



UDC 579.62.

DOI: 10.48077/scihor.25(11).2022.31-40

## Identification of the Bioaerosol Microbiota in Veterinary Clinics as the Key to Preventing Nosocomial Infection

Mykhailo Mocherniuk<sup>1</sup>, Mykola Kukhtyn<sup>2</sup>, Yulia Horiuk<sup>1\*</sup>, Liubov Savchuk<sup>1</sup>, Volodymyr Mizyk<sup>1</sup>

<sup>1</sup>Higher educational institution "Podillia State University"  
32300, 13 Schevchenko Str., Kamianets-Podilskyi, Ukraine

<sup>2</sup>Ternopil Ivan Puluj National Technical University  
46001, 56 Ruska Str., Ternopil, Ukraine

### Article's History:

Received: 10/02/2022

Revised: 11/11/2022

Accepted: 11/30/2022

### Suggested Citation:

Mocherniuk M., Kukhtyn, M., Horiuk, Yu., Savchuk, L., & Mizyk, V. (2022). Identification of the bioaerosol microbiota in veterinary clinics as the key to preventing nosocomial infection. *Scientific Horizons*, 25(11), 31-40.

**Abstract.** Nosocomial infections are important in veterinary medicine clinics, as they can contaminate surfaces and infect animals through bioaerosol and are the cause of ineffective antibiotic therapy. This paper presents a study of the effect of disinfection on the microbiota of bioaerozoic veterinary clinics. The purpose of this study was to establish the composition of the bioaerosol microbiota in veterinary clinic premises before and after disinfection with ultraviolet bactericidal lamps. Bioaerosol samples were taken in the premises of veterinary clinics by sedimentation method. Identification of the isolated microbiota was performed using classical methods involving commercial test systems for identifying microorganisms. It was established that the permanent microbiota of the bioaerosol of veterinary clinics includes the following representatives of gram-positive genera: *Staphylococcus* (coagulase-negative species), *Streptococcus spp.*, *Micrococcus spp.*, and *Corynebacterium spp.* These bacterial genera were present in the bioaerosol of all rooms in 100% of cases. Gram-negative species of bacteria were found in small quantities in the bioaerosol of such rooms as for the primary examination and the manipulation area with boxes for keeping sick animals. Representatives of gram-negative species were detected in a considerably larger number from the bioaerosol of the dental operating room during the day of the clinic. After disinfection with bactericidal lamps, pathogens of nosocomial infections (*S. aureus*, *S. pseudintermedius*, *Acinetobacter baumani*, *P. aeruginosa*) were released in the bioaerosol of such rooms as the primary examination, the manipulation area with boxes for keeping sick animals, and the dental operating room. This indicates that bioaerosol can serve as a medium for the spread of nosocomial infections among animals in veterinary clinics. Thus, conducting microbiological monitoring of bioaerosol in veterinary clinics will allow identifying pathogens of nosocomial infections and introducing preventive measures for the spread of pathogens

**Keywords:** air microflora, disinfection with ultraviolet lamps, nosocomial pathogens, pathogenic bacteria



Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

\*Corresponding author

## INTRODUCTION

The control of nosocomial infection in medical and veterinary institutions is crucial in the system of preventing the spread of antibiotic-resistant strains among patients and in the environment. Closed premises of veterinary clinics often create conditions for the circulation and transmission of nosocomial infection via airborne bioaerosol (Jeong *et al.*, 2022; Mocherniuk *et al.*, 2022; Zheng *et al.*, 2023). Nosocomial infection is considered a common cause of complications of the underlying disease among companion animals in veterinary clinics and quite often leads to mortality (Elnageh *et al.*, 2020). In addition, the infection of animals with nosocomial pathogens leads to a long stay in clinics, the use of a considerable range of antimicrobial preparations and the formation of pathogens with multi-resistance to antibiotics (Morgado-Gamero *et al.*, 2021). Therefore, it is important that the microflora circulating in the premises of veterinary clinics does not negatively impact the health of patients, especially those who have undergone surgery and are weakened (Sellera *et al.*, 2021). Therefore, microbiological monitoring of bioaerosol in veterinary clinics can provide information about the spread of nosocomial pathogens, transmission routes, and the state of anti-epidemic measures.

Microorganisms can spread indoors by air-droplet, contact, and transfer by organisms (fleas, ticks, other small animals) (Loncaric *et al.*, 2019). Transmission of an infection requires three elements: a source of pathogens, a susceptible organism, and a route of transmission (Berhilevych *et al.*, 2021; Kisera *et al.*, 2021). Such conditions are created in veterinary clinics, since bioaerosols are air pollutants. Bioaerosol is a collection of dead and living pathogenic and non-pathogenic microorganisms, epithelial cells, wool, hair, particles of plant origin, etc., which are suspended in the air (Tsay *et al.*, 2020). Exposure to bioaerosol from the premises of veterinary clinics poses a significant threat to the health of veterinary staff and patient animals, as it can transmit pathogens of airborne infections, acute toxic reactions, and allergies, according to (Chen *et al.*, 2022). Therefore, nosocomial pathogens can contaminate instruments, implants, equipment, environmental objects, and veterinary personnel by airborne droplets. In this regard, the airborne route of transmission of infections is an important one that needs to be addressed (Fahlgren *et al.*, 2010; Chai *et al.*, 2021).

