Acta Scientiae Veterinariae, 2021. 49: 1800.

RESEARCH ARTICLE
Pub. 1800

ISSN 1679-9216

Microbial Contamination and Antimicrobial Resistance Profiles Indicate Potential Risks of Infection at the Veterinary Medical Teaching Hospital - UFRGS, Porto Alegre, Brazil

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ABSTRACT

***Background*:** This study aimed to assess the level of bacterial contamination in the Small Animals Sector of the Veterinary Medical Teaching Hospital (HCV) of the Universidade Federal do Rio Grande do Sul (UFRGS). Firstly, a committee was invited to complete a questionnaire and to list critical sample sites for collection. With the identification of the places to be sampled, collections were made with sterile swabs on different surfaces of environments of the HCV. The identification of important bacteria in the veterinary area, in the different sampled environments, raises the concern for hygiene procedures in the veterinary hospital environment.

***Materials, Methods & Results*:** Sixteen samples were collected from these different areas, and microbiological analyses were performed. Standard counts of viable and strictly aerobic mesophilic microorganisms were realized. Collections were made to assess ambient air quality. With the microbiological analysis performed, bacteria of clinical importance were identified. To assess the resistance profile of the bacteria, the susceptibility test to antimicrobials was performed. MALDI-TOF/MS measurement identified 29 bacteria at the genus level and 10 bacteria at the species level and the antimicrobial susceptibility test was realized. Most of the isolates identified (60%) were bacteria of the genus *Staphylococcus* spp. Regarding antimicrobial susceptibility analysis the 10 bacteria identified at the species level were assessed. Test results showed that the isolates *S. aureus*, *S. epidermidis* and *S. haemolyticus* - collected from treatment room 2 - and *S. haemolyticus*, which had been isolated from samples from treatment room 2 of the cattery, presented multiresistance. *Pantoea ananatis* isolates from room 5 also showed a multiresistant profile for erythromycin, cephalothin, vancomycin and ampicillin. *Micrococcus luteus* isolates from the x-ray room and the kennel showed resistance to ceftazidime. *Staphylococcus equorum* isolates from room 4 were sensitive to all tested antimicrobials.

***Discussion*:** In Brazilian legislation there are no official microbiological parameters for surfaces in a veterinary hospital environment. The microorganisms present in the air are transient and variable, and the number and types of airborne agents is determined by the various sources of contamination in the environment. These microorganisms can be found in suspension, particulate matter and water droplets. Veterinary medical care tables are potentially contaminated by the animals handling, including those that sometimes defecate or urinate during their medical visit. Frequent handwashing is also known to be an important means of personal protection and disease prevention, although it is estimated that only 40% of practitioners do so routinely. Based on these results, we recommend a plan of bacterial control and disinfection that should be implemented to ensure more effective sanitary conditions. Microorganism counts were high in some of the veterinary hospital environments tested, indicating that current disinfection and hygiene practices are not sufficient to control the establishment of these microorganisms at the study sites. In view of this, it is reasonable to conclude that permanent monitoring and assessment of the effectiveness of hygiene protocols is needed in different sectors of the hospital. This may be an essential tool in a preventive approach to stop the spread of selectively resistant microorganisms, as well as cases of hospital infections. In addition, continuous staff training and awareness of the importance of personal and environmental hygiene is vital for minimizing the presence of these microorganisms in hospitals and avoid their transmission to patients. Finally, a more systematic hygiene guideline should be implemented in areas that showed higher counts.

Keywords: antimicrobial resistance, cats, dogs, bacteria, hospital infection, veterinary hospital.

DOI: 10.22456/1679-9216.108992

Received: 8 December 2020 Accepted: 10 March 2021 Published: 11 April 2021

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INTRODUCTION

Veterinary hospitals are similar to human hospitals in their unquestionable and considerable complexity. Thus, the increasing frequency of cases of hospital infections acquired by animals has obliged administrators, veterinarians and assistant staff to broaden their knowledge about this specific problem [15].

Infection control programs establish procedures aimed at preventing the spread of infections to patients, pet owners, veterinarians and other employees [6].

Routine practices should include effective and regular hand hygiene, the use of personal protective equipment, hospital cleaning and disinfection, and the appropriate management of environments and equipment. These procedures result in the removal of dirt, a reduction in microbial load and the elimination of multidrug-resistant strains. Nevertheless, it should be highlighted that, considering their purpose and how they are performed, these practices are clearly not intended to achieve a complete elimination of all microorganisms.

