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Research Article

Concentrations of mesophilic bacteria in a poultry farm over two fattening periods focusing on the presence of staphylococci and enterococci

Doris Haas [1,*], Tea Miskovic[1], Theresa Fritz[1], Herbert Galler[1], Juliana Habib[1], Sabine Köck[1], Michael Kropsch[2], Peter Pless[3], Martin Stonitsch[1], Eduard Zentner[2], Franz F. Reinthaler[1]

[1]D&R Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Neue Stiftingtalstraße 6, 8010 Graz, Austria
[2]AREC Raumberg-Gumpenstein, Institute for Livestock, Technology and Environment, Raumberg 38, 8952, Irdning-Donnersbachtal, Austria
[3]Animal Health Service of the Department of Veterinary Administration, Styrian Government, Friedrichgasse 9, 8010 Graz, Austria
*Corresponding author. D&R Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Neue Stiftingtalstraße 6, 8010 Graz, Austria. Tel: +43-316-385-73618; Fax: +43-316-385-79637. E-mail: doris.haas@medunigraz.at
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Abstract

The increasing animal numbers have a potential impact on the air quality of stables. The aim of this study was to assess the microbial load in the barn air from the day of entry of the chickens to the day of removal for slaughter. A total of 10 measurements in two fattening periods were conducted in a poultry farm with a capacity of 400 chickens in Styria, Austria. The samples were collected with an Air-Sampling Impinger for the investigation of mesophilic bacteria, staphylococci and enterococci. Chicken skin swab samples were collected to detect *Staphylococcus aureus*. The total colony forming units per cubic meter of mesophilic bacteria of the first measurement series of period I was 7.8×10^4 and increased to 1.4×10^8 at the end and at the fattening period II it increased from 2.5×10^5 to 4.2×10^7. In the measurement series of the fattening period I, the concentration of *Staphylococcus* spp. increased from 0 to 4.9×10^7 CFUs/m^3 and from 0 to 2.1×10^7 CFUs/m^3 in the fattening period II. *Staphylococcus aureus* could not be found on the chicken skin. An interesting finding was the increase of staphylococci while the intestinal enterococci were not detectable in the air of the barn toward the end of both fattening periods.

Keywords: airborne bacteria, *Enterococcus* spp., fattening period, intestinal enterococci, poultry farm, *Staphylococcus* spp.

Introduction

In recent years, livestock farming has undergone several changes. A trend toward more animals in larger farms with a simultaneously decreasing number of farmers has been observed in Austria since 2014 (Schlatzer and Lindenthal 2018). The increasing per capita consumption of poultry meat to 21.7 kg per person in 2018 has called for upgrades in smaller farms (Statistic Austria 2020).

Bioaerosols as well as odor, noise, dust emissions, and immissions in and around poultry houses have gained increasing importance with this changeover. Recent studies show that the necessary new technologies are being introduced to reduce emissions in order to protect not only animal health and the fattening performance of chickens, but also the health of farmers and neighboring communities (Jacobson et al. 2003, Hartung and Schulz 2007).

Airborne bacteria are the highest in the immediate vicinity of their sources (Burge 1995). Previous studies have shown that bioaerosols can travel across geographic barriers and long distances. The microbiological aerosols from animal houses can potentially be transported over distances of more than 100 m (Baykov and Stoyanov 1999, Duan et al. 2007). Another study reported that animal origin bacteria could be released into the ambient air and disperse to a distance of 10 km (Bai et al. 2022). A study performed in Netherlands found high concentrations of mesophilic bacteria including *Escherichia coli* at a distance of less than 200 m from farms and, *Staphylococcus* species were detected up to a distance of 400 m (de Rooij et al. 2019). The species *Staphylococcus aureus* can also spread over long distances (Zhong et al. 2009).

The air quality and limits of harmful air constituents at the national level are regulated by the Pollution Control Act (Immissionsschutzgesetz-Luft; IG-L, BGBl. I No. 115/1997) and by the Emissions-Amount Act (Emissionsgesetz-Luft; EG- 4 L, BGBl. I No. 75/2018). The VDI Guidelines describe methods for sampling and calculating emission factors of microorganisms as indicator parameters in livestock farming to specify the exact execution and planning of bioaerosols emission and immission measurements (VDI 4250 Part 1 2014, VDI 4255 Part 3 2016).

In large poultry farms, the air harbors a large number of microorganisms (Lonc and Plewa 2010). Bacteria originate from soil, feed, bedding, and from animals themselves. These include facultative pathogenic bacteria such as enterococci and staphylococci. The number of bacteria in the air depends on many factors such as the time of year and the location (Sanz et al. 2021). The bacterial genera *Staphylococcus* spp. and the intestinal enterococci are used to evaluate the emissions from livestock farming (Schulz et al. 2004, Clauß 2020). *Enterococcus* spp. belonging to the endogenous flora of humans and animals, are potentially pathogenic microorganisms and intrinsically resistant to various antibiotics (van den Bogaard et al. 2002). Dolka et al. (2017) reported infections caused by intestinal enterococci in chickens, turkeys, ducks,

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and ostriches. *Enterococcus cecorum* is considered as an emerging pathogen and can cause substantial losses in poultry (Jung and Rautenschlein 2014).

In addition to the mentioned air contaminations, the use of antibiotics in livestock farming and the concomitant risk of resistance development are of public interest. Agyare et al. (2018) stated that worldwide more than 60% of all produced antibiotics find their use in animal farming. For example, the methicillin-resistant *S. aureus* (MRSA) may colonize humans who are in contact with affected livestock animals. Studies have reported the occurrence of MRSA, especially livestock associated MRSA (LA-MRSA), in the air inside poultry farms and confirmed the colonization of staphylococci on the skin of chickens (Liu et al. 2012, Friese et al. 2013, Dahms et al. 2014). Recently, coagulase negative *Staphylococcus* species (e.g. *S. hyicus, S. arlettae*, or *S. felis*) have been increasingly linked to animal diseases and are major causes of hospital-acquired infections (Mascarenhas dos Santos et al. 2018, Peng et al. 2019). Members of the genus *Staphylococcus* frequently colonize the skin and upper respiratory tracts of human and animals (O'Sullivan et al. 2019), among which *S. aureus* is considered to be an important zoonotic pathogen (Fontana and Favaro 2018). The use of *S. aureus* as an indicator to study the origin and spread of airborne pathogens from chicken houses is potentially useful for enhancing public health and understanding the airborne epidemiology of this pathogen (Zhong et al. 2009).

The aim of the present study was to assess the microbial emissions by determining the concentrations of mesophilic bacteria, *Staphylococcus* spp., *Enterococcus* spp., and intestinal enterococci in the air of chicken houses over two fattening periods. In addition, the presence of *S. aureus* was examined on chicken skin as well as in the barn air with focus on MRSA.