Researchers (Horiuk *et al.*, 2020; Chueahiran *et al.*, 2021; Tamakan & Gocmen, 2022) state that bacteria inhabiting the mucous membranes of the nasal and oral cavity and the skin can be easily transmitted by airborne droplets, especially such dangerous bacteria as methicillin-resistant *Staphylococcus aureus* (*MRSA*). *MRSA* is one of the main organisms causing infections in hospitalized animal patients in veterinary clinics or animal holding boxes (Elnageh *et al.*, 2020). *MRSA* isolated from companion animals (dogs, cats) cannot be distinguished from *MRSA* isolated from different biotopes of

sick people. In addition, the authors (Chai *et al.*, 2021; Lee & Yoo, 2022) indicate that, apart from airborne transmission, veterinary staff in hospitals are also at risk for *MRSA* colonization and spreading this infection to other people. Researchers (Song *et al.*, 2021; Hamido *et al.*, 2022; Naziri *et al.*, 2022) indicate that microorganisms such as *S. aureus*, *Escherichia* spp., *Klebsiella* spp., *Salmonella* spp., *Serratia marcescens*, *Clostridium difficile* and *Acinetobacter baumannii*, were identified as pathogens of nosocomial infections in hospitalized dogs and cats. Specifically, they caused skin diseases, inflammation of the ears, diseases of the urinary system, and complicated the healing of wounds of various aetiologies.

Thus, the main pathogens of nosocomial infections of small animals are known and there are certain general recommendations for the prevention and control of nosocomial pathogens. However, the study of bioaerosol microflora of various objects in veterinary clinics will provide a better insight into the sources of infections, ways of transmission, and improve preventive measures for the spread of nosocomial pathogens, both among animals and veterinary personnel.

*The purpose of this study* was to determine the species composition of bioaerosol microbiota in different premises of veterinary clinics before and after disinfection using ultraviolet bactericidal lamps and to figure out the possible role of bioaerosol in the transmission of nosocomial pathogens.

## MATERIALS AND METHODS

The study was conducted during 2021-2022 in veterinary medicine clinics ( $n = 3$ ) in Chernivtsi and Kolomyia (Ukraine). 504 samples of bioaerosol from the premises of veterinary clinics were investigated to determine the effect of disinfection with bactericidal lamps on the amount of residual microflora. Sanitation in these clinics included daily evening wet cleaning of rooms with bactericidal lamps and once every three days disinfection with a disinfectant with active substances: N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine and N,N-didecyl-N,N-dimethylammonium chloride. Bactericidal lamps (15 W) were used for bioaerosol disinfection of premises, the duration of irradiation was 1 hour.

Bioaerosol (air) samples were taken in the premises of veterinary clinics by sedimentation method. For this, open Petri dishes with blood agar and selective media were placed in envelopes (four samples at the corners and the fifth in the centre) at 0.5 m from the wall and at a height of 1.6 m by 30 minutes, while the windows and doors in the rooms were closed. After 30 minutes of exposure, the dishes were closed, placed in a cooler bag, and delivered to the laboratory within 2 hours.

To isolate microorganisms, inoculation was carried out on the following media. Specifically, staphylococci and micrococci were isolated on blood agar containing 5% sodium chloride, enterococci – on *Bile*

*Esculin Azide Agar*, streptococci and corynebacteria – on *Streptococcus Selective Agar* and blood agar. Mushrooms were isolated on the Saburo medium. Enterobacteria (*Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, etc.) were grown on Endo, Ploskirev, and Levin media. *Pseudomonas* were isolated on a medium containing acetamide, other non-fermenting bacteria (*Acinetobacter* spp. and *Alcaligenes* spp.) – on MPA, with incubation at 20°C for 3 days. Cultures were incubated in a thermostat at 37 ± 1°C for 24-48 hours to isolate mesophilic microorganisms, and fungi at +28 ± 1°C for 5 days. Isolated cultures were identified according to morphological, tinctorial, cultural, biochemical properties and signs of pathogenicity, which are described in Bergey's Manual of Systematic Bacteriology (Vos et al., 2011).

Statistical processing was performed by analysis of variance using Fischer criteria (ANOVA). Data are presented as x±SD (mean±standard deviation). The significance of the obtained data was assessed by the F-criterion with a confidence level of P<0.05, P<0.01, P<0.001 (considering the Bonferroni correction).

## RESULTS AND DISCUSSION

Earlier studies (Mocherniuk et al., 2022) found that the quantitative content of mesophilic bacteria in the bioaerosol of the premises of veterinary clinics increases during the working day. Furthermore, there is a significant difference in the number of bacteria in different rooms of the clinic, which depends on the intensity of operation of the rooms and the therapeutic and preventive manipulations that are carried out in them. It was also found that microbial contamination of bioaerosol increases considerably in winter, which can cause the spread of nosocomial pathogens among patient animals that are overexposed around the clock. Therefore, to establish the most common types of bacteria in the bioaerosol of the premises of veterinary clinics, the isolated microbiota were identified. Therewith, bioaerosol samples were taken in the middle of the working day and after sanitary measures were carried out in the evening, as described above. The results of identification of isolated microorganisms before disinfection are presented in Table 1.

**Table 1.** Identification of bioaerosol microbiota of the premises of veterinary clinics (n = 3) selected during the working day, %

Microorganisms	Frequency of m/o release from indoor bioaerosol:						
	room for initial inspection of animals, n = 36	Ultrasound room, n = 36	X-ray room, n = 36	manipulation zone with boxes for keeping sick animals, n = 36	dental operating room, n = 36	operating room for soft tissue surgery, n = 36	orthopaedic operating room, n = 36
<b>Gram-positive:</b>							
CoNS	100	100	100	100	100	100	100
CoPS	16.3***	5.4***	2.7***	19.4***	27.7***	0***	0***
<i>Streptococcus</i> spp.	100	100	100	100	100	100	100
<i>Micrococcus</i> spp.	100	100	100	100	100	100	100
<i>Corynebacterium</i> spp.	100	100	100	100	100	100	100
<i>Enterococcus</i> spp.	11.1	2.7	0	13.8	16.6*	0	0
<i>Bacillus</i> spp.	27.7	5.4	5.4	30.5	38.9*	8.3	5.4
<b>Gram-negative:</b>							
<i>Escherichia</i> spp.	2.7	0	0	0	2.7	0	0
<i>Enterobacter</i> spp.	2.7	0	0	0	8.3*	0	0
<i>Acinetobacter</i> spp.	11.1	0	0	8.3	19.4*	0	0
<i>Pseudomonas</i> spp.	11.1	0	0	11.1	16.6*	0	0
Fungi	8.3	2.7	2.7	16.6	30.5*	0	0

