Scientific Works. Series C. Veterinary Medicine. Vol. LXVIII (1), 2022
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

INTERDEPENDENCE OF ORAL MICROBIOME-HABITAL MICROBIOME AND ITS ANTIBIOTIC RESISTANCE IN HEALTHY DOGS

Aurel Vasiu[1], Diana OLAH[1], Cătălina CORNEA, Gheorghiță DUCA[2], Marina SPÎNU[1], Monica Ioana SUĂTEAN[1], Emoke PALL[1]

[1]Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372, Cluj-Napoca, Romania
[2]Institute of Research and Development for Montanology Cristian, Sibiu, Romania

Corresponding author email: aurel.vasiu@usamvcluj.ro

Abstract

Antibiotic resistance is a growing phenomenon which involves a pronounced zoonotic risk. Healthy dogs can acquire antibiotic-resistant bacteria in their living environment, but also on occasional clinical examinations at the veterinary clinic where they are presented for consultation. In order to follow the way in which the microbiome transfer can be performed in a veterinary clinic to regular patients, saliva samples (n = 8) were collected from healthy dogs presented at a veterinary clinic in Cluj-Napoca. The bacterial population was also tested for resistance to antibiotics. The dogs were regular patients of the veterinary clinic, originating from different districts of Cluj-Napoca. Thus, the intersection between patients is performed only in the veterinary clinic. Samples were also collected from various surfaces in the consulting and waiting rooms. The samples were processed using classical microbiological methods and identified by rapid biochemical assays. The susceptibility to certain antibiotics was evaluated using agar diffusion method. In this study, bacteria of the same species were isolated from patients with different habitats, supporting the possible interchangeability of the microflora, probably in the case of repeated visits to the same office. The presence of a large number of strains involved in the oral microbiome associated with increased resistance to antimicrobials calls for the implementation of enhanced biosecurity measures.

Key words*: dogs, saliva, bacteria, veterinary clinic, antimicrobial resistance.*

INTRODUCTION

The subject of nosocomial infections in veterinary medicine is not very deeply studied, unlike human medicine. This is probably due to the fact that the animals do not have long-term contact with the environment of veterinary practices and veterinarians.

However, there are reasons why the possibility of transmitting bacteria in the veterinary office should not be ignored. One of these reasons, enough to study the subject, is the possibility of transmitting antibiotic-resistant bacteria. It is important to note that pets, especially dogs, have a specific research behavior. This increases the chances of the colonization of the skin and mucous membranes with bacteria. Also, one aspect that could increase the importance of the topic is the fact that in the veterinary office, unlike human hospitals, sick and healthy animals live in one space and are in direct contact. People, however, follow safety precautions to prevent the transmission of bacteria to other patients. The normal microflora of animals is directly proportional to environmental factors, so it is the main target of substances entering the body and is also involved in the transformation of natural and foreign substances to the body. This can lead to dysbiosis, changes in physiological, biochemical and immunological parameters, accumulation and selection of atypical strains and finally, the emergence of pathological processes (Yakshigulova, 2016).

The micro-environment of the veterinary office is considered a potential factor that can influence the composition of the oral microflora of pets. Direct contact between patients in waiting rooms, contact with surfaces and objects in the veterinary office, surgery and contact with the veterinarian are factors that lead to bacterial colonization and possibly to the appearance of nosocomial infections. In addition to these major risk factors are immunosuppression, antibiotic therapy and disease (Stull and Weese, 2015). The pathogens responsible for the occurrence of pathological processes, either transmitted from one patient to another or from staff to patient, may be

resistant to antimicrobials. Antibiotic-resistant bacteria that can be transmitted from veterinary clinics include *Escherichia coli*, *Clostridium* spp., *Enterobacter* spp., *Enterococcus* spp., *Staphylococcus* spp., *Acinetobacter baumannii*. These bacteria are a serious concern not only because of their virulence, but also because of their resistance to antibiotics. Infections associated with these bacteria are difficult to treat and can be associated with a serious prognosis (Weese, 2020).

The aim of this study was to highlight and characterize microorganisms isolated from the oral cavity of dogs and the environment where they intersect and to draw a conclusion about the possibility of bacteria transmission between dogs and the role of the environment in veterinary clinics on this transmission.

