PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 41:e06706, 2021
DOI: 10.1590/1678-5150-PVB-6706

Original Article
Small Animal Diseases
BY-NC

Detection of the main multiresistant microorganisms in the environment of a teaching veterinary hospital in Brazil[1]

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ABSTRACT.- Sfaciotte R.A.P., Parussolo L., Melo F.D., Bordignon G., Israel N.D., Salbego F.Z., Wosiacki S.R. & Ferraz S.M. 2021. **Detection of the main multiresistant microorganisms in the environment of a teaching veterinary hospital in Brazil**. *Pesquisa Veterinária Brasileira 41:e06706, 2021*. Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: sfaciotte@gmail.com

Contamination of the veterinary hospital environment with multiresistant pathogens endangers not only hospitalized animals, but also the workplace safety of veterinarians and nurses, animal guardians and, when in case of a teaching hospital, veterinary students. The objective of this study was to map the main points of bacterial contamination of a veterinary teaching hospital in Brazil to identify multiresistant microorganisms and their antimicrobial resistance genes. Samples were collected from 39 different locations of a veterinary school hospital which comprised a pool according to each hospital environment. In certain environments, more than one pool has been collected. All samples were collected in quadruplicates for the selective isolation of the main multiresistant microorganisms: methicillin-resistant *Staphylococcus* (MRS), vancomycin resistant *Enterococcus* (VRE), cephalosporinases and/or extended-spectrum beta-lactamase-producing Gram-negative bacteria (ESBL) and Carbapenemase-producing (CP). After isolation and identification of isolates, multiplex-PCR reactions were performed to detect the main genes for each microorganism and antimicrobial susceptibility tests with the main antibiotics used for each bacterial group according to CLSI. Of the 39 veterinary teaching hospital sites collected, all (100%) had at least one of the microorganisms surveyed, and 17.95% (n=7) of the sites were able to isolate the four pathogens. From the 94 pools collected, it was possible to isolate MRS in 81.91% (n=77), VRE in 12.77% (n=12), cephalosporinases and/or ESBL in 62.77% (n=59) and CP in 24.47%. (n=23). Regarding MRS, the *mec*A gene was detected in all isolates. All isolated VREs were identified as *Enterococcus faecalis* and presented the *van*A gene. Regarding cephalosporinases and/or ESBL, 89.83% (n=53) of the isolates presented the *bla*TEM gene, 57.63% (n=34) the *bla*OXA-1 gene, 37.29% (n=22) *bla*CTX-M gene from some group (1, 2, 9 ou 8/25) and 20.34% (n=12) the *bla*SHV gene. It was possible to identify the main microorganisms responsible for causing nosocomial infections in humans (VRE, MRS, ESBL and CP) in the veterinary hospital environment, suggesting a source of infection for professionals and students of veterinary medicine, placing a high risk for public health.

INDEX TERMS: Environment, ESBL, multiresistant, MRS, public health, Brazil.

[1] Received on April 29, 2021.
Accepted for publication on June 7, 2021.

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RESUMO.- [**Detecção dos principais microrganismos multirresistentes no ambiente de um hospital veterinário de ensino no Brasil.**] A contaminação do ambiente hospitalar veterinário com patógenos multirresistentes coloca em perigo não apenas os animais hospitalizados, mas também a segurança no local de trabalho de veterinários e enfermeiros, responsáveis por animais e, quando se tratar de um hospital de ensino, estudantes de veterinária. O objetivo deste estudo foi mapear os principais pontos de contaminação bacteriana de um hospital veterinário de ensino no Brasil, identificando microorganismos multirresistentes e seus genes de resistência antimicrobiana. As amostras foram coletadas em 39 locais diferentes de um hospital de escola veterinária, que compreendia um *pool* de acordo com o ambiente de cada hospital. Em certos ambientes, mais de um pool foi coletado. Todas as amostras foram coletadas em quadruplicados para o isolamento seletivo dos principais microorganismos multirresistentes: *Staphylococcus* resistente à meticilina (MRS), *Enterococcus* resistente à vancomicina (VRE), bactérias Gram-negativas produtoras de cefalosporinases e/ou beta-lactamase de espectro estendido (ESBL) e produtoras de carbapenemase (PC). Após o isolamento e identificação dos isolados, foram realizadas reações de PCR multiplex para detectar os principais genes de cada microorganismo e testes de susceptibilidade a antimicrobianos com os principais antibióticos utilizados para cada grupo bacteriano de acordo com o CLSI. Dos 39 locais do VCH coletados, todos (100%) possuíam pelo menos um dos microrganismos pesquisados e 17,95% (n=7) dos locais foram capazes de isolar os quatro patógenos. Dos 94 *pools* coletados, foi possível isolar MRS em 81,91% (n=77), VRE em 12,77% (n=12), ESBL em 62,77% (n=59) e carbapenemases em 24,47% (n=23). Em relação ao MRS, o gene *mec*A foi detectado em todos os isolados. Todos os VREs isolados foram identificados como *Enterococcus faecalis* e apresentaram o gene *van*A. Em relação às cefalosporinases e/ou ESBL, 89,83% (n=53) dos isolados apresentaram o gene *bla*TEM, 57,63% (n=34) o gene *bla*OXA-1, 37,29% (n=22) o gene *bla*CTX-M de algum grupo e 20,34% (n=12) o gene *bla*SHV. Foi possível identificar os principais microrganismos responsáveis por causar infecções nosocomiais em humanos (VRE, MRS, ESBL e CP) no ambiente hospitalar veterinário, sugerindo uma fonte de infecção para profissionais e estudantes de medicina veterinária, colocando alto risco para a saúde pública.

TERMOS DE INDEXAÇÃO: Ambiente, ESBL, multirresistentes, MRS, saúde pública, Brasil.