**Note:** \* – P<0.05, P<0.01 relative to the frequency of isolation of microorganisms from other rooms; \*\*\* – P<0.001 relative to the frequency of CoNS selection according to the F-criterion (considering the Bonferroni correction); CoNS – coagulase-negative staphylococci; CoPS – coagulase-positive staphylococci

**Source:** compiled by the authors

The results of Table 1 show that the autochthonous microbiota of the bioaerosol of veterinary clinics includes the following genera of gram-positive

bacteria: *Staphylococcus* (coagulase-negative species), *Streptococcus* spp., *Micrococcus* spp. and *Corynebacterium* spp. These genera of bacteria were present in the

bioaerosol of all rooms in 100% of cases. Although the above types of bacteria are mostly non-pathogenic, researchers (Naziri *et al.*, 2022) indicate that they can cause infections of the skin and genitourinary system in companion animals. The high frequency of detection of these microorganisms in the bioaerosol of all premises during the working day (Smith *et al.*, 2019) is associated with the fact that they are common inhabitants of the skin of dogs, cats, and people, and are easily released into the environment on exfoliated epithelial cells, wool.

*Bacillus spp.* bacteria, which were isolated from almost 30% of the examined samples of the rooms for the primary examination and the manipulation zone with boxes for keeping sick animals, were the second most important in the composition of the gram-positive microbiota of the bioaerosol. Most often, species of *Bacillus spp.* were emitted from the air during the working day, especially from the dental operating room in 38.9% of cases. Coagulase-positive species of staphylococci were considerably less frequently isolated in the bioaerosol of veterinary clinics ( $P < 0.001$ ) than coagulase-negative species. They were not isolated from the air of two operating rooms, while from the bioaerosol of the premises of the ultrasound room and the X-ray room, the frequency of their release was 5.4% and 2.7%, respectively. The most frequently isolated types of coagulase-positive staphylococci were present in the bioaerosol of the dental operating room – in 27.7% of cases. The frequency of detection of these species from the premises for primary examination and manipulation zone with boxes for keeping sick animals was 16.3% and 19.4%, respectively. Considering this fact, the presence of coagulase-positive species of staphylococci in the bioaerosol of veterinary clinics can be a serious source of spread of infection by airborne droplets. Specifically, the transmission of pathogens most often occurs in examination rooms, diagnostic rooms, intensive care, holding boxes for sick animals, as well as after contact with veterinary personnel (Feßler *et al.*, 2018; Krapf *et al.*, 2019; Elnageh *et al.*, 2020).

From the identified gram-positive coccal microflora, *Enterococcus spp.* were least often released from the bioaerosol of veterinary clinics during the working day. Thus, from the air of the X-ray room and two operating rooms, they were not released at all, and from the bioaerosol of the ultrasound room – in no more than 2.7% of samples. Within 11.1-13.8% of the examined samples, these types of bacteria were present in the bioaerosol of the rooms for primary examination and the manipulation area with boxes for keeping sick animals. The most frequently isolated types were present in the bioaerosol of the dental operating room – in 16.6% of samples.

The identification of gram-negative bioaerosol microbiota of all premises of veterinary clinics indicates that these microorganisms belong to the allochthonous microflora of this research object. Since there are no representatives of gram-negative bacteria that would exceed 20% in terms of detection frequency. That is, the frequency of detection of representatives of gram-negative bacteria is significantly ( $P < 0.01$ ) lower than that of gram-positive species. The most frequently identified gram-negative bacteria were *Acinetobacter spp.* species, which were present in approximately 10% of the bioaerosol samples of the premises for primary examination and the manipulation area with boxes for keeping sick animals. However, most often these species were identified from the bioaerosol of the dental operating room – in 19.4% of samples. Other representatives of gram-negative microbiota, namely *Escherichia spp.* and *Enterobacter spp.*, were detected only from the bioaerosol of the premises for the primary examination and the dental operating room in 2.7% and 8.3% of the samples, respectively. Types of *Pseudomonas spp.* were also found in a few examined samples – 11.1% of them were found in the primary examination room and manipulation area with boxes for keeping sick animals. However, most frequently – from the bioaerosol of the dental operating room (16.6% of samples).

The minimum amount of fungal microflora from the examined samples (2.7%) was detected from the bioaerosol of the ultrasound room and the X-ray room. An increase in the frequency of detection of fungi up to 8.3% was observed in the air of the room for primary examination and up to 16.6% in the manipulation area with boxes for keeping sick animals. Most often, these microorganisms were present in the bioaerosol of the dental operating room in 30.5% of the samples under study. However, fungal microflora was not detected in the bioaerosol of two operating rooms – orthopaedic and soft tissues.

Therefore, the data of the study indicate that the microbiota of the bioaerosol of veterinary clinics is mainly represented by species of gram-positive coccal microflora, which are isolated from almost all premises. However, gram-negative bacterial species are found in small amounts in the bioaerosol of such rooms as the room for primary examination and the manipulation area with boxes for holding sick animals. Representatives of gram-negative species are detected in a considerably larger number from the bioaerosol of the dental operating room during the day of the clinic. Table 2 shows the results of a study on the detection and identification of microbiota of bioaerosol of veterinary clinics after disinfection using bactericidal lamps.