Materials and methods

Sampling location

This study was carried out in a Styrian chicken farm, which is situated in a rural valley of a mountain region. The poultry house corresponds to a 28 m^2 closed housing system with forced ventilation. Ventilation fan takes place via a porous ceiling over the entire stable area and fresh air supply via south-facing eaves. The fan creates negative pressure in the compartment and fresh air enters the stable room through the porous ceiling. Exhaust air removed by fan in a chimney. Air exchange rate, depending on the age of the animals, between 0.8 m^3/h/animal and 6.3 m^3/h/per animal with a maximum weight of 1.5 kg (DIN NORM 18910).

The barn is equipped with two drinking pipeline tracks and semiautomatic feeders, which allow the chickens to access food *ad libitum*. Three-phase conventional fattening feed free of antibiotics. In each of the two fattening periods, 420 animals of 1-day-old Ross chicks were installed from the same breeding's supplier.

Starting temperature when stalling was 34°C and gradually decreased by 0.5°C/day over 28 days to reach 20°C. This temperature has been maintained until the chicken removed from the barn. The relative humidity was usually between 20% and 70%, the ammonia values were < 10 ppm in the barn throughout the fattening period according to DIN NORM 18910.

Wood shavings for bedding were of top quality, which was thermal sterilized and dusted nine times (https://www.happy-horse.at/). For each fattening period, the bedding was regularly changed and additionally renewed when damp spots were recognized.

Sample collection

A total of 10 bioaerosol measurements were carried out weekly over two fattening periods: period I ($n = 5$) was at winter months from November to December 2018 and period II ($n = 5$) from February to March 2019. The measurement series took place from the time of arrival until the evacuation of the chickens for slaughtering. According to the VDI standards (VDI 4252 Part 3 2008, VDI 4253 Part 3 2019), sampling was performed using a sterile Air-Sampling Impinger (ACE Glass Inc., Vineland, USA) at the central point of the poultry house, 1.5 m above the ground level with a flow rate of 12.5 l/min (cut-off diameter: 0.31 μm) for 30 min. The sampler was filled with 30 ml of sterile phosphate buffer mixed with saline solution (PBS) according to VDI 4257 Part 2 (2011) as a collection medium without replenishment during the measuring process (Fig. 1). The samples were transported in a sterile cool box with temperature of ± 4°C to the laboratory. During the time of air collection, the temperature and air humidity were recorded using Testo-Saveris sensor via radio (Testo, Wien, Austria).

Additionally, swab samples were taken from skin area of 2 cm^2 under the wings of six randomly selected chickens at fattening periods. The swab samples were collected on fattening day 32 in period I ($n = 3$) and on day 24 in period II ($n = 3$) using sterile COPAN-Transsystem® cotton swabs with liquid Amies to examine *S. aureus*. The microbiological investigation process of the air and swab samples were conducted within 6 h after the collection.

Quantitative and qualitative analysis

To process impinger fluid, various culture media were used for detection of mesophilic bacteria, staphylococci, and enterococci. Decimal dilution series up to 10^5 were conducted for the collected samples. From the appropriate dilution, 100 µl was inoculated in duplicate on Tryptic Soy Agar (TSA) with cycloheximide to determine the total concentrations of mesophilic bacteria; the selective medium Mannitol Salt Agar (MAN) and Slanetz Bartley Agar (SL) for counting and culturing the indicator parameters staphylococci and enterococci. MacConkey Agar (MC) was used to identify the Gram-negative bacteria (VWR International GmbH, Vienna, Austria). Subsequently, the agar plates were incubated at 37°C for 48 h. The visual determination of colony forming units (CFUs) was first performed for the quantitative analysis. After the 48 h incubation period, all colonies grown on the culture media were counted according to VDI guidelines (VDI 4253 Part 3 2019).

The identification of the colonies was performed based on the typical colony color and their growth characteristics according to the manufacturer's instructions. The culture media MAN and SAIDE chromID™ *S. aureus* Elite Agar (SAIDE; bioMérieux, Marcy-l'Etoile, France) were used for the selective identification of *S. aureus*. Yellow-colored colonies for *S. aureus* were counted on MAN agar media. The SAIDE agar produced by chromogenic conversion, a purple–red coloration specific to *S. aureus*.

To distinguish the intestinal enterococci from the other enterococci, the red–brown colonies grown on SL agar were transferred on Bile Aesculine Azide Agar (BAA), which was incubated at 44°C for 2 h (VWR International GmbH). After incubation, the black colonies were counted as intestinal enterococci. On MC agar, only the pink or colorless colonies were identified as Gram-negative bacteria.

For the qualitative evaluation, a maximum of five characteristic colonies per culture media were transferred onto BD™ Columbia Agar with 5% Sheep Blood (COL) to obtain pure cultures for identification of the selected bacteria and subsequently incubated at 37°C for 24 h (Becton Dickinson GmbH, Heidelberg, Germany).