INTRODUCTION

The use of antimicrobial agents is essential for the treatment of bacterial infections in human and veterinary medicine, and indiscriminate and often reckless use in veterinary medicine is an additional risk factor for selective pressure and the emergence of multiresistant microorganisms. Most of multiresistant animal pathogens can colonize and/or infect humans by direct or indirect contact through the environment (Costa et al. 2013). In view of this, the World Health Organization recognizes bacterial multidrug resistance to antimicrobials as an essential and important public health issue, since human health is closely linked to the health of animals and the environment in which they live (Robinson et al. 2016).

Hospital infections (HI) are those acquired by the patients during the hospitalization period, whether human or animal patient. In human medicine there is already a lot of studies about HI, with an estimated 5% of patients developing a hospital-acquired infection each year, however, in veterinary, these numbers are scarce (Klevens et al. 2007, Stull & Weese 2015). For Stull & Weese (2015), the greatest challenges of animal hospital infections are associated with patient hygiene, wound licking and less established infection control programs.

The environment has a strong connection with cases of nosocomial infection in human medicine, but in veterinary medicine, although there are some studies describing the environmental contamination associated with hospital infections, there is not much information about this link, since no effective data about these infections (Schaer et al. 2010, Ekiri et al. 2010), especially in Brazil.

Some critical points of contamination are: door handles, light switches, computers, cell phones (Fraser & Girling 2009, Bender et al. 2012), cage doors, stethoscopes, thermometers, mouth gags (Ghosh et al. 2012) and especially the hands of doctors and staff, who, when sanitation and disinfection fail, increase the chances of nosocomial infections (Weese 2012).

Contamination of the veterinary hospital environment with multi-resistant pathogens endangers not only hospitalized animals, but also safety in the workplace of veterinarians and nurses, animal tutors and, in the case of a teaching hospital, veterinary students (Walther et al. 2014). This risk is due to the fact that most of these pathogens are transmissible between animals and humans, according to Wieler et al. (2015).

As in human medicine, the main pathogens associated with the hospital infections in veterinary medicine are: methicillin-resistant *Staphylococcus* (MRS), vancomycin-resistant *Enterococcus* (VRE), as well as Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* producing cephalosporinases and/or extended spectrum beta-lactamases (ESBL) and carbapenemase producers (CP) (Müller et al. 2014, Wieler et al. 2015, Stull & Weese 2015). Thus, the objective of this study was to map the main points of bacterial contamination of a teaching veterinary hospital in Brazil, identifying multiresistant microorganisms and their antimicrobial resistance genes.

MATERIALS AND METHODS

Ethics statement. The experiment was conducted at the "Centro de Diagnóstico Microbiológico Animal" (CEDIMA) of the "Universidade do Estado de Santa Catarina" (UDESC), Lages/SC, Brazil, in compliance with the Ethics Principles in Animal Experimentation, being approved by the Ethics Committee on Animal Experimentation (CEUA-UDESC) (Protocol 2042290617).

The Brazilian veterinary medical teaching hospital of "Universidade do Estado de Santa Catarina" (CAV-UDESC) treats approximately 5000 animals per year. Responsible for attending the Veterinary Clinic Hospital (VCH) are teachers and veterinarians, as well as residents of small animals' clinic and surgery assisted by interns, fellows and nurses. In its structure, the VCH is basically composed of the reception, outpatient clinics, emergency room, kennel, solarium, cattery, feline care office, vaccine room, X-ray room, report room, postoperative, preoperative and trichotomy, physiotherapy rooms, sterilization, operating rooms, kitchen, anesthesia room, men's and women's locker room, men's and women's restroom, clinical analysis laboratory and corridors.

Sample characterization. The collection was performed in September 2017, in a single day, and the samples were collected with swabs moistened with sterile distilled water. Each sample comprised a pool according to each hospital environment. In certain environments, more than one pool was collected, which was defined taking into account the size of the environment and the flow of animals and people from the site. The pools included: surface samples (procedure table, floor, cabinets, scale), instruments (stethoscopes, otoscope, thermometers, tracheotubes), door handles, sinks, among others (feed and water pot, collars, muzzles, Elizabethan collar, cages) according to each environment. In total 94 pools from 39 distinct VCH environments were collected. All samples were collected in quadruplicate for selective isolation of the main multiresistant microorganisms: methicillin resistant *Staphylococcus* (MRS), vancomycin-resistant *Enterococcus* (VRE), Gram negative cephalosporinase-producing and/or extended-spectrum beta lactamase (ESBL) bacteria and Carbapenemase-producing bacteria.

Methicillin resistant *Staphylococcus* (MRS). One of the environmental swab samples were placed in a tube containing 2ml of BHI with 7.5% NaCl and incubated at 37°C for 24 h. After incubation, samples were seeded on Mueller Hinton agar containing 4% NaCl and 6mg/L oxacillin and incubated again at 37°C for 24 h. Disk diffusion test including oxacillin and cefoxitin was performed with the MRS isolates obtained. Those resistant to oxacillin and/or cefoxitin were phenotypically characterized by Gram stain, catalase, oxidase, rabbit plasma coagulase, urease, Voges-Proskauer (VP) test, sucrose and trehalose fermentation and polymyxin B resistance. To confirm the identification of coagulase-positive *Staphylococcus* species, the multiplex PCR technique was performed as described by Sasaki et al (2010).

To detect the beta-lactam resistance genes (*mec*A, *mec*C and *bla*Z) present in MRS isolates, Nakadomari et al. (2019) multiplex PCR technique was performed. *Staphylococcus aureus* ATCC 43330 reference strain was used as a positive control to all genes.