In Veterinary Medicine, this issue still hasn't been a subject of robust research, which makes any new study important for the implementation of more efficient preventive measures - such as the control, identification and measurement of the causes of infections - with a view towards reducing contamination. The present study aimed to assess the level of bacterial contamination in the Small Animals Sector of one Veterinary Medical Teaching Hospital (HCV). The data collected was to be later used in making decisions to lower the number of cases of infection as well as to improve planning for hospital health and safety guidelines.

MATERIALS AND METHODS

Institution and data collection site

The Veterinary Medical Teaching Hospital (HCV) of the Universidade Federal do Rio Grande do Sul (UFRGS), which receives an average of 20,000 patient visits per year, was the object of study of this research. It is a teaching hospital that is run by staff composed by Professors, DVM, residents, interns, students and other employees, that are directly in contact with animals and the facilities in which they are treated or kept. In recent years, the HCV-UFRGS has registered some cases of bacterial infections arising from or following admission to the hospital environment. Consequently, a Disinfection Commission was established in 2016 to plan and oversee the control of hospital infections as well as pest and rodent problems.

The study began with the completion of a questionnaire by 4 members of the Disinfection Commission (three veterinarians and one collaborator) through which they were asked to assess the hygienic and sanitary status of the VH. They should also identify the most critical contamination sites, based on the history of infectious diseases available in Sector of Medical Archives (SMA), animal transit within the institution and the appearance of facilities in terms of hygiene. After analysing their answers, specific sites were chosen for being more critical for sample collection, in order to assess the level of environmental microbial contamination.

Sampling procedures for microbiological analysis

Environmental samples were taken from surfaces and air sedimentation. For surfaces, sterile swabs were moistened in 0.1% peptone water[1] and rubbed against them three times in different directions, within an area of 100 cm^2. The collected swabs were packed in 9 mL of 0.1% sterile peptone water[1], refrigerated and transported to the laboratory. As for the sedimentation of air, Plate Count Agar (PCA)[2] plates were used. These were left in each room for 20 min of direct exposure, then collected and sent to the laboratory.

Standard counting of viable strict and non-strict aerobic mesophilic microorganisms and collection by spontaneous sedimentation

For the plate count of surface swabs, we used the spread-plate technique by surface seeding 0.1 mL of each dilution (10-1 to 10-5) and spreading it evenly over the middle surface with a Drigalski handle. Plates were incubated at 36°C for 48 h. For the evaluation of the microbiological quality of air by spontaneous sedimentation, plates were incubated at 36ºC for 48 h. For reading, all PCA[2] plates containing between 25 and 250 colonies were selected for counting. Results of samples collected by swabbing were expressed in colony forming units per square centimetre (CFU/cm^2), whereas samples of air sedimentation were expressed in CFU per plate (CFU/plate).

Selection and identification of isolates via Matrix-Associated Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF/MS)

The criterion for bacteria selection was the presence of different colonial morphotypes on the

same culture plate; all morphotypes observed on each plate were used for the subsequent analyses. Selected colonies were seeded on Tryptic Soy Agar (TSA)[1] and incubated at 36ºC for 24 h. From this growth, one colony of each isolate was resuspended in 300 µL of ultrapure water and 900 µL of absolute ethanol. Initially, the samples were centrifuged and the supernatant discarded. After, 70% formic acid was added to the pellet and a new centrifugation step was performed. Subsequently, 1 µL of supernatant was pipetted onto a stainless steel plate and allowed to dry at room temperature. Subsequently 1 µL of the matrix was added. The analyses were performed with the MALDI Biotyper 4.0, MBT OC software[3].

Antimicrobial susceptibility test

For this test, the agar disk diffusion method was adopted, in accordance with the recommendations of the Clinical and Laboratory Standards Institute [3]. The assays were performed with cultures of the TSA[1] agar isolates obtained after incubation at 36°C for 24 h. From this growth, bacterial suspensions matching the turbidity of a McFarland 0.5 standard were seeded on the surface of plates containing Mueller-Hinton agar[1]. The tested antibiotics were: Ceftazidime 30 µg (CAZ 30)[4], Tetracycline 30 µg (TET 30)[4], Amikacin 30 µg (AMI 30)[4], Cephalothin 30 µg (CFL 30)[4], Vancomycin 30 µg (VAN 30)[4], Imipenem 10 µg (IPM 10)[4], Chloramphenicol 30 µg (CLO 30)[4], Ampicillin 10 µg (AMP 10)[4], Meropenem 10 µg (MER 10)[4] Plates were incubated at 36°C for 18 h, followed by the interpretation of the diameters of inhibition halos.