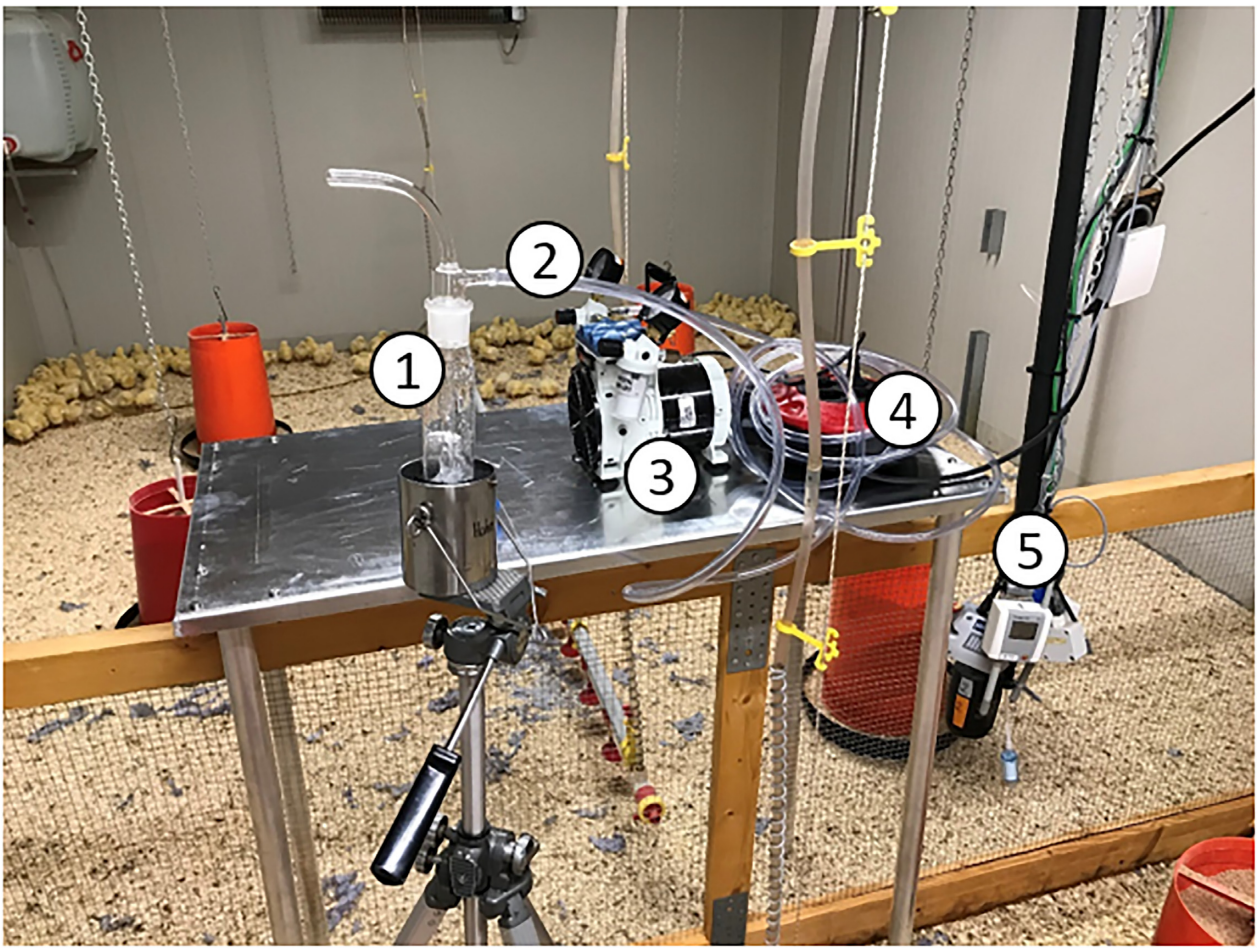

Figure 1. Sampling device used inside the chicken barn (1) ACE Glass impinger, (2) vacuum tube, (3) pump, (4) power source, and (5) sensors for gases and humidity). The impinger is connected to a pump powered by electricity. Simultaneously, gases, temperature, and humidiy are recorded during air sampling.

To isolate *S. aureus* from the smears taken from the chicken skin ($n = 6$), the swabs were streaked directly onto MAN and SAIDE. On SAIDE agar, only the strong redviolet-colored colonies were selected for the qualitative identification of *S. aureus*. Based on the morphological criteria, the colonies were subsequently subcultured on COL Agar.

Following successful pure cultivation, individually obtained colonies were qualitatively analyzed and identified by means of VITEK® MS (bioMérieux), a MALDI-TOF mass spectrometry system (Neumeister et al. 2009). All identifications displaying a single result with a confidence value of 99.9% were considered acceptable for Vitek MS. Isolates yielding single or multiple results without acceptable confidence level or no identification, were retested (Neumeister et al. 2009, Kärpänoja et al. 2014). The retesting of bacterial identification was done using 16S rRNA PCR and sequences were compared with those available in the GenBank, EMBL, and DJB databases using the gapped BLASTN 2.10.1 program through the National Center for Biotechnology Information server (Relman 1993, Altschul et al. 1997).

Antimicrobial susceptibility testing and *spa*-typing

The susceptibility to antibiotics of the identified *S. aureus* isolates was tested using the BD BBL™ SensiDisc™ Agar Diffusion Assay (BD, USA). The following antibiotics were tested: penicillin (P), cefoxitin (FOX), tetracycline (TE), clindamycin (CC), erythromycin (E), norfloxacin (NOR), mupirocin (GM), linezolid (LZD), rifampicin (RA), fusidic acid (FA), sulfamethoxazole and trimetoprim (SXD), and gentamicin (GM). The obtained diameter of the inhibition zones was compared with breakpoint table of the European Committee of Antimicrobial Susceptibility Testing; EUCAST V9.0 (2019) according to Fritsche (2016) and Mutschler et al. (2012).

For further differentiation of the identified *S. aureus* isolates, molecular genetic *spa*-typing was performed by means of polymerase chain reaction (PCR) using the forward primer *spa*-1113f and the reverse primer *spa*-1514r (Zhang et al. 2005, Ruppitsch et al. 2006). The protein A gene of *S. aureus* is used for amplification and allows a precise assignment of the desired gene sequence to already defined *spa*-types by repeating recognition sequences using Ridom StaphType™ (Vogel et al. 2005).

Results

At the beginning of each of the two fattening periods, 420 animals entered the barn of which 402 in period I and 399 chicken in period II left the barn for slaughter after 39 days of fattening.

Quantitative analysis—measurement series of fattening period I

Bioaerosol measurements were taken weekly during fattening period I. The barn temperature, air humidity, and the average weight throughout the fattening period are shown in Table 1. The temper-

Table 1. The sampling parameter and concentration of bioaerosols (CFU/m^3) of fattening periods I and II.

	Sampling parameters[a]				Bacterial concentrations (CFU/m^3)[b]		
Fattening day	Weight (g)	T (°C)	RH (%)	Total bacteria	*Staphylococci*	*Enterococci*	Intestinal enterococci
Period I							
4	91	31	33	7.8×10^4	0	4.9×10^4	3.2×10^4
10	265	29	29	4.8×10^4	5.2×10^2	3.0×10^4	1.7×10^4
17	635	26	37	6.4×10^4	5.0×10^3	2.8×10^4	9.8×10^3
25	1218	21	62	1.2×10^8	1.1×10^8	1.1×10^8	3.9×10^4
32	1858	21	65	1.4×10^8	4.9×10^7	4.4×10^7	0
Period II							
4	97	32	27	2.5×10^5	0	7.1×10^4	5.0×10^4
9	258	29	29	6.5×10^4	0	5.3×10^4	3.8×10^4
18	701	24	63	7.8×10^6	7.4×10^6	4.8×10^6	3.9×10^2
24	1136	22	57	4.9×10^7	3.3×10^7	2.0×10^6	3.9×10^2
32	1748	21	57	4.2×10^7	2.1×10^7	1.1×10^7	0

[a]The sampling parameters were recorded at five measuring days in each fattening period. Weight: the mean weight of the chicken; T: temperature, and RH: relative humidity.
[b]Bacterial concentration (CFU/m^3) values counted on different agar media: total mesophilic bacteria on TSA: tryptic soy agar; staphylococci on MAN: mannitol salt agar, enterococci on SL: Slanetz Bartley Agar, and intestinal enterococci on BAA: bile aesculine azide agar.

ature in the barn decreased from fattening days 4 to 32 from 31 to 21°C. Air humidity increased from 33% to 65% along with the weight of the chicken from 91 to 1858 g toward the end of the fattening period.

The five measurements of period I took place between days 4 and 32 and bacterial changes in the barn air were observed. On the fourth day of the fattening period I, the mean of the total concentration of mesophilic bacteria was 7.8×10^4 CFUs/m^3. Neither *Staphylococcus* spp. nor Gram-negative bacteria were found. In the first half of the fattening period *Enterococcus* spp. and intestinal enterococci could be detected. The total concentration of mesophilic bacteria increased over time, whereas the number of intestinal enterococci declined from the 25th day of fattening until they were barely detectable in the air.

