos; C - control

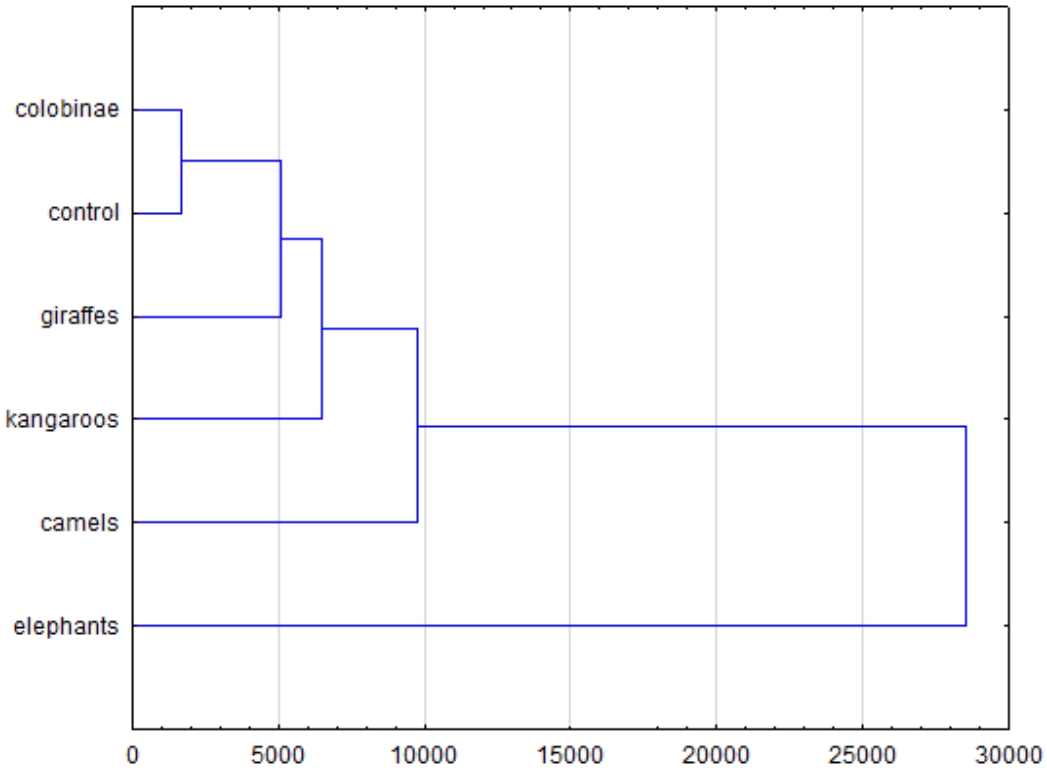

Figure 2

Cluster analysis for shares of fractions of bacterial bioaerosol in animal shelters in the zoo in Chorzów