Isolates confirmed as MRS were subjected to vancomycin resistance testing using Etest® (Biomerieux) strips for possible identification of vancomycin resistant *Staphylococcus* (VRS) or vancomycin intermediate resistance *Staphylococcus* (VIS).

Vancomycin-resistant *Enterococcus* (VRE). One of the swab collected from the environment was placed in Enterococcosel Broth with 6µg/mL vancomycin and incubated at 37°C for 48 h. After the incubation period, the sample was seeded on Blood agar containing vancomycin discs and incubated overnight at 35°C. Colonies that showed growth inside the inhibition zone were selected for Gram and catalase testing to confirm *Enterococcus* isolation.

Two Multiplex PCR techniques described by Depardieu et al (2004) were performed, one for identification of the two main species of *Enterococcus* (*E. faecium* and *E. faecalis*) and another for detection of vancomycin resistance genes (*van*A, *van*B, *van*C, *van*D, *van*E e *van*G). *Enterococcus faecium* (ATCC 51299) and *Enterococcus faecalis* (ATCC 700221) reference strains were used to assure quality control for the detection (Fundação Oswaldo Cruz - Fiocruz). *Escherichia coli* ATCC 25922 reference strain was used as a negative control.

Gram negative cephalosporinase-producing and/or extended-spectrum beta lactamase (ESBL). Another swab collected from the environment was placed in 15mL of BHI Broth containing ceftriaxone disc (30mcg) and incubated at 37°C for 24 h. Afterwards, the samples were plated on MacConkey agar and incubated at 37°C for 24 h. Then, we screened positive samples for bacterial growth. In order to detect cephalosporinase-producing and/or ESBL-producing isolates, we performed disk approximation testing according to Souza-Junior et al. (2004) recommendations. Positive isolates to cephalosporinase-producing and/or ESBL-producing were identified with Gram-negative Bactray system kit (Laborclin®, Vargem Grande, Brazil).

Isolates that exhibited positive cephalosporinase-producing and/or ESBL-producing phenotypic profile were further investigated to verify the presence of resistance genes. For that, we performed two conventional multiplex PCRs: one to detect the genes Bla_{TEM} (and variants), Bla_{SHV} (and variants), and Bla_{OXA-1}; and the other to disclose Bla_{CTX-M} genes of Groups 1, 2, and 9. In addition, a conventional PCR was conducted to identify Bla_{CTX-M} genes of groups 8 and 25, using specific primers. All reactions were according to the Dallenne et al. (2010) protocols. *Klebsiella pneumoniae* CCBH5991 and *K. pneumoniae* CCBH15948 reference strains were used to assure quality control for the detection of Bla_{TEM+}, Bla_{SHV+}, Bla_{CTX-M+}, and Bla_{OXA+} genes (Fundação Oswaldo Cruz - Fiocruz). *Escherichia coli* ATCC 25922 reference strain was used as a negative control.

Gram negative carbapenemase-producing. To detect carbapenemases, swabs collected from the environment were placed in 10mL of BHI Broth with ertapenem and ampicillin discs and incubated at 37°C for 12 to 16 h. Then the samples were seeded on MacConkey agar where ertapenem discs were placed at the beginning and end of the streak and incubated at 37°C for 24 h.

Colonies growing within the ertapenem zone diameter of 27mm or less were identified with Gram-negative Bactray system kit (Laborclin®, Vargem Grande, Brazil). To confirm carbapenemase-producing Enterobacteria, tests were performed according to Technical Note No. 01/2013 of the "Agência Nacional de Vigilância Sanitária" (ANVISA 2013). For microorganisms not belonging to the Enterobacteriaceae family, the antimicrobial susceptibility test was performed with the main carbapenem discs (meropenem, ertapenem and imipenem).

The isolates suggestive of carbapenemase production were submitted to conventional multiplex PCR techniques to detect *GES, IMP, VIM, KPC* and *OXA-48* genes according to Dallenne et al. (2010), as well as for *SPM* gene detection according to Sader et al. (2005) and the *NDM* gene. CCBH5991 *Klebsiella pneumoniae* (KPC), CCBH23559 *K. pneumoniae* (OXA-48), CCBH15948 *K. pneumoniae* (NDM) and CCBH23579 *Pseudomonas aeruginosa* (SPM) reference strain were used as a positive control. In addition, detection of *amp*C genes (FOX, MOX, ACC, CMY, DHA, LAT, CIT and EBC) was performed according to Dallenne et al. (2010).

Resistance profile. After isolation of multiresistant microorganisms, antimicrobial susceptibility testing was performed by disk diffusion test with the antibiotics used for each microorganism group according to the animal Clinical and Laboratory Standards Institute VET08 ED4 (CLSI 2018) and the human CLSI M100 ED29 (CLSI 2019) to establish the resistance profile of each of them. *E. coli* ATCC 25922 reference strain was used as quality control to determine susceptibility to antimicrobial agents.

Bacterial DNA extraction. Genomic DNA extraction of the isolates and reference strains was performed in accordance to previous protocol for Parussolo et al. (2019). DNA concentration measurements were performed on Nanodrop (Thermofisher, Waltham, USA). To perform PCR tests, DNA concentration was adjusted to 15-100ng.

RESULTS

Of the 39 VCH sites that were collected from the environment, all (100%) had at least one of the microorganisms surveyed (MRS, VRE, ESBL and carbapenemase), and 17.95% (n=7) of the sites were able to isolate the four pathogens, as shown in Table 1. In four places, only one microorganism was identified: teachers' room (ESBL), outpatient 3 (MRS), operating room 1 (MRS) and the operating room corridor 1 (MRS).

From the 94 pools collected, it was possible to isolate MRS in 81.91% (n=77), VRE in 12.77% (n=12), ESBL in 62.77% (n=59) and CP in 24.47%. (n=23). The number of bacterial isolates may differ from the number of microorganisms per environment, since several pools were collected from each of the 39 veterinary hospital locations, according to size. The identification of microorganisms is shown in Table 2.