On fattening day 25, an increase occurred in the concentration of mesophilic bacteria, *Enterococcus* spp., and *Staphylococcus* spp. of about four and five times, respectively. Only few Gram-negative bacteria were detected in the second half of the fattening period.

Quantitative analysis—measurements series of period II

In order to compare fattening period I with period II, the same measurement procedures were carried out. Table 1 gives an overview of the barn air temperature and humidity as well as the number and weight of chickens during fattening period II. The temperature decreased from 32 to 21°C while the humidity increased from 27% to 57% toward the end of the fattening period. On the fourth day of measurement, the chicks had a weight of 97 g. The fattened chickens reached a weight of 1748 g on the 32nd day of measurement.

The pictures in Fig. 2 show the growth of the animals and the change of the color as result of the intensity of the bedding material during fattening period II. The progress of chicken age and weight lead to minimize the place and activity for each chicken.

At the beginning of the second measurement series, the mean concentration of the total mesophilic bacteria was 2.5×10^5 CFUs/m^3 and reached a value of 4.2×10^7 CFUs/m^3 in the subsequent measurements. However, *Staphylococcus* spp. were not detected at the beginning of fattening period II but it reached 7.4×10^6 CFUs/m^3 on fattening day 18 and 3.3×10^7 CFUs/m^3 on fattening day 24.

The concentration values of *Enterococcus* spp. and intestinal enterococci were at the same range of 10^4 CFUs/m^3 in the first two measurements. Intestinal enterococci values dropped gradually toward the end of fattening period II and totally disappeared in the last measurements. Gram-negative bacteria were scattered along period II. The relevant concentrations of identified bacteria during measurement series II are summarized in Table 1.

Qualitative analysis of bioaerosols during fattening periods I and II

The results show that there was a relatively low level of microbial contamination in the barn air at the beginning of the fattening process and only the intestinal enterococci were detectable in both periods. Identical is the enormous increase of the concentrations of total mesophilic bacteria, *Enterococcus* spp. and *Staphylococcus* spp. in the third and fourth measurements in both fattening periods. The identified bacterial species were also similar within the two fattening periods. A comparison between the investigated bacterial colonies ($n = 162$) in fattening periods I and II, the identified 16 genera and 31 species as well as their frequency are listed in Table 2.

Figure 3 shows that the majority of the investigated bacterial colonies was identified as *Enterococcus hirae* (20%) followed by *Staphylococcus xylosus* (18%), and *Staphylococcus saprophyticus* (13%). Bacterial species grouped under "other" occurred only once or twice in both fattening periods and represent a proportion of 21%. These included *Acinetobacter radioresistens*, bacilli (eg. *Lactobacillus salivarius*, *Bacillus altitudinis*), *E. coli*, *Enterococcus gallinarum*, *Enterococcus casseliflavus*, *Klebsiella pneumoniae*, *Macrococcus caseolyticus*, *Microbacterium paraoxydans*, *Neisseria flava*, *Pantoea agglomerans*, *Proteus mirabilis*, *Rothia dentocariosa*, and streptococci (eg. *Streptococcus sanguinis*). *Staphylococcus aureus* was isolated and identified in the air only once in the third measurement of fattening period I.

Chicken skin swab samples

Staphylococci were found in the smear samples, which were taken from the chicken skin. A total of 10 selected colonies of *Staphylococcus* spp. were identified as *S. xylosus* and *Staphylococcus arlettae* in the fifth and last measurement of period I and *S. xylosus* and *Staphylococcus sciuri* in the fourth measurement of period II.

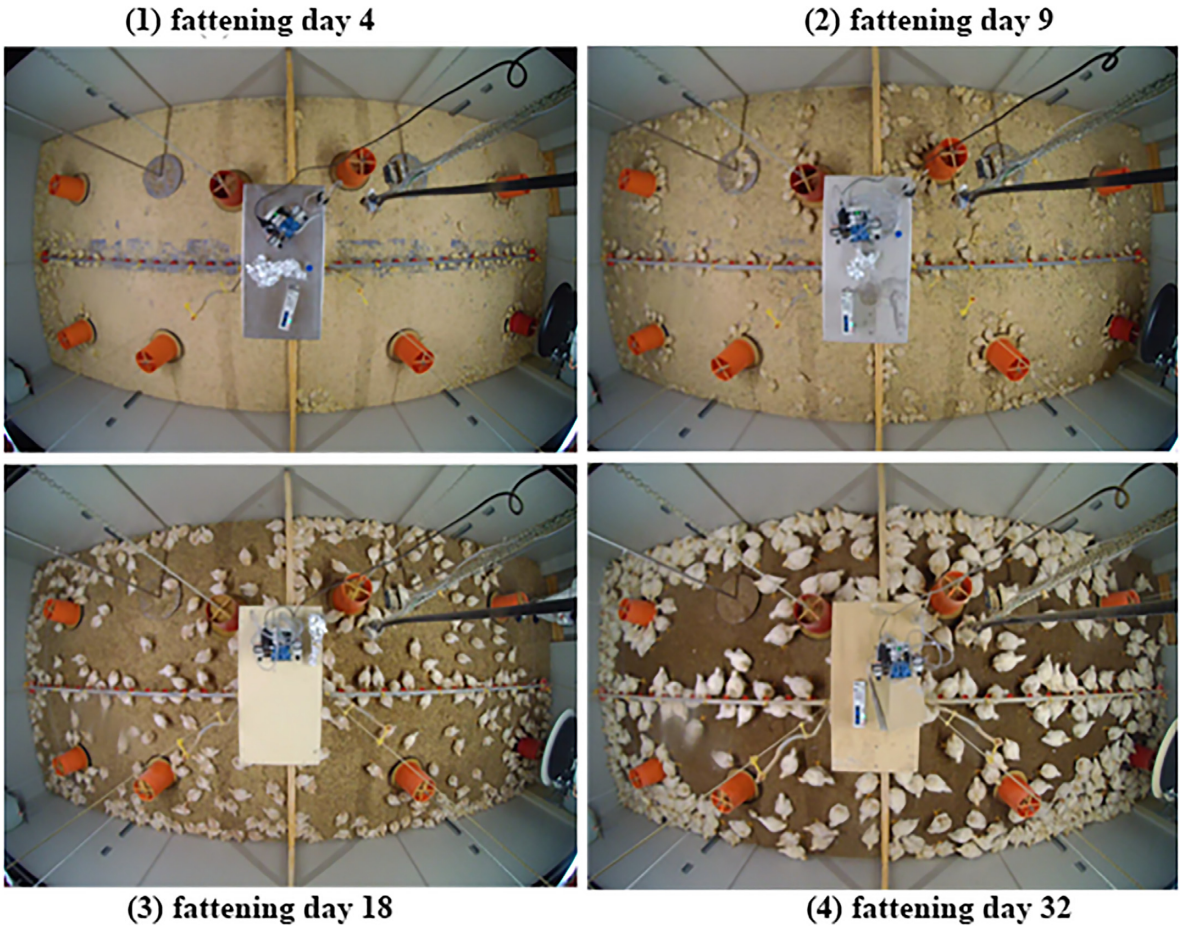

Figure 2. Chicken growth and change of bedding material during period II. (1) The fourth fattening day of the chicks (97 g), which were installed as 1 day old on fresh bright bedding material; (2) after nine fattening days, the bedding material was darkened by the feces. (3) On the 18th day of fattening, the animals already have a weight of 701 g. (4) From days 18 to 32, the weight of the chickens doubled (1748 g) and the bedding has become completely dark due to the feces.