RESULTS

During the visit to the HCV it was noted that some hospital sectors have a greater volume of movement of employees, veterinarians, tutors and animals, such as the hospitalization sectors and the kennel treatment room. Hygiene procedures on the surfaces of tables in these places were done the correct way. However, in other sectors, no disinfection of surfaces after use was observed, except in service room 3. In service room 1 of the cattery, staff reported the execution of hygiene protocols on the examination table after each patient.

Positive points were identified through the field visit, in addition to the analysis of the questionnaire answers, namely: i) existence of the Disinfection Commission, ii) training of hospital staff; and iii) the standardization of products used for the cleaning and disinfection of all sectors of the HCV. Nevertheless, some negative points were also identified, such as: i) inconsistent periodicity of training, ii) difficulty in checking cleaning practices, iii) inconsistent maintenance of good hygiene practices and iv) a failure to carry out hygiene protocols in some environments after use. According to the responses to the questionnaire, these points are also considered important challenges by the Disinfection Commission.

After this analysis, we chose 16 sites for sample collection on the surfaces of various VH sectors. Facilities with higher numbers of patient visits and more intense animal transit were taken into consideration. In general, the VH was considered to be under good hygienic-sanitary conditions at the time of data collection (Table 1).

We have performed the mesophilic microorganisms count, which provide data that the VH is an important environmental reservoir of pathogens. There were no patients being attended in the treatment rooms 1 (SA1M), 2 (SA2M) at the time of data collection. In both these spaces, the examination table (DIM) and cage (DIGa) of the isolation room (DI), the cattery cage (GATGa) and the examination table of the x-ray (RXM), were all visually clean and sanitised. In Table 1 we can see that in SA1M and SA2M the counts from surface samples were higher while air sedimentation counts were within acceptable ranges, in accordance with the American Public Health Association [1]. These results show that an absence of animals and people in a particular area is associated with lower levels of microbial growth. On the other hand, it is also possible to conclude that cleaning practices on surfaces in these rooms were not performed satisfactorily. This is a different finding from those of other areas of which surface and sedimentation sample counts registered within acceptable standards. In treatment room 3 (SA3), and in a service room 1 of the cattery (GATSA1), the hygiene of the examination tables (SA3M) had been performed by the veterinarian after the last visit, and samples from these places revealed low counts (Table 1).

The examination tables of rooms 4 and 5 (SA4M and SA5M), and service room 2 of the cattery (GATSA2), as well as in the general kennel and cattery sections, sample collection was carried out while the veterinarians were attending animal patients. Nevertheless, the kennel (CANM) and cattery (GATMT) examination tables were not in use at the time of sample collec-

tion. The cleaning staff had sanitised these local after the last patient. While air sedimentation counts were high, surface scores were not; this data demonstrates that although the disinfection of surfaces was satisfactory, the ongoing patient visits that were underway in those environments could have contributed to the microbial growth gauged via sedimentation (Table 1). The examining tables of the ultrasound (US) and x-ray (RXM) rooms were not in use, but these had not been sanitised before sample collection. Thus, high counts were observed: 17.83 CFU/cm^2 and 43.59 CFU/cm^2, respectively. One can see that even surfaces which had been reported as sanitised presented microbial growth, even if most still remained within the standard of up to 2 CFU/cm^2 [1]. This fact may suggest that the products used for hygiene, as well as the adopted procedures, are proving to be efficient. In the analyses of air quality was observed high bacterial growth as observed in Table 1, except in the cattery facilities.

From the isolation of bacterial colonies with different morphotypes, 55 isolates were selected for identification. Of the 55 analysed isolates, 46 were identified and 10 of these were further precisely identified at the species level (Table 2), as well as one type of yeast. The other 29 were identified at the gender level and the remaining six were impossible to identify more precisely according to MALDI-TOF criteria.

In general, most of the isolates identified (60%) were bacteria of the genus *Staphylococcus* spp. Regarding antimicrobial susceptibility analysis the 10 bacteria identified at the species level were assessed. Test results showed that the isolates *S. aureus*, *S. epidermidis* and *S. haemolyticus* - collected from treatment room 2 - and *S. haemolyticus*, which had been isolated from samples from treatment room 2 of the cattery, presented multiresistance. *Pantoea ananatis* isolates from room 5 also showed a multiresistant profile for erythromycin, cephalothin, vancomycin and ampicillin. *Micrococcus luteus* isolates from the x-ray room and the kennel showed resistance to ceftazidime. *Staphylococcus equorum* isolates from room 4 were sensitive to all tested antimicrobials.