Regarding MRS, the *mec*A gene was detected in all phenotypically identified isolates (n=77) and in 31.17% (n=24) the *bla*Z gene (three methicillin-resistant *Staphylococcus aureus* - MRSA, and 21 methicillin-resistant *Staphylococcus pseudintermedius* - MRSP). The *mec*C gene was not identified in any isolate. The antimicrobials nitrofurantoin (10.39%, n=8), chloramphenicol (11.36%, n=9), amikacin (15.58%, n=12), doxycycline (19.48%, n=15) and rifampicin (20.78%, n=16) had the lowest resistance indices in MRS isolates, unlike erythromycin (88.31%, n=68), norfloxacin (66.23%, n=51), sulfazotrim (63.64%, n=49), enrofloxacin (62.34%, n=48) and ciprofloxacin (61.04%, n=47) which had the highest rates. Two MRSP isolates showed phenotypically clindamycin-induced resistance with halo formation in "D" along with erythromycin. No MRS isolates showed resistance to vancomycin by Etest.

All isolated VRE (n=12) were identified as *Enterococcus faecalis* and all presented the *van*A gene. One of the isolates, detected in kennel 2, showed resistance to all antimicrobials tested (n=16), including the high concentration of gentamicin, being identified as high-level aminoglycoside resistance (HLAR). The fluorquinolone class presented the highest resistance against VRE (between 50 and 66.67%), while penicillin G and ampicillin had the lowest indices (8.33%, n=1).

Regarding cephalosporinases and/or ESBL, 89.83% (n = 53) of the isolates presented the *bla*TEM gene, 57.63% (n=34) the *bla*OXA-1 gene, 37.29% (n=22) *bla*CTX-M gene from some group and 20.34% (n=12) the *bla*SHV gene. No isolates of *Pseudomonas aeruginosa* and *E. coli* presented the *bla*SHV gene.

Carbapenemase-positive isolates are arranged in Table 3 according to bacterial identification, the site the microorganism was identified, the resistance genes detected, and the antimicrobial classes to which the pathogen was resistant. Table 1 shows the number of places contaminated with carbapenemase-producing bacteria and Table 3 shows how many isolates. The largest amount of microorganisms in Table 3 is because in some environments more than one microorganism was isolated.

Table 1. Teaching veterinary hospital environments that were collected and the microorganisms isolated in each one of them

Site	MRS	VRE	ESBL	CP
Kennel 1	+	+	+	+
Kennel 2	+	+	+	+
Kennel corridor	+	N.F	+	+
Maternity	+	N.F	+	N.F
Physiotherapy room	+	N.F	+	N.F
Postoperative room	+	N.F	+	+
Medication room	+	N.F	+	N.F
Cattery 1 (doctor's office)	+	+	+	+
Cattery 2 (internment)	+	+	+	+
Solarium	+	+	N.F	N.F
Stitching room	+	N.F	+	N.F
Kitchen	+	+	+	+
Ladies room	+	N.F	+	+
Men's room	+	N.F	+	+
Reception hallway	+	+	+	N.F
Hallway teachers rooms	N.F	N.F	+	N.F
Vaccine room	+	N.F	+	N.F
Women's locker room	+	N.F	+	N.F
Men's locker room	+	N.F	+	N.F
Clinical pathology corridor	+	N.F	+	+
Preoperative room	+	+	+	+
Hall sterilization room	+	N.F	+	N.F
Emergency office	+	N.F	+	N.F
Ambulatory 1	+	N.F	+	+
Ambulatory 2	+	+	+	N.F
Ambulatory 3	+	N.F	N.F	N.F
Ambulatory 4	+	N.F	+	+
Outpatient corridor	+	N.F	+	+
Surgical technique	+	N.F	+	+
X-ray room	+	N.F	+	N.F
X-ray report room	+	N.F	+	N.F
Anesthesiology room	+	N.F	+	N.F
Sterilization room	+	N.F	+	+
Operating room 1	+	N.F	N.F	N.F
Operating room 2	+	N.F	+	+
Operating room 3	+	N.F	+	N.F
Operating room corridor 1	+	N.F	N.F	N.F
Operating room corridor 3	+	N.F	+	N.F
Reception	+	+	+	+
TOTAL	38	10	35	19

MRS = methicillin resistant *Staphylococcus,* VRE = vancomycin resistant *Enterococcus,* ESBL = extended spectrum beta-lactamase, CP = carbapenemase, N.F = microorganism not found; (+) microorganism found.

Table 2. Identification of microorganisms isolated from teaching veterinary hospital environments

MDR	Microorganism	Sites (%) n = 39	Pool (%) n = 94
MRS	*Staphylococcus pseudintermedius*	11 (28.20%)	11 (11.70%)
	Staphylococcus aureus	37 (94.87%)	66 (70.21%)
	Total	38 (9744%)	77 (81.91%)
VRE	*Enterococcus faecalis*	10 (25.64%)	12 (12.77%)
	Total	10 (25.64%)	12 (12.77%)
ESBL	*Acinetobacter baumannii*	8 (25.31%)	10 (10.64)
	Escherichia coli	5 (12.82%)	5 (5.32)
	Pseudomonas aeruginosa	17 (43.59%)	18 (19.15%)
	Serratia sp.	18 (46.15%)	26 (27.66%)
	Total	35 (89.74%)	59 (62.77%)
CP	*Acinetobacter baumannii*	14 (35.90%)	15 (15.96%)
	Pseudomonas aeruginosa	5 (12.82%)	5 (5.32%)
	Serratia sp.	3 (7.69%)	3 (3.19%)
	Total	19 (48.72%)	23 (24.47%)

MDR = multidrug resistente, MRS = methicillin resistant *Staphylococcus,* VRE = vancomycin resistant *Enterococcus,* ESBL = extended spectrum beta-lactamase, CP = carbapenemase.