MATERIALS AND METHODS

Samples from the oral cavity were collected from 8 (n = 8), clinically healthy dogs aged between 5 months and 7 years. The purpose of the visit to the vet was routine internal and/or external deworming, vaccination. The dogs subjected to the study only intersect in the veterinary office where the samples were collected. The owners of each animal were informed accordingly about the details of the study and the agreement signed by them was obtained for the collection of samples from the oral cavity. Prior to sampling, each dog underwent objective clinical examination, and animals with various pathologies were excluded from the study. All dogs are constant patients of this veterinary office, which intersect with each other and have contact with the environment of the office (walls, floor, etc.).

In addition to the dog population, samples were taken from the veterinary office. Samples were collected from the angle of 2 walls (consultation room) and from the angle between a wall and the floor (waiting room). For initial microbiological analyses, the samples were inoculated in nutrient broth and nutrient agar (both from Oxoid) in aerobic conditions at 37°C for 24 hours. After obtaining the isolated colonies, the catalase test and the oxidase test were performed. Bacterial strains identification were performed by standard microbiological methods adopted from the Clinical and Laboratory Standards Institute (CLSI) guideline. The identification of microorganisms was performed using GP 24 (Diagnostics) for the identification of Gram-positive bacteria and GN 24 (Diagnostics) kits for the identification of Gram-negative bacteria (Figure 1).

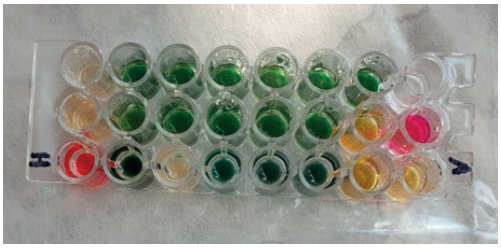

Figure 1. The kit used to characterize bacterial strains

The results obtained in the biochemical tests together with those obtained in the oxidase test were evaluated using the microID software.

The antimicrobial sensitivity patterns of the isolated strains were evaluated using the standard Kirby-Bauer disk diffusion method according to the CLSI guidelines. The strains were tested towards 15 antimicrobials: GEN-gentamicin; AK-amikacin; TE-tetracycline; DOX-doxycycline; CIP-ciprofloxacin; LEV-levofloxacin; TOC-trimethoprim; C-chloramphenicol; A/S-ampicillin/sulbactam; CRO-ceftriaxone; TOB-tobramycin; AMC-amoxicillin; CD-clindamycin and E-erythromycin; P-penicillin. The antibiotic assay discs were purchased from Oxoid. The results were evaluated based on the growth inhibition zone diameters (mm) and were classified as sensitive (S), Intermediate resistant (I) and resistant (R) (according to the standard values of CLSI 2020). The multiple antibiotic resistance index was also calculated according to Krumperman (Krumperman 1983).

RESULTS AND DISCUSSIONS

In this study, eight dogs were sampled from a private veterinary clinic in Cluj-Napoca. The dogs were of different breeds, sizes, sexes and ages. Following the objective clinical examination performed before collecting the samples, it was proved that all the dogs were clinically healthy. Samples were also collected from the veterinary office where these dogs are regularly checked.

After transferring bacterial culture sampes from nutrient broth to agar, several types of colonies developed. Thus, for each sample collected from dogs, a minimum of 2 and a maximum of 3 types of colonies were identified for each sample.
In the samples collected from the environment of the veterinary office, 1 or 2 types of colonies developed.
After examination of the pure cultures, the presence of 13 Gram-negative and 12 Gram-positive bacteria was observed. Out of a total of 9 Gram-positive bacteria, identified in 6 dogs, 3 are members of the genus *Staphylococcus* (*S. arlettae, S. epidermidis, S. acidominimus*), and 2 are members of the genus *Actinomyces* (*A. radingae, A. turicensis*).
In the case of a sample collected from the dog, bacterial strains were identified identical to those isolated from the samples collected from the veterinary clinic.
Of the 3 bacteria identified in the veterinary clinic samples, one is sporulated (*Clostridium difficile*) along with *Aerococcus viridans* and *Facklamia sourekii* strains (Table 1).