**Table 2.** Identification of bioaerosol microbiota of the premises of veterinary clinics (n = 3), after disinfection with ultraviolet lamps, n = 36

Microorganisms	Frequency of m/o release from indoor bioaerosol:						
	room for initial inspection of animals, n = 36	Ultrasound room, n = 36	X-ray room, n = 36	manipulation zone with boxes for keeping sick animals, n = 36	dental operating room, n = 36	operating room for soft tissue surgery, n = 36	orthopaedic operating room, n = 36
<b>Gram-positive:</b>							
CoNS	100	83.3	83.3	100	100	86.1	83.3
CoPS	2.8	0	0	8.4	8.4*	0	0
<i>Streptococcus</i> spp.	0	0	0	5.4	5.4*	0	0
<i>Micrococcus</i> spp.	100	100	100	100	100	100	100
<i>Corynebacterium</i> spp.	100	0	0	100	100	0	0
<i>Enterococcus</i> spp.	0	0	0	2.8	5.4*	0	0
<i>Bacillus</i> spp.	8.3	2.7	0	5.5	8.3*	0	0
<b>Gram-negative:</b>							
<i>Escherichia</i> spp.	0	0	0	0	0	0	0
<i>Enterobacter</i> spp.	0	0	0	0	0	0	0
<i>Acinetobacter</i> spp.	2.8	0	0	0	5.5*	0	0
<i>Pseudomonas</i> spp.	0	0	0	0	2.7*	0	0
Mushrooms	0	0	0	8.3	11.1*	0	0

**Note:** \* –  $P < 0.05$  relative to the frequency of isolation of microorganisms from other premises according to the F-criterion (considering the Bonferroni correction); CoNS – coagulase-negative staphylococci; CoPS – coagulase-positive staphylococci

**Source:** compiled by the authors

It was established (Table 2) that after disinfection of premises with ultraviolet lamps, the frequency of detection of microorganisms from bioaerosol significantly decreased, or bacteria were not isolated at all. Furthermore, gram-positive microorganisms of bioaerosol are more resistant to the influence of ultraviolet rays of lamps, compared to gram-negative bacteria. Therewith, among gram-positive bacteria, *Micrococcus* spp. bacteria were in the first place in terms of frequency of isolation, which were detected in 100% of cases from the bioaerosol of all premises after disinfection. Coagulase-negative staphylococci, which were isolated in 83.3-100% of samples, ranked second in frequency of detection from bioaerosol after exposure to ultraviolet rays. At the same time, coagulase-positive Staphylococcus species were detected much less frequently in air samples. Specifically, these species were found in 2.7% of samples in the bioaerosol of rooms for primary examination and 8.3% of samples in the air of the manipulation area with boxes for keeping sick animals and the dental operating room. Bacteria of the species *Corynebacterium* spp. were isolated in 100% of the samples under study from the bioaerosol of the rooms for the primary examination, the manipulation area with boxes for sick animals and the dental operating room. They were not

emitted from the air of other premises of veterinary clinics. The proposed disinfection with ultraviolet rays had a rather harmful effect on bacteria of *Streptococcus* spp. and *Enterococcus* spp., which were isolated only from the bioaerosol of two rooms of the manipulation area with boxes for keeping sick animals and the dental operating room in 2.8-5.4% of samples. Spore-forming bacteria species *Bacillus* spp. were released from the bioaerosol of four premises with a frequency of no more than 10%. Thus, bacteria were released from the air of the room for the primary examination of animals and the dental operating room in 8.3% of samples, and from the bioaerosol of the ultrasound cabinet and manipulation area with boxes for keeping sick animals – in 2.7% and 5.5% of samples, respectively.

Among gram-negative bacteria from the bioaerosol of veterinary clinics after disinfection with bactericidal lamps, only *Acinetobacter* spp. from two rooms: for primary examination and dental operating room, with a frequency of 2.8% and 5.5% of samples, respectively. *Pseudomonas* spp. species were also isolated from the bioaerosol of the dental operating room in the minimum amount from the tested samples – 2.7%. Fungal microflora was present after treatment with ultraviolet lamps only in the bioaerosol of the manipulation area

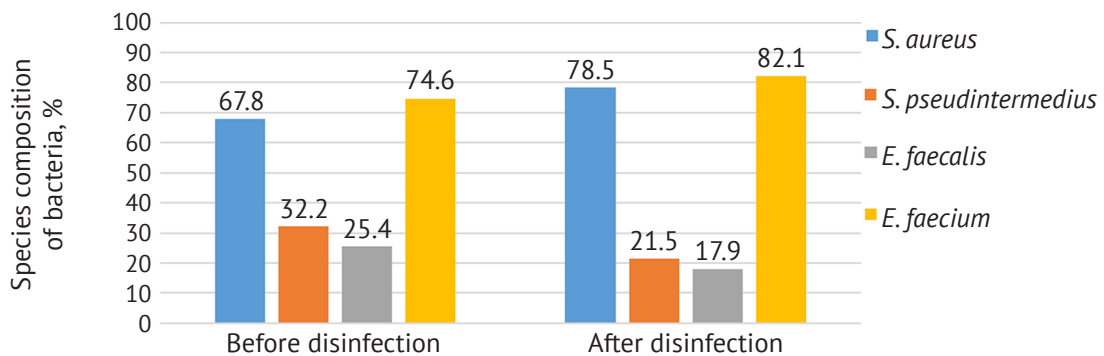


with boxes for keeping sick animals and in the dental operating room in 8.3 and 11.1% of samples, respectively. No fungi were found in the air of other rooms.