DISCUSSION

Due to the very close proximity of dogs and cats to humans, care for these animals has significantly increasing their life quality and expectation. With this proximity, these animals become important sources of transmission of antimicrobial resistance genes to humans either directly or even indirectly through the environment (Hordijk et al. 2013, De Briyne et al. 2014),

The environment has a great connection with cases of hospital infection in human medicine, but in veterinary medicine, there is not much information about this connection even though it exists (Schaer et al. 2010, Ekiri et al. 2010). Although this study also does not show the relationship between environment and nosocomial infections, the identification of the main microorganisms responsible for these infections throughout the veterinary hospital environment in the present study, raises a warning regarding the spread of these pathogens to humans.

The flow of people inside a hospital is very large and the daily routine makes the rotation of patients frequent, which favors a heterogeneous contamination of the environment. In a veterinary school hospital there are still two other aggravating factors that contribute to this pathogen heterogeneity a) the patient is always accompanied by his guardian, who, due to the emotional attachment to the animal, is accompanied by other family members and b) for being a hospital school, student turnover (from various regions of the state and even the country) is regular. The presence of different microorganisms with different antimicrobial resistance genes identified in the environment of this study may suggest this heterogeneous contamination from different sources.

The influx of multiresistant microorganisms within a veterinary hospital varies significantly according to their location, size, range of services offered and group of admitted patients (Donker et al. 2012), i.e. referral hospitals, as in the case of VCH have a higher risk of contamination by these resistant pathogens.

According to Table 3, if we look at the pools to which carbapenemase-producing microorganisms have been identified, most of them have been isolated from door handles, cabinet knobs and light switches, which are considered critical points of contamination according to Bender et al. al. (2012), which suggest contamination through people's hands. According to Weese (2012), when there is no proper hygiene and disinfection of the hands of health professionals (and in this case students and owners too), the chances of environmental contamination and possible hospital infections increase.

The environment of the veterinary hospital not only serves as a vector for contaminating the animals hospitalized there, but also as a source of infection for veterinarians, staff, students and animal guardians, being a major public health problem. According to Walther et al. (2017), people who work in the veterinary hospital have great potential to acquire diseases of animal patients (zoonoses), besides being colonized by multiresistant microorganisms present in the environment.

Schaufler et al. (2015), detected ESBL-producing *Escherichia coli* strains in feces of clinically infected dogs in the vicinity of the hospital, showing environmental contamination and

Table 3. Identification of carbapenemase-producing microorganisms in the teaching veterinary hospital environment associated with antimicrobial susceptibility profile and detection of carbapenemic resistance genes

Site	Pool	Microorganism	Antimicrobial resistance	Genes
Post operative	Table, door handle, bracket and switch	*Acinetobacter baumannii*	BL/AG/TET/NIT/SUL/AMP	OXA-48
Post operative	Floor	*A. baumannii*	BL/NIT/SUL/AMP	OXA-48
Inpatient cattery	Table, bottles and cabinets	*A. baumannii*	BL/AG/NIT/SUL	KPC and ampC (ACC)
Kitchen	Floor and handles	*A. baumannii*	BL/AG/NIT	OXA-48 and ampC (ACC)
Kitchen	Table, sink, fridge and stove	*A. baumannii*	BL/AG/FQ/TET/NIT	OXA-48 and ampC (MOX)
Clinical pathology corridor	door handle, switch and cabinets	*A. baumannii*	BL/AG/NIT	OXA-48 and ampC (FOX)
Ambulatory corridor	door handle, cabinets and switch	*A. baumannii*	BL/FQ/NIT/SUL/AMP	OXA-48 and ampC (FOX)
Ambulatory 1	Table, door handle, sink and cabinets	*A. baumannii*	BL/NIT/AMP	OXA-48 and ampC (FOX)
Dog kennel	Cages	*A. baumannii*	BL/AG/FQ/NIT/SUL/AMP	NDM
Men's room	Sink, door handle and switch	*A. baumannii*	BL/NIT/SUL	KPC and ampC (ACC)
Ladies room	Sink, door handle and switch	*A. baumannii*	BL/AG/FQ/TET/NIT/SUL	OXA-48 and ampC (MOX)
Emergency	Table, sink, cabinet and door handle	*A. baumannii*	BL/AG/FQ/TET/NIT/SUL/AMP	OXA-48
Ambulatory 4	Table and countertops	*A. baumannii*	BL/AG/TET/NIT	OXA-48 and ampC (FOX)
Surgical technique	Tables, sink, cabinets and air control	*A. baumannii*	BL/FQ/NIT/SUL/AMP	OXA-48
Operating room 2	Floor	*A. baumannii*	BL/FQ/NIT/SUL/AMP	OXA-48 and ampC (MOX)
Kennel 2	Pots of water and feed	*Pseudomonas aeruginosa*	BL/AG/FQ/TET/NIT/SUL/AMP	ampC (LAT)
Kennel corridor	Switch and door handle	*P. aeruginosa*	BL/FQ/NIT/SUL/AMP	OXA-48 and ampC(MOX)
Cattery (office)	Table, sink and bottles	*P. aeruginosa*	BL/FQ/NIT/SUL/AMP	KPC and ampC (ACC)
Sterilization	Sink and cabinets	*P. aeruginosa*	BL/FQ/NIT/SUL/AMP	ampC (DHA)
Reception	Floor	*P. aeruginosa*	BL/AG/FQ/TET/NIT/SUL/AMP	KPC and ampC (ACC)
Kennel 2	Table, cabinet handles	*Serratia* sp.	BL/AG/FQ/NIT/SUL/AMP	NDM and ampC (MOX)
Kennel corridor	Floor	*Serratia* sp.	BL/AG/FQ/TET/NIT/SUL/AMP	NDM and ampC (MOX)
Preoperative	Sink, faucet, dryer	*Serratia* sp.	BL/AG/FQ/TET/NIT/SUL/AMP	OXA-48 and ampC (AAC)