Staphylococcus aureus could not be detected on the skin of the six randomly selected animals.

Antibiotic resistance testing

The *S. aureus* isolate detected in the air of the investigated poultry farm was sensitive to all antibiotics tested except penicillin. This isolate was *spa*-type t012 and thus an MSSA strain.

Discussion

The results of the present study provide an estimation of the bioaerosol load in the air of a poultry farm over two fattening periods and show almost the same course regarding the concentrations of total mesophilic bacteria, *Staphylococcus* spp. and *Enterococcus* spp. The similar findings could be explained by the housing conditions, which hardly differed during the two fattening periods. In spite of a slight deviation in the sampling days, the two fattening periods could be compared and information about the culturable bioaerosols were obtained.

In the current study, the highest values of the total concentration of mesophilic bacteria measured by impingement were between 4.9×10^7 and 1.4×10^8 CFUs/m^3. Such high concentrations of mesophilic bacteria were also determined in other studies (Terzieva et al. 1996, Chi and Li 2006, Schulz et al. 2011). However, the concentration of mesophilic bacteria and *Staphylococcus* spp. may not depend on the barn capacity. The present study investigated a poultry farm where 420 chickens were housed during each fattening period. This study recorded higher mesophilic bacterial concentrations than that of Vučemilo et al. (2007). They recorded a concentration of 1.8×10^5 CFUs/m^3 in a barn with approximately 5300 chicken using the impactor MAS-100 NT. Schulz et al. (2011), using an AGI-30 impinger determined concentrations of *Staphylococcus* spp. of 1.0×10^6 and 1.0×10^7 CFUs/m^3 at a farm occupied by 40 000 chickens which are similar to the present study. A clear statement whether chicken farms with a higher number of animals have a higher bacterial load cannot be made due to the small sample size of the present study. However, poultry houses were presumed to be heavily contaminated by staphylococci between 0 and 1.4×10^4 CFUs/m^3 (Plewa and Lonc 2011).

In a short fattening time, chickens increased enormously in size, weight, and surface, which lead to a spread of skin bacteria into the barn air. This rapid growth can explain the enormous increase of total concentrations of mesophilic bacteria in fattening periods I and II. Lawniczek-Walczyk et al. (2013) found that bacterial aerosol concentrations in examined poultry houses varied greatly at different stages of the production cycle. A clear association between the increase of culturable bacteria due to concomitant change in skin flora composition the rising age of chickens was reported by Lippmann et al. (2016). Baykov and Stoyanov (1999) showed that the emission of microorganisms increased proportional to the age of the chickens. Oppliger et al. (2008) could

Table 2. Frequency of the identified bacteria genera and species in fattening periods I and II. Microorganisms identified from air in period I ($n = 106$) in relation to period II ($n = 56$). No *S. aureus* could be found in the air of the barn.

Genera and species of bacteria ($n = 162$)	Period I	Period II
Staphylococcus		
S. arlettae	2	5
S. aureus	1	-
S.capitis	-	1
S. lugdunensis	1	-
S. saprophyticus	9	13
S. sciuri	-	3
S. warneri	-	1
S. xylosus	20	3
Enterococcus		
E. casseliflavus	5	-
E. faecalis	6	-
E. faecium	12	1
E. gallinarum	-	1
E. hirae	21	13
Aerococcus		
A. viridans	2	5
(Lacto-)Bacillus		
B. altitudinis/pumilus	3	-
B. megaterium	3	-
Lactobacillus salivarius	2	-
Streptococcus		
S. sanguinis	2	-
S. vestibularis/S. salivarius ssp. thermophilus	-	
Micro- und Marcococcus		
Macrococcus caseolyticus	2	-
Micrococcus luteus	3	-
Gram-negative bacteria		
Acinetobacter radioresistens	4	-
E. coli	3	3
Klebsiella pneumoniae	-	2
Neisseria flava/perflava/subflava	-	1
Proteus mirabilis	3	-
Stenotrophomonas maltophilia	-	1
Other species		
Microbacterium paraoxydans	-	1
Pantoea agglomerans	-	1
Rothia dentocariosa	-	1
Total	**106**	**56**

also show a significant increase of bioaerosol levels during the fattening period of chickens due to the resulting emissions of skin debris, broken feather barbules, aerosolized feed, and poultry excreta. The settled bioaerosols on the barn floor spread into stable air due to chicken activity and could be collected with the impingement or impaction method.

In the present study, increasing of *Staphylococcus* spp. concentrations may also be due to increasing biomass, body surface area, chicken activity through pecking, and scratching of the bedding. *Staphylococcus* spp. were initially not detectable but increased abruptly as weight doubled. Brodka et al. (2012) also obtained the lowest concentrations of mesophilic bacteria in animal housings where 1-day-old chickens has been kept. Martin et al. (2012) and Vučemilo et al. (2007) were able to confirm an increase in bioaerosol with increasing age and weight of the animals.

A further aspect was the bedding, which was only disposed of once after each fattening period before cleaning the barn. The organic material of the animals such as excrement products, dander, and feather components on the bedding explain the detection of nonairborne microorganisms such as Gram-negative *E. coli* (Gärtner et al. 2017, VDI 4253 Part 3 2019). A study by Whyte et al. (2001) shows that levels of airborne Enterobacteriaceae especially *E. coli* were significantly higher in samples recovered from the defeathering and evisceration stages of poultry slaughtering. Other studies describe a low incidence of enterobacteria as they die off rapidly in the air (Zhao et al. 2016). Duan et al. (2007) calculated median concentrations of airborne *E. coli* between 9 and 63 CFUs/m^3 measured by an Andersen sampler in the air of five different chicken houses. The present study identified a small number of 4 CFUs of *E. coli* in the second week of measurement in period I. In addition to the increase of the total mesophilic bacterial concentrations and *Staphylococcus* spp., an increase of the *Enterococcus* spp. also occurred. In contrast, a decrease of intestinal enterococci concentrations was observed in both investigated fattening periods. At the beginning of the period I measurements, *Enterococcus* spp. and intestinal enterococci were detected and they remained constant during the fattening days. At the end of this period, the enterococci continued to rise and intestinal enterococci dropped to 0 CFUs/m^3. A similar course was documented in period II measurements, whereby a slight reduction in CFUs/m^3 was observed from the second week of measurement. Although intestinal enterococci such as *E. faecalis* are resistant to external