Therefore, the identification of bioaerosol microbiota of the premises of veterinary clinics after disinfection with ultraviolet lamps established a significant decrease in the frequency of detection of all microorganisms. At the same time, in the bioaerosol of such premises as the primary examination, the manipulation area with boxes for keeping sick animals and the dental operating room, types of gram-positive bacteria were distinguished, especially coagulase-positive staphylococci and fungi. This suggests that under strong microbial contamination, bacteria can survive in the suspended phase of bioaerosol

and continue to be a source of infection. Therefore, the authors of this paper believe that added disinfection measures should be taken to reduce the risk of the spread of nosocomial pathogens in premises with an intensive movement of animals, where frequent diagnostic manipulations or dental treatments are carried out.

In general, gram-positive and gram-negative genera of opportunistic pathogens, which can be a source of nosocomial infection, were isolated from the bioaerosol of the premises of veterinary clinics. Figure 1 shows the results of a study of the identification of gram-positive opportunistic microbiota of the bioaerosol of the premises of veterinary clinics before and after disinfection using bactericidal ultraviolet lamps.



**Figure 1.** Identification of gram-positive opportunistic microorganisms from the bioaerosol of the premises of veterinary clinics ( $n = 3$ )

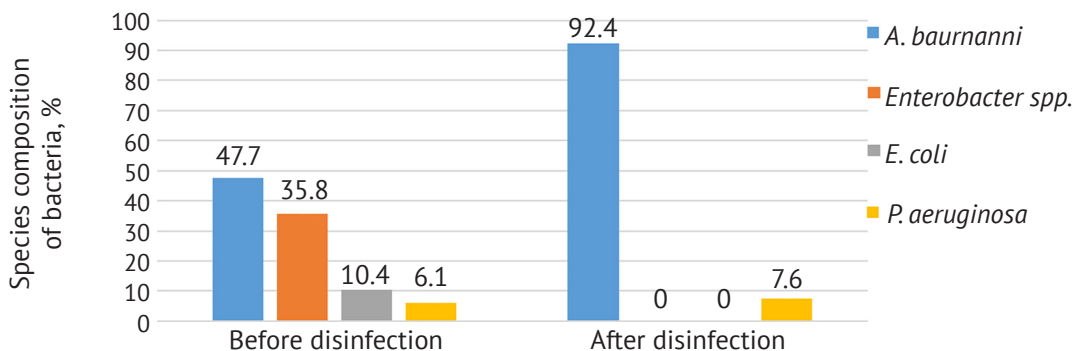
**Source:** compiled by the authors

As Figure 1 shows, among the isolated coccal bacteria from the bioaerosol before disinfection, coagulase-positive staphylococci were represented by two species: *S. aureus* and *S. pseudintermedius*. Therewith, *S. aureus* accounted for  $67.8 \pm 2.1\%$ , and *S. pseudintermedius* –  $32.2 \pm 0.8\%$ . According to (Murray *et al.*, 2018), *S. pseudintermedius* is a species that is mainly isolated from dogs and cats and is the cause of diseases such as pyoderma, otitis externa, etc. At the same time, *S. aureus* is a species that colonizes biotopes, both human and animal ones (Berhilevych *et al.*, 2021). Enterococci before disinfection in the bioaerosol were mainly represented by the *E. faecium* species, which accounted for

$74.6 \pm 2.4\%$  of the isolated cultures, and  $25.4 \pm 0.7\%$  belonged to *E. faecalis*.

Disinfection did not significantly change the ratio between bacterial species, although greater resistance of *S. aureus* and *E. faecium* to the action of ultraviolet radiation was noted. The share of these species in disinfected air increased by 10.7% and 7.5%, respectively. The detection of coagulase-positive staphylococci and enterococci in the bioaerosol of veterinary clinics after the action of bactericidal lamps indicates the imperfection of the introduced system of combating infectious agents in these clinics.

The identification of gram-negative opportunistic bacteria isolated from the bioaerosol is presented in Fig. 2.



**Figure 2.** Identification of gram-negative opportunistic microorganisms from the bioaerosol of the premises of veterinary clinics ( $n = 3$ )

**Source:** compiled by the authors

It was established (Fig. 2) that among the bacteria isolated from the bioaerosol before disinfection, the largest share was *Acinetobacter baumani* –  $47.7 \pm 1.3\%$ . *Enterobacter spp.* accounted for  $35.8 \pm 1.1\%$  of identified cultures, while the least were found for *E. coli* and *P. aeruginosa* –  $10.4 \pm 0.4\%$  and  $6.1 \pm 0.2\%$ , respectively.

After a one-hour exposure to ultraviolet lamps, *E. coli* and *Enterobacter spp.* cultures were not released from the indoor bioaerosol, which indicates the bactericidal effect of this disinfection regimen on these types of bacteria. At the same time, the cultures of *Acinetobacter baumani* stayed resistant to the action of ultraviolet rays, the share of which was  $92.4 \pm 3.8\%$  of the isolated gram-negative bacteria. In addition, *P. aeruginosa* was isolated from the bioaerosol of some rooms after disinfection, which accounted for  $7.6 \pm 0.3\%$  of cultures.

Therefore, the obtained data clearly shows that representatives of gram-negative microflora are more often released from the bioaerosol of the premises of veterinary clinics with an intensive movement of animals (examination room), where manipulations are carried out with the possible splashing of tissue particles, tooth enamel (dental operating room), in boxes for keeping sick animals, i.e., all this is connected with biological secretions from animals. Such results are consistent with research by (Sitkowska et al., 2015), that the microbiota of the bioaerosol of veterinary clinics is based on microorganisms that are representatives of the normal microbiota of closed premises of the housing stock, offices, i.e., gram-positive genera. Gram-negative microflora is present in the air in most cases due to violations of hygiene requirements, and in veterinary clinics in the premises of long-term or permanent stay of animals, for instance, in boxes after surgical intervention (Pertegal et al., 2022).