Table 1. Isolated Gram-positive bacteria

Bacterial strain ID	Bacterial strain
1.1	*Staphylococcus arlettae*
3.1	*Micrococcus spp.*
4.2	*Aerococcus viridans*
4.3	*Enterococcus hirae/dispar*
6.1	*Actinomyces radingae*
6.2	*Actinomycens turicensis*
6.3	*Staphylococcus epidermidis*
9.2	*Staphylococcus acidominimus*
10.1	*Bacillus mesentericus*
11	*Clostridium difficile (clinic)*
12.1	*Aerococcus viridans (clinic and patient)*
12.2	*Facklamia sourekii*

Among the isolated Gram-positive bacteria, some have zoonotic potential, such as Bacillus or *Clostridium* species, in which case the control is difficult, given their sporulated character. Due to the isolation of the genus *Clostridium* from the clinical microclimate, it is important that those responsible for mandatory disinfection re-evaluate the zoonotic risks posed by it and propose periodic disinfection using broad-spectrum disinfectants.
The results of the oxidase test showed that out of 13 Gram-negative bacteria, 10 are oxidase-positive and 3 are oxidase-negative (Table 2).

Table 2. Isolated Gram-negative bacteria

Bacterial stain ID	Bacterial strain
1.2	*Alcaligenes faecalis*
1.3	*Acinetobacter lwofii*
3.2	*Escherichia coli*
3.3	*Brevundimonas diminuta*
5.1	*Delftia acidovorans*
5.2	*Achromobacter xylosoxidans*
8.1	*Yersinia aldovae*
8.2	*Pseudomnas aeruginosa*
8.3	*Myroides odoratimimus*
9.1	*Escherichia coli*
9.3	*Brevundimonas diminuta*
10.2	*Aeromonas hydrofila*
10.3	*Bordetella bronchisepica*

The evaluation of antibiotic sensitivity/ resistance indicated very different patterns, depending on the tested strain.
Various classes of antibiotics have been used, including penicillins, aminoglycosides, tetracyclines, cephalosporins and fluoroquinolones.
After identifying the susceptibility of each bacterium, it was found that most bacterial strains are sensitive to the used antibiotimicrobials (Table 3).

Table 3. Susceptibility of bacterial strains to antibiotics

Strains	GEN	AK	TE	DOX	CIP	LEV	COT	C	A/S	CRO	TOB	AMC	CD	E	P
Staphylococcus arlettae	S	-	R	-	S	-	S	S	-	-	-	-	-	-	-
Aerococcus viridans	S	-	S	S	S	S	-	-	I	R	-	S	-	-	R
Staphylococcus epidermidis	S	-	R	S	S	S	-	-	-	-	-	-	I	I	R
Yersinia aldovae	S	S	I	S	S	S	-	-	I	R	S	S	-	-	R
Escherichia coli	S	S	S	-	S	S	-	-	S	S	S	S	-	-	R
Staphilococcus acidominimus	S	S	-	S	S	S	-	-	-	-	S	-	-	R	S
MAR index			0.4							0.66					0.8

I - intermediate resistant, R - resistant, S - sensible. MAR - multiple antibiotic resistance index
GEN-gentamicin; AK-amikacin; TE-tetracycline; DOX-doxycycline; CIP-ciprofloxacin; LEV-levofloxacin; TOC-trimethoprim; C-chloramphenicol; A/S-ampicillin/sulbactam; CRO-ceftriaxone; TOB-tobramycin; AMC-amoxicillin; CD-clindamycin; E-erythromycin; P-penicillin.

Figure 2 shows an increase in the MAR index in some of the antibiotics, which indicates a broad resistance to at least some of the tested antimicrobials and argues for their increased pathogenicity. *Pseudomonas aeruginosa, Escherichia coli, Acinetobacter lwofii, Staphylococcus eidermidis, Achromobacter xylosoxidans* and *Clostridium difficile* are considered antibiotic-resistant bacteria, according to many studies (Cheung, 2017). Some studies show that *Yersinia aldovae* is resistant to tetracycline, ciprofloxacin, ampicillin and amoxicillin and penicillin (Jamali et al., 2014).