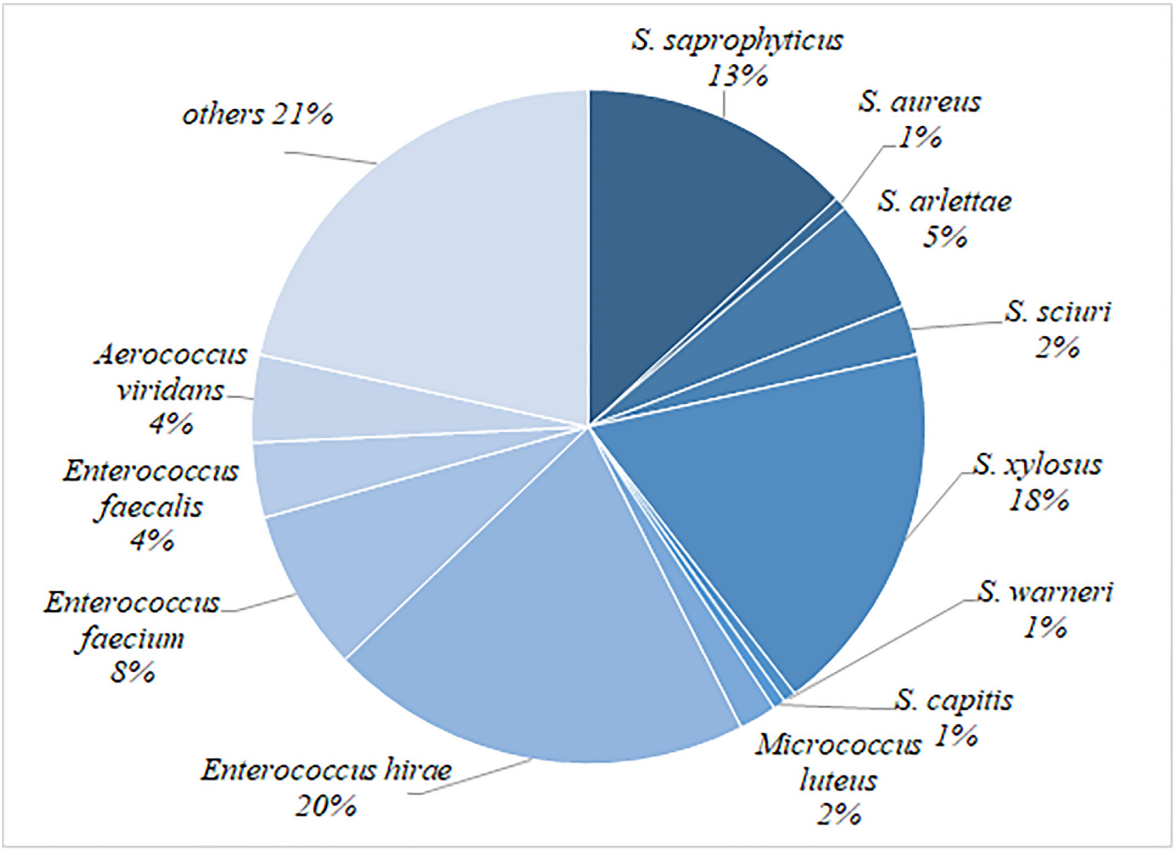

Figure 3. Spectrum of detected bacteria in fattening periods I and II ($n = 162$). The majority of examined bacterial colonies were of *E. hirae* and staphylococci. The most common representatives being *S. xylosus* with 18% and *S. saprophyticus* with 13%. Those summarized under "other" bacterial species, some of which only identified once or twice in both measurement periods a percentage of 21%.

influences such as increasing humidity from 37% to 62% and decreasing temperature from 26 to 21°C in the first fattening period I, a reduction in concentration was observed. Byappanahalli et al. (2012) have shown that enterococci are widely distributed in a variety of environmental habitats, in which temperatures are variable. In the present study, it is possible that the intestinal enterococci adhere to the bedding material during the fattening period and, therefore, do not get into the barn air, whereas other enterococci remain constantly in the air. A study of broiler chickens by Dolka et al. (2017) reported a high prevalence of *Enterococcus* species such as *E. faecalis*, *E. cecorum*, *E. faecium*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*, and *E. durans*, whereby the age of the animals had an influence on the species diversity of the staphylococci. Brodka et al. (2012) found *E. faecalis* and *E. faecium* among the identified bacteria and the concentration of *Enterococcus* spp. ranged from 1.5×10^4 to 1.1×10^7 CFUs/m^3. In the study of Gärtner et al. (2011), the concentrations of enterococci between 10^4 and 10^5 CFUs/m^3 showed no recognizable dependency during the poultry fattening period (Gärtner et al. 2011).

The results of the present study concerning the detected bacterial spectrum were similar to the species found by other researchers. The most common species verified among *Staphylococcus* spp. were *S. xylosus* and *S. saprophyticus*. Schulz et al. (2011) reported that the most frequently identified *Staphylococcus* species were *S. cohnii*, *S. saprophyticus*, *S. arlettae*, and *S. xylosus*, which are similar to the present study findings. *Staphylococcus saprophyticus* can cause human urinary tract infections (Hahn et al. 2005). *Staphylococcus xylosus* is a component of the human and animal skin flora and may rarely lead to urinary tract infections, endocarditis and corneal infections (Vela et al. 2012). In the air of poultry slaughterhouses where 6000 animals per hour were hung onto moving rails, Haas et al. (2005) detected coagulase-negative staphylococci as well as *S. xylosus*, *S. warneri*, *S. sciuri*, *S. lentus*, and *S. equorum*. These are predominantly bacteria of the risk group 1. *Aerococcus viridans*, *E. hirae*, *E. faecium*, *E. faecalis*, and *S. saprophyticus* belong to risk group 2 and are, thus potentially endangering bacteria for employees working with biological agents (TRBA 466 2015). These bacteria were identified by Martin et al. (2012) as well as in the present study. Vučemilo et al. (2007) defined *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., and *Bacillus* spp. as well as *E. coli* and other enterobacteria among the most frequently encountered species. Similar to the present study, Gram-positive cocci from *Staphylococcus* spp. and *Enterococcus* spp. predominated the identified bacteria in the study of Lawniczek-Walczyk et al. (2013).

MRSA strains were not detected either in the air or on the skin of chicken investigated in the present study. In this regard, other authors also did not identify MRSA in the air of livestock houses (van Cleef et al. 2011, Wendlandt et al. 2013, El-Adawy et al. 2016, Pauly et al. 2019).