Considering the data obtained, the authors of this paper believe that the more frequent release of gram-positive microflora from indoor bioaerosol, compared to gram-negative, is explained by their greater resistance to the action of ultraviolet rays. This fact is confirmed by (Fahlgren et al., 2010), who found increased resistance of gram-positive microflora to strong sunlight during long-term exposure, compared to gram-negative. However, the survival of coagulase-positive staphylococci, enterococci, species of *Acinetobacter spp.*, *Pseudomonas spp.* after air treatment with ultraviolet lamps should be noted for such premises as the primary examination room, the manipulation area with boxes for keeping sick animals, and the dental operating room. That is, bacteria that, according to (Hritcu et al., 2020; Elnageh et al., 2020), are the cause of outbreaks of nosocomial infections among animal patients in veterinary clinics.

Outbreaks of nosocomial infection in hospitalized animals caused by the multiresistant species *Acinetobacter baumani* are reported by researchers (Kempf & Rolain, 2012). Therefore, it is necessary to pay attention to this type of bacteria in the practical activities of

veterinary clinics. Researchers (Giannouli et al., 2013) point to increased resistance to drying and the action of ultraviolet radiation in *Acinetobacter baumani*, as they can form dense biofilms on various surfaces and thereby survive in the hospital environment. The obtained studies indicate that some strains of opportunistic bacteria survive in the bioaerosol of the premises of veterinary clinics after exposure to ultraviolet rays of bactericidal lamps. This indicates that bioaerosol can serve as a medium for the spread of nosocomial infections among animals in veterinary clinics.

Therefore, today there is a considerable number of recommendations for the prevention and control of nosocomial infections in humane medicine, which are based on practical experience (Zheng et al., 2023). At the same time, there are no generally accepted recommendations or instructions for the supervision and control of nosocomial pathogens in veterinary clinics in Ukraine. This makes the system of preventive measures not too strong, since there are no stable, defined indicators that need to be focused on, and accordingly prevent the spread of nosocomial infection. Therefore, the authors of the present study believe that the development and implementation of a system for monitoring nosocomial pathogens in veterinary medicine clinics in Ukraine should complement the national strategy for combating antibiotic resistance of microorganisms. This will, firstly, reduce infection with resistant strains of animals, secondly, reduce the use of broad-spectrum antibiotics, and thirdly, prevent the spread of antibiotic-resistant bacteria in the environment of veterinary clinics between animals, between animals and veterinary and service personnel, and between personnel and other people and animals.

## CONCLUSIONS

It was established that bacteria of the *Micrococcus spp.*, *Staphylococcus* (coagulase-negative species), *Streptococcus spp.* and *Corynebacterium spp.* species were released from the bioaerosol of veterinary clinics during the working day in 100% of cases. Coagulase-positive species of staphylococci were present in indoor bioaerosols in 5.4-27.7% of samples. Gram-negative species of bacteria: *Escherichia spp.*, *Enterobacter spp.*, *Acinetobacter spp.* and *Pseudomonas spp.* were present in the bioaerosol of the dental operating room in 2.7-9.4% of the samples, and these types of bacteria were practically not isolated from the bioaerosol of other rooms. Thus, gram-positive bacterial species make up the permanent microflora of bioaerosol in veterinary clinics.

Disinfection with ultraviolet lamps ensured the destruction of gram-negative bacteria, which were practically not released from the bioaerosol of the premises, except for the dental operating room, where up to 5.5% of *Acinetobacter spp.* and *Pseudomonas spp.* bacteria were released from the air. Gram-positive microbiota was more resistant to the effects of ultraviolet

rays, as bacteria of the *Micrococcus spp.*, *Staphylococcus* (coagulase-negative species) and *Corynebacterium spp.* species were isolated from the bioaerosol in almost 100% of the samples.

The causative agents of nosocomial infections were identified from the indoor bioaerosol: *S. aureus* and *S. pseudintermedius*, *Acinetobacter baumani* and *P. aeruginosa*. This indicates that bioaerosol can serve as

a medium for the spread of pathogenic bacteria among animals in veterinary clinics. In addition, this provides a basis for the development of added sanitary measures to combat the spread of nosocomial pathogens. Prospects for further research lie in determining the sensitivity of the isolated microflora to antibacterial preparations and establishing the presence of antibiotic resistance plasmids.