BL = beta-lactam antibiotics, AG = aminoglycosides, FQ = fluoroquinolones, TET = tetracycline, SUL = sulfonamide, AMP = amphenicols, NIT = nitrofurans.

possible risk factors for contamination of other patients. Although this study does not relate these environmental pathogens to clinically infected animals, the isolation not only of ESBL, but also of MRS, VRE and CP evidences the concern with the cleaning and disinfection of the hospital environment. In Brazil, veterinary hospitals, unlike human hospitals, do not have the obligation of a hospital infection control committee, which would have as its function, detect these pathogens in the environment and draw up an appropriate disinfection protocol.

MRSP and cephalosporinase and/or ESBL producing *E. coli* are the main multiresistant microorganisms often isolated in veterinary medicine (Perreten et al. 2010, Rubin & Pitout 2014), but pathogens such as *Pseudomonas aeruginosa* and *Enterococcus*, as well as MRSA (Vincze et al. 2014), carbapenemase-producing enterobacteria (Abraham et al. 2014) and *Acinetobacter baumannii* (Endimiani et al. 2011) are emerging as major causes of nosocomial infection in in small animal medicine. In the present study, all these microorganisms were detected in the hospital environment, either in environments with high flow of sick animals or even in places where animals do not have access, suggesting a spread of these pathogens within the hospital itself.

Generally, the bacteria involved in nosocomial infections have multidrug resistance to antimicrobials, especially those most commonly used in the hospital in question, which further complicates the treatment and improvement of the patient increasing the chances of procedures such as euthanasia in the animal (Stull & Weese 2015). In addition, public health problems increase this concern about these microorganisms due to their transmission to humans and the increasing use of antimicrobials in animals that are restricted to human use, such as carbapenemics (Guardabassi & Prescott 2015). The isolation of carbapenemase-producing microorganisms in 48.72% (n=19) of VCH environments and 24.47% of all samples collected evidenced this concern with the dissemination of these pathogens to exposed humans and their possible transmission to the community.

The high consumption of antimicrobials from the beta-lactam and fluorquinolone classes establish a selective pressure on the microorganisms, especially in MRSA, MRSP and ESBL (Damborg et al. 2011, Guardabassi et al. 2013), however these antimicrobials are indispensable for the treatment of urinary and skin infections in animals (Hillier et al. 2014). In the present study, all groups of microorganisms (MRS, VRE, ESBL and CP), the fluorquinolone class antimicrobials were one of the most resistant, which can be explained by the high use of enrofloxacin in patients, corroborating the above.

Despite the need for further research on environmental contamination research in veterinary hospitals in Brazil, the present study suggests intrinsic contamination with a diversity of multiresistant microorganisms in this type of environment. According to Stull & Weese (2015), the variety of animal species treated within a veterinary hospital is large, and each species has a specific microbiota and distinct contamination risks, which increases the heterogeneity of microorganisms contaminating the environment.

One of the main limiting factors of the research was not being able to carry out a genetic mapping of the microorganisms isolated in each environment to know the diversity of bacterial clones present and to try to trace a possible route of contamination of the entire hospital environment.

Therefore, it is essential to establish procedures to prevent the spread of multiresistant microorganisms in the hospital environment, such procedures include: an effective disinfection protocol that respects animal welfare (Burgess & Morley 2015), screening of patients for colonization multiresistant pathogens, decolonization of veterinarians, teachers and students, and epidemiological surveillance of the environment. According to this study, the lack of these protocols associated with the lack of knowledge on the subject, neglect the spread of antimicrobial resistance genes and hinder the control of these bacteria.

CONCLUSIONS

It was possible to identify the main microorganisms responsible for causing nosocomial infections in humans (VRE, MRS, ESBL and CP) in the studied veterinary hospital environment, suggesting a source of infection for veterinary professionals and students, placing a great risk to public health.

The most isolated microorganism was MRS, followed by ESBL, CP and VRE.

All hospital environments had at least one MDR, showing the difficulty of controlling the spread of these pathogens in the environment even more when knowledge and information about the subject is lacking in the area.

Thus, lack of disinfection protocols for multidrug-resistant bacteria can facilitate their spread, making it difficult to treat infected animals and favoring the colonization of human, as well as favoring their spread to the community.

Acknowledgements.- We thank "Fundação Oswaldo Cruz" (Fiocruz) and, the "Instituto Adolfo Lutz" for providing the reference strains.

Funding.- This work was supported by the "Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina" (FAPESC).

Conflict of interest statement.- The authors have no competing interests.

REFERENCES

Abraham S., Wong H.S., Turnidge J., Johnson J.R. & Trott D.J. 2014. Carbapenemaseproducing bacteria in companion animals: a public health concern on the horizon. J. Antimicrob. Chemother. 69(5):1155-1157. <https://dx.doi.org/10.1093/jac/dkt518> <PMid:24398342>

ANVISA 2013. Medidas de prevenção e controle de infecções por enterobactérias multiresistentes. Nota Técnica n° 1/2013, Agência Nacional de Vigilância Sanitária, Brasília, DF. Available at <http://portal.anvisa.gov.br/documents/33852/271858/Nota+t%C3%A9cnica+n%C2%BA+01+de+2013/5be89853-7eca-4b4b-98e4- 5096b9f5a2ec> Accessed on Dec. 20, 2019.