Table 1. Selected sample collection points at the Veterinary Medical Teaching Hospital (HCV) of the Universidade Federal do Rio Grande do Sul (UFRGS) and results of the standard count of strict and non-strict aerobic mesophilic microorganisms via surface swabs and spontaneous air sedimentation expressed in CFU/cm^2 and CFU/plate.

Sample site code	Facility/object of sample collection	Surface Swabs	Spontaneous air sedimentation
SA1M	Table Room 1	4 x 10^2	17.83
SA2M	Table Room 2	3.5 x 10^3	7.92
SA3M	Table Room 3	0	9.90
SA4M	Table Room 4	0	21.79
SA5M	Table Room 5	< 25*	99.07
DIM	Infectious disease room: treatment table	0	11.88
DIGa	Cage for hospitalization of animals with infectious diseases	0	-
RXM	X-ray room examination table	< 25*	43.59
RXA	Apron used in the X-ray room	< 25*	-
US	Ultrasound room examination table	0	17.83
CANM	Kennel treatment table	0	63.41
Cane	Kennel for hospitalization	< 25*	-
GATSA1	Examination table of room 1 of the cattery	0	5.94
GATSA2	Examination table of room 2 of the cattery	< 25*	5.94
GATMT	Treatment table of the cattery	< 25*	0
Cat	Cage for hospitalization at the cattery	0	-

*< 25: growth less than 25 CFUs. (-) Collection points which were not sampled.

Table 2. Identification of isolates at species-level by MALDI-TOF/MS.

Site and Collection Method	Microorganism Identification
Surface SA1M-1	*Staphylococcus haemolyticus*
Surface SA2M-3	*Staphylococcus aureus*
Surface SA2M-4	*Staphylococcus epidermidis*
SA2-4 sedimentation	*Staphylococcus epidermidis*
SA4-1 sedimentation	*Staphylococcus equorum*
SA5-1 sedimentation	*Pantoea ananatis*
RXM-2 surface swab	*Micrococcus luteus*
CAN-2 sedimentation	*Micrococcus luteus*
GATSA1-2 sedimentation	*Leclercia adecarboxylata*
GATSA2-3 surface swab	*Staphylococcus haemolyticus*

Database analysis performed by Biotyper 4.0 software MBT OC.

DISCUSSION

Regarding the analyses of the microbiological quality of the air rooms where there was greater circulation of people and animals, the growth of bacteria by sedimentation of the air was greater, except in the cattery facilities (Table 1). This data shows that microorganisms circulate in greater numbers where there is greater volume of human and animal transit. As recommendation for simple sedimentation plates is up to 30 CFU/cm^2, the results obtained in SA5M and in CANM are outside of acceptable standards [1].

It is important to note that in Brazilian legislation there are no official microbiological parameters for surfaces in a veterinary hospital environment. However, there are regulations for the microbiological quality of air in other environments, which forces institutions to adopt Resolution No. 9 as a guide for air quality studies [2]. The microorganisms present in the air are transient and variable, and the number and types of airborne agents is determined by the various sources of contamination in the environment. These microorganisms can be found in suspension, particulate matter and water droplets. Veterinary medical care tables are potentially contaminated by the animals handling, including those that sometimes defecate or urinate during their medical visit. Frequent handwashing is also known to be an important means of personal protection and disease prevention, although it is estimated that only 40% of practitioners do so routinely

Staphylococci are Gram-positive bacteria, being the higher abundant bacterial taxon in the skin microbiota. *Staphylococcus* spp. are resistant to variations in pH and desiccation, especially in exudates, and may remain present for weeks in a given environment, which could explain the high identification level of this genera in the present study [14].

The skin microbiota of pets is highly complex with large inter-individual variability and differences among skin sites [4]. Environmental variations could provide populational instability allowed important disease, such as folliculitis, otitis, pyoderma, opportunistic infections, urinary tract infections, impetigo and endocarditis.

Veterinary nosocomial infections have been recognised as increasing in frequency and, as in human medicine, the most common pathogens are *Staphylococcus* spp., *Enterococcus* spp., members of the *Enterobacteriaceae* family, *Clostridium difficile*, *Acinetobacter* spp. and *Pseudomonas* spp. [12].