## REFERENCES

- [1] Berhilevych, O., Kasianchuk, V., Kukhtyn, M., Shubin, P., & Butsyk, A. (2021). Comparison of cell sizes of methicillin-resistant *Staphylococcus aureus* with Presence and absence of the *MecA* Gene. *Microbiological Journal*, 83(1), 68-77. doi: 10.15407/microbiolj83.01.068.
- [2] Chai, M.H., Sukiman, M.Z., Liew, Y.W., Shapawi, M.S., Roslan, F.S., Hashim, S.N., & Ghazali, M.F. (2021). Detection, molecular characterization, and antibiogram of multi-drug resistant and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from pets and pet owners in Malaysia. *Iranian Journal of Veterinary Research*, 22(4), 277-287. doi: 10.22099/ijvr.2021.39586.5752.
- [3] Chen, P., Guo, X., & Li, F. (2022). Antibiotic resistance genes in bioaerosols: Emerging, non-ignorable and pernicious pollutants. *Journal of Cleaner Production*, 348, article number 131094. doi: 10.1016/j.jclepro.2022.131094.
- [4] Chueahiran, S., Yindee, J., Boonkham, P., Suanpairintr, N., & Chanchaithong, P. (2021). Methicillin-resistant *Staphylococcus aureus* clonal complex 398 as a major MRSA lineage in dogs and cats in Thailand. *Antibiotics*, 10(3), article number 243. doi: 10.3390/antibiotics10030243.
- [5] Elnageh, H.R., Hiblu, M.A., Abbassi, M.S., Abouzeed, Y.M., & Ahmed, M.O. (2020). Prevalence and antimicrobial resistance of *Staphylococcus* species isolated from cats and dogs. *Open Veterinary Journal*, 10(4), 452-456. doi: 10.4314/ovj.v10i4.13.
- [6] Fahlgren, C., Hagström, A., Nilsson, D., & Zweifel, U.L. (2010). Annual variations in the diversity, viability, and origin of airborne bacteria. *Applied and Environmental Microbiology*, 76(9), 3015-3025. doi: 10.1128/aem.02092-09.
- [7] Feßler, A.T., Schuenemann, R., Kadlec, K., Hensel, V., Brombach, J., Murugaiyan, J., Oechtering, G., Burgener, I.A., & Schwarz, S. (2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) among employees and in the environment of a small animal hospital. *Veterinary Microbiology*, 221, 153-158. doi: 10.1016/j.vetmic.2018.06.001.
- [8] Giannouli, M., Antunes, L.C., Marchetti, V., Triassi, M., Visca, P., & Zarrilli, R. (2013). Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I-III and to the emerging genotypes ST25 and ST78. *BMC Infectious Diseases*, 13(1), article number 282. doi: 10.1186/1471-2334-13-282.
- [9] Hamido, A.J., Sirika, N.B., & Omar, I.A. (2022). Literature review on antibiotics. *Clinical Medicine and Health Research Journal*, 2(4), 174-182. doi: 10.18535/cmhrj.v2i4.65.
- [10] Horiuk, Y., Kukhtyn, M., Horiuk, V., Kernychnyi, S., & Tarasenko, L. (2020). Characteristics of bacteriophages of the *Staphylococcus aureus* variant *bovis*. *Veterinary Medicine-Czech*, 65, 421-426. doi: 10.17221/55/2020-VETMED.
- [11] Hritcu, O.M., Schmidt, V.M., Salem, S.E., Maciucă, I.E., Moraru, R.F., Lipovan, I., & Timofte, D. (2020). Geographical variations in virulence factors and antimicrobial resistance amongst staphylococci isolated from dogs from the United Kingdom and Romania. *Frontiers in Veterinary Science*, 7, 1-10. doi: 10.3389/fvets.2020.00414.
- [12] Jeong, S.B., Ko, H.S., Heo, K.J., Shin, J.H., & Jung, J.H. (2022). Size distribution and concentration of indoor culturable bacterial and fungal bioaerosols. *Atmospheric Environment: X*, 15, article number 100182. doi: 10.1016/j.aeaoa.2022.100182.
- [13] Kempf, M., & Rolain, J.-M. (2012). Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: Clinical impact and therapeutic options. *International Journal of Antimicrobial Agents*, 39(2), 105-114. doi: 10.1016/j.ijantimicag.2011.10.004.
- [14] Kiserá, Y., Bozhyk, L., Grynevych, N., & Martyniv, Y. (2021). Species composition of circulation microflora and its resistance to antibacterial drugs in the conditions of the impulse veterinary clinic of the city of Lviv. *Scientific Bulletin of Veterinary Medicine*, 2(168), 65-71. doi: 10.33245/2310-4902-2021-168-2-65-71.