Bender J.B., Schiffman E., Hiber L., Gerads L. & Olsen K. 2012. Recovery of staphylococci from computer keyboards in a veterinary medical center and the effect of routine cleaning. Vet. Rec. 170(16):414. <https://dx.doi.org/10.1136/vr.100508> <PMid:22447457>

Burgess B.A. & Morley P.S. 2014. Veterinary hospital surveillance systems. Vet. Clin. N. Am., Small Anim. Pract. 45(2):235-242. <https://dx.doi.org/10.1016/j.cvsm.2014.11.002> <PMid:25534534>

CLSI 2018. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals. 5th ed. CLSI standard VET01, Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI 2019. Performance standards for antimicrobial susceptibility testing. 29th ed. CLSI Supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA.

Costa P.M., Loureiro L. & Matos A.J.F. 2013. Transfer of multidrug-resistant bacteria between intermingled ecological niches: The interface between humans, animals and the environment. Int. J. Environ. Res. Publ. Health 10(1):278-294. <https://dx.doi.org/10.3390/ijerph10010278> <PMid:23343983>

Dallenne C., Da Costa A., Decré D., Favier C. & Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother. 65(3):490-495. <https://dx.doi.org/10.1093/jac/dkp498> <PMid:20071363>

Damborg P., Gaustad I.B., Olsen J.E. & Guardabassi L. 2011. Selection of CMY-2 producing *Escherichia coli* in the faecal flora of dogs treated with cephalexin. Vet. Microbiol. 151(3/4):404-408. <https://dx.doi.org/10.1016/j.vetmic.2011.03.015> <PMid:21497459>

De Briyne N., Atkinson J., Pokludova L. & Borriello S.P. 2014. Antibiotics used most commonly to treat animals in Europe. Vet. Rec. 175(13):325. <https://dx.doi.org/10.1136/vr.102462> <PMid:24899065>

Depardieu F., Perichon B. & Courvalin P. 2004. Detection of the van alphabet and identification of Enterococci and Staphylococci at the species level by multiplex PCR. J. Clin. Microbiol. 42(12):5857-5860. <https://dx.doi.org/10.1128/JCM.42.12.5857-5860.2004> <PMid:15583325>

Donker T., Wallinga J., Slack R. & Grundmann H. 2012. Hospital networks and the dispersal of hospital-acquired pathogens by patient transfer. PLoS One 7(4):e35002. <https://dx.doi.org/10.1371/journal.pone.0035002> <PMid:22558106>

Ekiri A.B., Morton A.J., Long M.T., MacKay R.J. & Hernandez J.A. 2010. Review of the epidemiology of and infection control aspects of nosocomial *Salmonella* infections in hospitalized horses. Equine Vet. Educ. 22(12):631-641. <https://dx.doi.org/10.1111/j.2042-3292.2010.00144.x>

Endimiani A., Hujer K.M., Hujer A.M., Bertschy I., Rossano A., Koch C., Gerber V., Francey T., Bonomo R.A. & Perreten V. 2011. *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. J. Antimicrob. Chemother. 66:2248-2254. <https://dx.doi.org/10.1093/jac/dkr289>

Fraser M.A. & Girling S.J. 2009. Bacterial carriage of computer keyboards in veterinary practices in Scotland. Vet. Rec. 165(1):26-27. <https://dx.doi.org/10.1136/vetrec.165.1.26> <PMid:19578192>

Ghosh A., Kukanich K.S., Brown C.E. & Zurek L. 2012. Resident cats in small animal veterinary hospitals carry multidrug resistant enterococci and are likely involved in cross-contamination of the hospital environment. Front. Microbiol. 3:1-14. <https://dx.doi.org/10.3389/fmicb.2012.00062> <PMid:22363334>

Guardabassi L. & Prescott J.F. 2015. Antimicrobial stewardship in small animal veterinary practice: from theory to practice. Vet. Clin. N. Am., Small Anim. Pract. 45(2):361-376. <https://dx.doi.org/10.1016/j.cvsm.2014.11.005> <PMid:25721619>

Guardabassi L., Larsen J., Weese J.S., Butaye P., Battisti A., Kluytmans J., Lloyd D.H. & Skov R.L. 2013. Public health impact and antimicrobial selection of methicillin-resistant staphylococci in animals. J. Glob. Antimicrob. Resist. 1(2):55-62. <https://dx.doi.org/10.1016/j.jgar.2013.03.011> <PMid:27873579>

Hillier A., Lloyd D.H., Weese S.J., Blondeau J.M., Boothe D., Breitschwerdt E., Guardabassi L., Papich M.G., Rankin S., Turnidge J.D. & Sykes J.E. 2014. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). Vet. Dermatol. 25(3):163-e43. <https://dx.doi.org/10.1111/vde.12118> <PMid:24720433>

Hordijk J., Schoormans A., Kwakernaak M., Duim B., Broens E., Dierikx C., Mevius D. & Wagenaar J.A. 2013. High prevalence of fecal carriage of extended spectrum -lactamase/AmpC-producing Enterobacteriaceae in cats and dogs. Front. Microbiol. 4:242. <https://dx.doi.org/10.3389/fmicb.2013.00242>

Klevens R.M., Edwards J.R., Richards Jr C.L., Horan T.C., Gaynes R.P., Pollock D.A. & Cardo D.M. 2007. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. Publ. Health Rep. 122(2):160-166. <https://dx.doi.org/10.1177/003335490712200205> <PMid:17357358>

Müller S., Janssen T. & Wieler L.H. 2014. Multidrug resistant *Acinetobacter baumannii* in veterinary medicine-emergence of an underestimated pathogen? Berl. Munch. Tierarztl. Wochenschr. 127(11/12):435-446. <https://dx.doi.org/10.2376/0005-9366-127-435> <PMid:25872253>