Regarding antimicrobial susceptibility analysis the 10 bacteria identified at the species level were assessed. Test results showed that the isolates *S. aureus*, *S. epidermidis* and *S. haemolyticus* - collected from treatment room 2 - and *S. haemolyticus*, which had been isolated from samples from treatment room 2 of the cattery, presented multiresistance. That is to say resistance to 3 or more antimicrobial categories [10]. *Pantoea ananatis* isolates from room 5 also showed a multiresistant profile for erythromycin, cephalothin, vancomycin and ampicillin. To date, there have been no reports in the literature about *P. ananatis* being resistant to vancomycin; yet nosocomial infections in animals caused by *Enterococcus* spp. resistant to vancomycin, as well as the identification of animal carriers have already been described [7]. The detection of these bacteria resistant to this drug in veterinary hospitals is very worrisome and serves as a warning about the risks

to public health, since species such as *P. ananatis* may play an important role in the maintenance and spreading of factors which provide resistance to vancomycin. *Micrococcus luteus* isolates from the x-ray room and kennel showed resistance to ceftazidime. *Staphylococcus equorum* isolates from room 4 were sensitive to all tested antimicrobials.

These results revealed that the resistance frequency is higher in isolates of the genus *Staphylococcus* spp. The multiresistance of *Staphylococcus* is extremely important, both from a clinical and microbiological point of view, since bacterial resistance causes difficulties in the treatment of animals and multiresistant bacteria can spread to the wider environment [13]. There was an even greater prevalence of resistance to erythromycin (44%), tetracycline (33%), the ampicillin (55%) and ceftazidime (66%). The presence of the *Staphylococcus* spp. in different hospital environments is another point of concern, since it shows that these microorganisms have become persistent in these environments. Moreover, their antimicrobial resistance profiles demonstrate the difficulty to be faced in reducing their numbers [14].

It is important to highlight that in this study resistant bacteria were isolated from the environment and not from animals under treatment. This is a compelling observation because it draws attention to the movement of environmental bacteria with multidrug resistance patterns. In turn, this raises the alarm against the indiscriminate use of antimicrobials, which allows for the natural selection of resistant bacteria. Furthermore, the abuse and indiscriminate use of antimicrobial agents in human and veterinary clinical practice, especially in hospital settings, promote the natural selection of antibiotic resistant strains, such as *Staphylococcus* spp. *S. aureus* and *S. pseudointermedius* in particular are extremely versatile in developing resistance to antimicrobial agents. This supports their survival in hospital settings and its diffusion among patients [11]. The multiresistant profile that was observed in some isolates becomes even more alarming when antibiotics such as ceftazidime fail to be effective, since it is a third generation antimicrobial, highlighting the increase of more scarce therapeutic options [5,8].

The consequences of inappropriate antimicrobial use in small animals do not differ from those in human medicine: the quantities and standards of use determine the rate of appearance of resistant strains. Several retrospective studies have reported an increased prevalence of resistance in different bacteria isolates from companion animals [7,12,15]. In London, Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 17.9% of the employees of a veterinary hospital and in 9% of hospitalised dogs; these samples were resistant and related to isolates from human hospitals [9]. In Germany, 869 samples from small animals at a veterinary medical school were studied and MRSA was detected in 18 dogs, four cats, a guinea pig, a rabbit, an aquatic turtle and a bat [16].

Microorganism counts were high in some of the veterinary hospital environments tested, indicating that current disinfection and hygiene practices are not sufficient to control the establishment of these microorganisms at the study sites. In view of this, it is reasonable to conclude that permanent monitoring and assessment of the effectiveness of hygiene protocols is needed in different sectors of the hospital. This may be an essential tool in a preventive approach to stop the spread of selectively resistant microorganisms, as well as cases of hospital infections. In addition, continuous staff training and awareness of the importance of personal and environmental hygiene is vital for minimizing the presence of these microorganisms in hospitals and avoid their transmission to patients. Finally, a more systematic hygiene guideline should be implemented in areas that showed higher counts.

CONCLUSION

With this work it was possible to identify mandatory and important points of contamination within one Veterinary Medical Teaching Hospital. *Staphylococcus* spp. were isolated in various environments and the resistance profile was observed. The existence of a Standing Committee for infection control is necessary in Veterinary Hospitals in order to stablish guidelines and recommendations according to each particular situation.

MANUFACTURERS

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Acknowledgements. The research team would like to thank the Veterinary Hospital of Universidade Federal do Rio Grande do Sul, for allowing us to carry out our study at their institution.

Declaration of interest. The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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