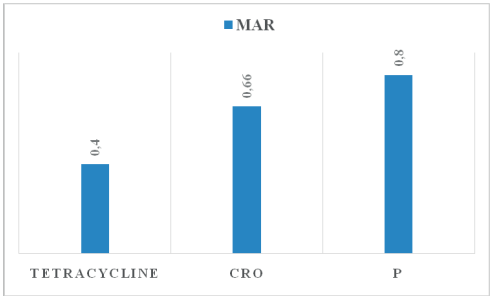

Figure 2. Multiple antibiotic resistance -MAR index in isolated strains

Dogs are part of the category of pets that live in close contact with humans. The oral cavity of clinically healthy dogs of different ages, sexes, breeds and management systems are colonized with multidrug-resistant bacteria which can act as an important source of infection for their owners and/or handlers. The most common bacterial strains isolated from the oral cavity of dogs are *Klebsiella pneumoniae* ssp. *pneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacter freundii, Enterobacter cloacae, Acinetobacter calcoaceticus* and *Pasteurella* species, which can be transmitted by biting or licking. The predominant species of bacteria involved in the bite wound infection are *Staphylococcus aureus, Pasteurella multocida, E. coli, Moraxella, Pasteurella canis, Enterobacter cloacae* (Kasempimolporn et al., 2003). Multiple studies demonstrate the presence of pathogenic, zoonotic or multidrug-resistant bacteria in the oral cavity of dogs that can serve as a possible source of transmission to humans through direct contact or bite (Bata et al., 2020). Bacterial strains isolated from some of the dogs represent the normal microflora present in the skin and / or mucous membranes, digestive tract, urine, respiratory tract or are pathogenic, present in dermatitis. During the study, bacteria of the same species with different habitats, respectively patient and clinic, were isolated, supporting the interchangeability of the bacterial microflora, probably in the case of repeated visits to the same clinic of the same patient. The results of the study draw attention to the multitude of bacterial species present in the oral cavity in dogs and which, in combination with an increased resistance to antibiotics may pose a danger to staff, but also to owners or even patients.

CONCLUSIONS

According to the results obtained in this study, in order to reduce the risk of nosocomial infections, it is important to ensure a rigorous asepsis. It is important to regularly disinfect the surfaces of the clinics, including the waiting room, as this is a place where the animals stay the longest and have contact with the walls, floor and surrounding objects. Veterinarians must follow hygiene rules to prevent the spread of bacteria to patients. Given the possibility of transmitting antibiotic-resistant bacteria, a preventative measure is the correct diagnosis and choice of medication and therapeutic protocols.

REFERENCES

Bata S.I., Andua O.A., Maimadu A., Sabo J.A., Mayowa O. Waziri I.A. (2020). Oral cavities multidrug resistant bacteria colonization in apparently healthy dogs in jos, plateau state, Nigeria, *Science World Journal*, vol. 15(no 1).

Cheung Ka Tik (2017). Evaluation of domestic dogs and cats oral flora in relationship of oral hygiene and detection of multi resistant bacterial strains, Medical Microbiology diagnosis, London.

Jamali H., Paydar M., Radmehr, B. and Ismail, S. (2014). Prevalence, characterization, and antimicrobial resistance of Yersinia species and Yersinia enterocolitica isolated from raw milk in farm bulk tanks. *J. Dairy Sci*., 12.

Kasempimolporn S, Benjavongkulchai M, Saengseesom W, Sitprija V. (2003). Oral bacterial flora of dogs with and without rabies: a preliminary study in Thailand. *J Med Assoc Thai.* Dec; 86(12):1162-6.

Krumperman P. H. (1983). Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. *Applied and environmental microbiology*, *46*(1), 165–170.

Stull, J. W., & Weese, J. S. (2015). Hospital-associated infections in small animal practice. *The Veterinary clinics of North America. Small animal practice*, *45*(2), 217–v.

Weerse J.S. (2020). *Clostridium* (*Clostridioides*) *difficile* in animals. *J Vet Diagn Invest*. Mar;32(2):213-221.

Yakshigulova L.Z. (2016). Features of microbiocenosis of the oral cavity of dogs in conditions of technogenic impact in the Moscow and Bryansk regions, Orenburg.