Conclusion

The bioaerosol investigation over two fattening periods in an Austrian poultry farm show almost identical course of bacteria. It was found that the concentrations of mesophilic bacteria, *Staphylococcus* spp. and *Enterococcus* spp. in the barn air increased toward the end of both fattening periods, whereas the concentrations of in-

testinal enterococci disappeared. The sudden decrease in intestinal enterococci concentrations within the scope of the measurement series could not be clarified, therefore, further investigation is required. The concentrations of microbial loads depend on the age and weight of chickens but not on the number of animals in the barn.

Institutional review board statement

This study was conducted in a stable, which is normally used for research purposes of microbiological examinations and animal welfare; therefore, there is no need for ethical permission.

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Supplementary data

Supplementary data are available at *FEMSMC* online.

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References

Agyare C, Boamah VE, Zumbi CN. *et al.* Antibiotic use in poultry production and its effects on bacterial resistance. Kumar Y. Intech Open. In: *Antimicrobial Resistance-A Global Threat*. London, UK. 2018;DOI: 10.5772/intechopen.79371.

Altschul SF. TL Madden AA Schaeffer *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.

Bai H, He LY, Wu DL. *et al.* Spread of airborne antibiotic resistance from animal farms to the environment: dispersal pattern and exposure risk. *Environ Int* 2022;**158**:1–12.

Baykov B, Stoyanov M. Microbial air pollution caused by intensive broiler chicken breeding. *FEMS Microbiol Ecol* 1999;**29**:389–92.

Bródka K, Kozajda A, Buczyńska A. *et al.* The variability of bacterial aerosol in poultry houses depending on selected factors. *Int J Occup Med Environ Health* 2012;**25**:281–93.

Burge HA. *Bioaerosols*. Boca Raton: CRC Press Inc, 1995.

Byappanahalli MN, Nevers MB, Korajkic A. *et al.* Enterococci in the environment. *Microbiol Mol Biol Rev* 2012;**76**:685–706.

Chi MC, Li CS. Analysis of bioaerosols from chicken houses by culture and non-culture method. *Aerosol Sci Technol* 2006;**40**:1071–9.

Clauß M. Emission of bioaerosols from livestock facilities-methods and results from available bioaerosol investigations in and around agricultural livestock farming. Thünen Working Paper 138a. Braunschweig: Johann Heinrich von Thünen-Institut, 2020;1–124.

Dahms C, Hübner NO, Cuny CH. *et al.* Occurrence of methicillin-resistant *Staphylococcus aureus* in farm workers and the livestock environment in Mecklenburg-Western Pomerania, Germany. *Acta Vet Scand* 2014;**56**:2–8.

De Rooij MMT, Hoek G, Schmitt H. *et al.* Insights into livestock-related microbial concentrations in air at residential leval in a livestock dense area. *Environ Sci Technol* 2019;**53**:7746–58.

Dolka B, Gołebiewska-Kosakowska M, Krajewski K. *et al.* Occurence of *Enterococcus* spp. in poultry in Poland based on 2014-2015 data. *Med Weter* 2017;**73**:220–4.

Duan H, Chai T, Zhang X. *et al.* Microbiological aerosols in poultry houses and its ambience. In: *Proceedings: XIII International Congress in Animal Hygiene, ISAH 17-21 June*, Tartu, 2007.

El-Adawy H, Ahmed M, Hotzel H. *et al.* Characterization of methicillin-resistant *Staphylococcus aureus* isolated from healthy turkeys and broilers using DNA microarrays. *Front Microbiol* 2016;**2019**:1–11.

Emissionsgesetz-Luft. 2018. EG-L: BGBl. I No. 75/2018.

EUCAST. 2019. http://www.eucast.org/clinical_breakpoints/. (March 2019, date last accessed).

Fontana C, Favaro M. Coagulase-positive and coagulase-negative staphylococci in human disease. Chapter 3. In: Savini V (ed.), *Pet-to-Man Travelling Staphylococci. A World in Progress*. London: Academic Press 2018 Elsevier Inc., 2018, 25–42.

Friese A, Schulz J, Zimmermann K. *et al.* Occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* in turkey and broiler barns and contamination of air and soil surfaces in their vicinity. *Appl Environ Microbiol* 2013;**79**:2759–66.

Fritsche O. *Mikrobiologie*. Berlin, Heidelberg: Springer-Verlag, 2016.

Gärtner A, Gessner A, Martin E. *et al.* Emissionsmessungen von Mikroorganismen aus hähnchenmastanlagen – aktuelle messergebnisse und vergleichende untersuchungen von drei verschiedenen ställen. *Gefahrstoffe – Reinhaltung der Luft* 2011;**71**:362–6.

Gärtner A, Gessner A, Geueke KJ. *et al.* Emissionen aus Hähnchenmastanlagen. *Gefahrstoffe – Reinhaltung der Luft* 2017;**77**:249–56.

Haas D, Posch J, Schmidt S. *et al.* A case study of airborne culturable microorganisms in a poultry slaughterhouse in Styria, Austria. *Aerobiologia* 2005;**21**:193–201.

Hahn H, Miksits K, Gatermann S. Medizinische mikrobiologie und infektiologie. In: Hahn H, Falke D, Kaufmann SHE, Ulmann U. (eds.), *Staphylokokken*. Berlin, Heidelberg: Springer-Lehrbuch, 2005.

Hartung J, Schulz J. *Risks Caused by Bio-Aerosols in Poultry Houses*. Hannover: Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour of the University of Veterinary Medicine Hannover, 2007;1–11.

Immissionsschutzgesetz-Luft, IG-L: BGBl. I No. 115. 1997.

Jacobson LD, Bicudo JR, Schmidt DR. *et al.* Air emissions from animal production buildings. In: *Proceedings of the XI International Congress in Animal Hygiene, ISAH 23-27 February*, Mexico City, 2003.

Jung A, Rautenschlein S. Comprehensive report of an *Enterococcus cecorum* infection in a broiler flock in northern Germany. *BMC Vet Res* 2014;**10**:1–8.

Kärpänoja P, Harju I, Rantakokko-Jalava K. *et al.* Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of viridans group streptococci. *Eur J Clin Microbiol Infect Dis* 2014;**33**:779–88.

Lawniczek-Walczyk A, Gorny RL, Golofit-Szymczak M. *et al.* Occupational exposure to airborne microorganisms, endotoxins and β-glucans in poultry houses at different stages of the production cycle. *Ann Agric Environ Med* 2013;**20**:259–68.

Lippmann J, Mietke-Hofmann H, Deichmann J. *et al.* Bioaerosole aus anlagen der geflügelhaltung. *Schriftenreihe des LfULG* 2016;**13**:1–105.

Liu D, Chai T, Xia X. *et al.* Formation and transmission of *Staphylococcus aureus* (including MRSA) aerosols carrying antibiotic-

resistant genes in a poultry farming environment. *Sci Total Environ* 2012;**426**:139–45.