- [15] Krapf, M., Müller, E., Reissig, A., Slickers, P., Braun, S.D., Müller, E., & Monecke, S. (2019). Molecular characterisation of methicillin-resistant *Staphylococcus pseudintermedius* from dogs and the description of their SCCmec elements. *Veterinary Microbiology*, 233, 196-203. doi: 10.1016/j.vetmic.2019.04.002.
- [16] Lee, G., & Yoo, K. (2022). A review of the emergence of antibiotic resistance in bioaerosols and its monitoring methods. *Reviews in Environmental Science and Biotechnology*, 21, 799-27. doi: 10.1007/s11157-022-09622-3.
- [17] Loncaric, I., Lepuschitz, S., Ruppitsch, W., Trstan, A., Andreadis, T., Bouchlis, N., & Spargser, J. (2019). Increased genetic diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from companion animals. *Veterinary Microbiology*, 235, 118-126. doi: 10.1016/j.vetmic.2019.06.013.
- [18] Mocherniuk, M.M., Kukhtyn, M.D., Horiuk, Y.V., Horiuk, V.V., Tsvigun, O.A., & Tokarchuk, T.S. (2022). Microflora of boxes for holding veterinary patients in clinics. *Regulatory Mechanisms in Biosystems*, 13(3), 257-264. doi: 10.15421/022233.
- [19] Morgado-Gamero, W.B., Parody, A., Medina, J., Rodriguez-Villamizar, L.A., & Agudelo-Castañeda, D. (2021). Multi-antibiotic resistant bacteria in landfill bioaerosols: Environmental conditions and biological risk assessment. *Environmental Pollution*, 290, article number 118037. doi: 10.1016/j.envpol.2021.118037.
- [20] Murray, A.K., Lee, J., Bendall, R., Zhang, L., Sunde, M., Schau Slette-meås, J., Gaze, W., Page, A.J., & Vos, M. (2018). *Staphylococcus cornubiensis* sp. nov., a member of the *Staphylococcus intermedius* Group (SIG). *International Journal of Systematic and Evolutionary Microbiology*, 68(11), 3404-3408. doi: 10.1099/ijsem.0.002992.
- [21] Naziri, Z., Poormaleknia, M., & Ghaedi Oliyaei, A. (2022). Risk of sharing resistant bacteria and/or resistance elements between dogs and their owners. *BMC Veterinary Research*, 18(1), 1-8. doi: 10.1186/s12917-022-03298-1.
- [22] Pertegal, V., Lacasa, E., Cañizares, P., Rodrigo, M.A., & Sáez, C. (2022). Understanding the influence of the bioaerosol source on the distribution of airborne bacteria in hospital indoor air. *Environmental Research*, 216(1), article number 114458. doi: 10.1016/j.envres.2022.114458.
- [23] Sellera, F.P., Da Silva, L.C., & Lincopan, N. (2021). Rapid spread of critical priority carbapenemase-producing pathogens in companion animals: A one health challenge for a post-pandemic world. *Journal of Antimicrobial Chemotherapy*, 76(9), 2225-2229. doi: 10.1093/jac/dkab169.
- [24] Sitkowska, J., Sitkowski, W., Sitkowski, L., Lutnicki, K., Adamek, L., & Wilkolek, P. (2015). Seasonal microbiological quality of air in veterinary practices in Poland. *Annals of Agricultural and Environmental Medicine*, 22(4), 614-624. doi: 10.5604/12321966.1185763.
- [25] Smith, A., Wayne, A.S., Fellman, C.L., & Rosenbaum, M.H. (2019). Usage patterns of carbapenem antimicrobials in dogs and cats at a veterinary tertiary care hospital. *Journal of Veterinary Internal Medicine*, 33(4), 1677-1685. doi: 10.1111/jvim.15522.
- [26] Song, L., Wang, C., Jiang, G., Ma, J., Li, Y., Chen, H., & Guo, J. (2021). Bioaerosol is an important transmission route of antibiotic resistance genes in pig farms. *Environment International*, 154, article number 106559. doi: 10.1016/j.envint.2021.106559.
- [27] Tamakan, H., & Gocmen, H. (2022). Genetic characterization of methicillin resistant *Staphylococcus pseudintermedius* in dogs and cats in Cyprus: Comparison of MRSP and MRSA results. *Pakistan Journal of Zoology*, 54(4). doi: 10.17582/journal.pjz/20211101121137.
- [28] Tsay, M.D., Tseng, C.C., Wu, N.X., & Lai, C.Y. (2020). Size distribution and antibiotic-resistant characteristics of bacterial bioaerosol in intensive care unit before and during visits to patients. *Environment International*, 144, article number 106024. doi: 10.1016/j.envint.2020.106024.
- [29] Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., & Whitman, W.B. (Eds.). (2011). *Bergey's manual of systematic bacteriology*. New York: Springer Science & Business Media. doi: 10.1007/b92997.
- [30] Zheng, Y., Dong, H., Wang, S., Zhang, Y., & Cong, Q. (2023). A new air cleaning technology to synergistically reduce odor and bioaerosol emissions from livestock houses. *Agriculture, Ecosystems & Environment*, 342, article number 108221. doi: 10.1016/j.agee.2022.108221.

## Ідентифікація мікробіоти біоаерозолі ветеринарних клінік – запорука профілактики нозокоміальної інфекції

Михайло Михайлович Мочернюк<sup>1</sup>, Микола Дмитрович Кухтин<sup>2</sup>,  
Юлія Вікторівна Горюк<sup>1</sup>, Любов Броніславівна Савчук<sup>1</sup>, Володимир Павлович Мізик<sup>1</sup>

<sup>1</sup>Заклад вищої освіти «Подільський державний університет»  
32316, вул. Шевченка, 12, м. Кам'янець-Подільський, Україна

<sup>2</sup>Тернопільський національний технічний університет імені Івана Пулюя  
46001, вул. Руська, 56, м. Тернопіль, Україна

**Анотація.** Внутрішньо лікарняні інфекції мають суттєве значення у клініках ветеринарної медицини, оскільки можуть контамінувати поверхні і заражати тварин через біоаерозоль та є причиною неефективної антибіотикотерапії. У роботі наведено дослідження впливу дезінфекції на мікробіоту біоаерозолі ветеринарних клінік. Мета роботи полягала у встановленні складу мікробіоти біоаерозолі приміщень ветеринарних клінік до та після знезараження ультрафіолетовими бактерицидними лампами. Проби біоаерозолі відбирали в приміщеннях ветеринарних клінік седиментаційним методом. Ідентифікацію виділеної мікробіоти проводили класичними методами із застосуванням комерційних тест-систем для ідентифікації мікроорганізмів. Встановлено, що до постійної мікробіоти біоаерозолі ветеринарних клінік можна віднести наступні представники грам позитивних родів: *Staphylococcus* (коагулазонегативні види), *Streptococcus spp.*, *Micrococcus spp.* та *Corynebacterium spp.* Дані роди бактерій були наявні в біоаерозолі всіх приміщень у 100 % випадків. Грамнегативні види бактерій зустрічалися в незначній кількості в біоаерозолі таких приміщень, як для первинного огляду та маніпуляційної зони з боксами для перетримування хворих тварин. У значно більшій кількості представники грамнегативних видів виявлялися з біоаерозолі стоматологічної операційної протягом дня роботи клініки. Після дезінфекції бактерицидними лампами у біоаерозолі таких приміщень, як первинного огляду, маніпуляційної зони із боксами для перетримування хворих тварин та стоматологічної операційної виділялися збудники нозокоміальних інфекцій (*S. aureus*, *S. pseudintermedius*, *Acinetobacter baumani*, *P. aeruginosa*). Це вказує на те, що біоаерозоль може слугувати середовищем для розповсюдження збудників нозокоміальних інфекцій серед тварин ветеринарних клінік. Отже, проведення мікробіологічного моніторингу біоаерозолі у ветеринарних клініках дозволить виявити збудників нозокоміальних інфекцій та запровадити профілактичні заходи щодо розповсюдження патогенів

**Ключові слова:** мікрофлора повітря, дезінфекція ультрафіолетовими лампами, внутрішньолікарняні збудники, патогенні бактерії