Nakadomari G.H., Charalo A.C., Pavan A.C.L., Vignoto V.K.C., Sfaciotte R.A.P. & Wosiacki S.R. 2019. Multiplex-PCR for detection of beta-lactam resistance in *Staphylococcus* spp. Revta Ciênc. Vet. Saúde Públ. 6(2):262-275. <https://dx.doi.org/10.4025/revcivet.v6i2.45050>

Parussolo L., Sfaciotte R.A.P., Dalmina K.A., Melo F.D., Costa U.M. & Ferraz S.M. 2019. Detection of virulence genes and antimicrobial resistance profiles of *Escherichia coli* isolates from raw milk and artisanal cheese in Southern Brazil. Semina, Ciênc. Agrárias 40(1):163-178. <https://dx.doi.org/10.5433/1679-0359.2019v40n1p163>

Perreten V., Kadlec K., Schwarz S., Andersson U.G., Finn M., Greko C., Moodley A., Kania S.A., Frank L.A., Bemis D.A., Franco A., Iurescia M., Battisti A., Duim B., Wagenaar J.A., van Duijkeren E., Weese J.S., Fitzgerald J.R., Rossano A. & Guardabassi L. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J. Antimicrob. Chemother. 65(6):1145-1154. <https://dx.doi.org/10.1093/jac/dkq078> <PMid:20348087>

Robinson T.P., Bu D.P., Carrique-Mas J., Fèvre E.M., Gilbert M., Grace D., Hay S.I., Jiwakanon J., Kakkar M., Kariuki S., Laxminarayan R., Lubroth J., Magnusson U., Ngoc P.T., Van Boeckel T.P. & Woolhouse M.E. 2016. Antibiotic resistance is the quintessential One Health issue. Trans. R. Soc. Trop. Med. Hyg. 110(7):377-380. <https://dx.doi.org/10.1093/trstmh/trw048> <PMid:27475987>

Rubin J.E. & Pitout J.D.D. 2014. Extended-spectrum b-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. Vet. Microbiol. 170(1/2):10-18. <https://dx.doi.org/10.1016/j.vetmic.2014.01.017>

Sader H.S., Reis A.O., Silbert S. & Gales A.C. 2005. IMPs, VIMs and SPMs: the diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in a brazilian hospital. Clin. Microbiol. Infect. 11(1):73-76. <https://dx.doi.org/10.1111/j.1469-0691.2004.01031.x> <PMid:15649310>

Sasaki T., Tsubakishita S., Tanaka Y., Sakusabe A., Ohtsuka M., Hirotaki S., Kawakami T., Fukata T. & Hiramatsu T. 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J. Clin. Microbiol. 48(3):765-769. <https://dx.doi.org/10.1128/JCM.01232-09> <PMid:20053855>

Schaer B.L.D., Aceto H. & Rankin S.C. 2010. Outbreak of salmonellosis caused by *Salmonella enterica* serovar Newport MDR-AmpC in a large animal veterinary teaching hospital. J. Vet. Intern. Med. 24(5):1138-1146. <https://dx.doi.org/10.1111/j.1939-1676.2010.0546.x> <PMid:20584143>

Schaufler K., Bethe A., Lübke-Becker A., Ewers C., Kohn B., Wieler L.H. & Guenther S. 2015. Putative connection between zoonotic multiresistant extendedspectrumbeta-lactamase (ESBL)-producing *Escherichia coli* in dog feces from a veterinary campus and clinical isolates from dogs. Infect. Ecol. Epidemiol. 5:25334. <https://dx.doi.org/10.3402/iee.v5.25334> <PMid:25656467>

Souza-Junior M.A., Ferreira E.S. & Conceição G.C. 2004. Betalactamases de espectro ampliado (ESBL): um importante mecanismo de resistência bacteriana e sua detecção no laboratório clínico. NewsLab 63:152-174.

Stull J.W. & Weese J.S. 2015. Hospital-associated infections in small animal practice. Vet. Clin. N. Am., Small Anim. Pract. 45(2):217-233. <https://dx.doi.org/10.1016/j.cvsm.2014.11.009> <PMid:25559054>

Vincze S., Stamm I., Kopp P.A., Hermes J., Adlhoch C., Semmler T., Wieler L.H., Lübke-Becker A. & Walther B. 2014. Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010-2012. PLoS One. 9(1):e85656. <https://dx.doi.org/10.1371/journal.pone.0085656> <PMid:24465637>

Walther B., Luebke-Becker A., Stamm I., Gehlen H., Barton A.K., Janssen T., Wieler L.H. & Guenther S. 2014. Suspected nosocomial infections with multi-drug resistant *E. coli* including extended-spectrum beta-lactamase (ESBL)-producing strains, in an equine clinic. Berl. Munch. Tierarztl. Wochenschr. 127(11/12):421-427. <PMid:25872251>

Walther B., Tedin K. & Lübke-Becker A. 2017. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet. Microbiol. 200:71-78. <https://dx.doi.org/10.1016/j.vetmic.2016.05.017> <PMid:27291944>

Weese J.S. 2012. Monitoring for surgical infection, p.170-179. In: Tobias K.M. & Johnston S.A. (Eds), Veterinary Surgery, Small Animals. Elsevier Saunders, Missouri.

Wieler L.H., Walther B., Walther S., Guenther S. & Lübke-Becker A. 2015. Infections with multidrug-resistant bacteria: has the post-antibiotic era arrived in companion animals? p.433-452. In: Sing A. (Ed.), Zoonoses - Infections Affecting Humans and Animals: focus on public health aspects. Springer, Dordrecht. <https://dx.doi.org/10.1007/978-94-017-9457-2_17>