Lonc E, Plewa K. Microbiological air contamination in poultry houses. *Pol J Environ Studies* 2010;**19**:15–19.

Martin E, Gessner A, Gärtner A. *et al.* Molekularbiologische charakterisierung luftgetragener bakterien in emissionsproben aus hähnchenmastanlagen. *Gefahrstoffe – Reinhaltung der Luft* 2012;**72**:99–103.

Mascarenhas Dos Santos AC, Jie R, Antunes Godoy H. *et al.* Complete genome sequences of the potential zoonotic pathogens *Staphylococcus felis* and *Staphylococcus kloosii*. *Genome Announc* 2018;**6**:1–2.

Mutschler E, Geisslinger G, Kroemer HK. *et al.* Arzneimittelwirkungen. In: *Lehrbuch der Pharmakologie und Toxikologie*. Stuttgart: Wissenschaftliche Verlagsgesellschaft, 2012.

Neumeister B, Geiss H, Braun R. *et al. Mikrobiologische Diagnostik: Bakteriologie - Mykologie - Virologie - Parasitologie*. 2nd edn. Stuttgart: Georg Thieme Verlag, 2009.

O'Sullivan JN, Rea MC, O'Connor PM. *et al.* Human skin microbiota is a rich source of bacteriocin-producing staphylococci that kill human pathogens. *FEMS Microbiol Ecol* 2019;**95**:1–10.

Oppliger A, Charrière N, Droz PO. *et al.* Exposure to bioaerosols in poultry houses at different stages of fattening; use of real-time PCR for airborne bacterial quantification. *Ann Occup Hyg* 2008;**52**:405–12.

Pauly N, Wichmann-Schauer H, Ballhausen B. *et al.* Detection and quantification of methicillin-resistant *Staphylococcus aureus* in fresh broiler meat at retail in Germany. *Int J Food Microbiol* 2019;**292**:8–12.

Peng P, Baldry M, Gless BH. *et al.* Effect of co-inhabiting coagulase negative staphylococci on *S. aureus* agar quorum sensing, host factor binding, and biofilm formation. *Front Microbiol* 2019;**10**:1–14.

Plewa K, Lonc E. Analysis of airborne contamination with bacteria and moulds in poultry farming: a case study. *Pol J Environ Studies* 2011;**20**:725–31.

Relman DA. The identification of uncultured microbial pathogens. *J Infect Dis* 1993;**168**:1–8.

Ruppitsch W, Indra A, Stöger A. *et al.* Classifying *spa*-types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2006;**44**:2442–8.

Sanz S, Olarte C, Hidalgo-Sanz R. *et al.* Airborne dissemination of bacteria (enterococci, staphylococci and enterobacteriaceae) in a modern broiler farm and its environment. *Animals* 2021;**11**:1–11.

Schlatzer M, Lindenthal T. Analyse der landwirtschaftlichen tierhaltung in Österreich – Umwelt- und Tierschutzaspekte. Vienna: Synthesebericht über Kernfragen und Kernaspekte BOKU Vienna. 2018, 7–21,

Schulz J, Hartung J, Seedorf J. *et al.* Staphylococci as an indicator for bacterial emissions from broiler house. Saint-Malo: International Society for Animal Hygiène, 2004, 75–77.

Schulz J, Formosa L, Seedorf J. *et al.* Measurement of culturable airborne staphylococci downwind from a naturally ventilated broiler house. *Aerobiologia* 2011;**27**:311–8.

Statistic Austria. Agriculture and forestry, supply balances. 2020. (January 2020, date last accessed). http://www.statistik.at/wcm/idc/idcplg?IdcService=GET_NATIVE_FILE&RevisionSelectionMethod=LatestReleased&dDocName=022376.

Terzieva S, Donnelly J, Ulevicius V. *et al.* Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. *Appl Environ Microbiol* 1996;**62**;2264–72.

TRBA 466. Einstufung von prokaryonten (bacteria und archaea) in risikogruppen. Malta: GMBl, 2015, 46–50.

Van den Bogaard AE, Willems R, London N. *et al.* Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. *J Antimicrob Chemother* 2002;**49**:497–505.

Van Cleef BAGL, Monnet DL, Voss A. *et al.* Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. *Emerg Infect Dis* 2011;**17**:502–5.

VDI 4250 Part 1. *Bioaerosols and Biological Agents - Risk Assessment of Source-Related Ambient Air Measurements in the Scope of Environmental Health - Effects of Bioaerosol Pollution on Human Health*. Berlin: Beuth-Verlag, 2014,1–23.

VDI 4252 Part 3. *Measurement of Airborne Microorganisms and Viruses in Ambient Air - Active Sampling of Bioaerosols - Separation of Airborne Bacteria with Impingers Using the Principle of Critical Nozzle*. Berlin: Beuth-Verlag, 2008,1–26.

VDI 4253 Part 3. *Measurement of Airborne Microorganisms and Viruses in Ambient Air - Culture Based Method for the Quantitative Determination of Bacteria in Air - Method After Separation in Liquids*. Berlin: Beuth-Verlag, 2019,1–60.

VDI 4255 Part 3. *Bioaerosols and Biological Agents - Emission Factors for Poultry*. Berlin: Beuth-Verlag, 2016,1–29.

VDI 4257 Part 2. *Bioaerosols and Biological Agents – Emission Measurement. Sampling of Bioaerosols and Sampling in Liquids*. Berlin: Beuth Verlag, 2011,1–60.

Vela J, Hildebrandt K, Metcalfe A. *et al.* Characterization of *Staphylococcus xylosus* isolated from broiler chicken barn bioaerosol. *Poult Sci* 2012;**91**:3003–12.

Vogel U, Kurzai O, Claus H. *et al.* Spa-typisierung von methicillin-resistenten *Staphylococcus aureus* stämmen. *Univ Clin Würzburg Mikrobiol* 2005;**15**:131–5.

Vučemilo M, Matković K, Vinković B. *et al.* The effect of animal age on air pollutant concentration in a broiler house. *Czech J Anim Sci* 2007;**52**:170–4.

Whyte P, Collins JD, McGill K. *et al.* Distribution and prevalence of airborne microorganisms in three commercial poultry processing plants. *J Food Prot* 2001;**64**:388–91.

Wendlandt S, Kadlec K, Fessler AT. *et al.* Transmission of methicillin-resistant *Staphylococcus aureus* isolates on broiler farms. *Vet Microbiol* 2013;**167**:632–7.

Zhang K, McClure JA, Elsayed S. *et al.* Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005;**43**:5026–33.

Zhao Y, Zhao D, Ma H. *et al.* Environmental assessment of three egg production systems – Part III: airborne bacteria concentrations and emissions. *Poult Sci* 2016;**95**:1473–81.

Zhong Z, t Chai, Duan H. *et al.* REP-PCR tracking of the origin and spread of airborne *Staphylococcus aureus* in and around chicken house. *Indoor Air* 2009;**19**:511–6.