Original

Isolation of bacteria present in the veterinary necropsy room and health risks

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ABSTRACT

Objective. The aim of this study was to characterize the bacteria of a veterinary autopsy room, their antimicrobial susceptibility profile and testing the efficiency of two sanitizers against these microorganisms. **Materials and methods.** Three points of the room that professionals do not normally wear personal protective equipment (PPE) getting in direct contact with these bacterias. Anaerobic and aerobic mesophilic count were performed before and after disinfection with hypochlorite and sodium alkylbenzene sulfonate then, isolates were identified by their morphotintorials and biochemical characteristics and their antibiotic susceptibility. **Results**. Preliminary results indicated that the hypochlorite was the sanitizing agent of choice for surface disinfection and against the most frequent potential pathogenic bacterials isolated such as *Staphylococcus* spp (75%), *E. coli* and *Klebsiella* spp (15%), and *Pseudomonas* spp (10%). In addition, 25% of the staphylococci were resistant to at least one antimicrobial tested and *Klebsiella* spp, *E. coli,* and *Pseudomonas* spp were taken into consideration, wide antimicrobial resistance tested were observed. **Conclusions**. The characterization of these bacteria found in the autopsy room is important to alert professionals about the biological risks they are exposed to, as well as the precautions they should take.

Keywords: Sodium alkylbenzene sulfonate; antimicrobials; hypochlorite; microbiology; pathology; veterinary (*Source: DeCS*).

RESUMEN

Objetivo. El objetivo de este estudio fue caracterizar las bacterias de una sala de autopsias veterinarias, su perfil de susceptibilidad antimicrobiana y probar la eficacia de dos desinfectantes frente a estos microorganismos. **Materiales y métodos.** Tres puntos de la sala en los que los profesionales normalmente no usan equipo de protección personal (EPP) al entrar en contacto directo con estas bacterias. Antes y después de la desinfección con hipoclorito y alquilbencenosulfonato sódico se realizaron recuentos anaeróbicos y aeróbicos mesófilos, luego se identificaron los aislamientos por sus características morfotintoriales y bioquímicas y su susceptibilidad antibiótica. **Resultados.** Los resultados preliminares indicaron que el hipoclorito fue el agente desinfectante de elección para la

desinfección de superficies y contra las bacterias patógenas potenciales aislados más frecuentes como *Staphylococcus* spp (75%), *E. coli* y *Klebsiella* spp (15%) y *Pseudomonas* spp (10%). Además, el 25.0% de los estafilococos fueron resistentes a al menos un antimicrobiano probado y se tomaron en consideración *Klebsiella* spp, *E. coli* y *Pseudomonas* spp, se observó una amplia resistencia antimicrobiana probada. **Conclusiones.** La caracterización de estas bacterias encontradas en la sala de autopsias es importante para alertar a los profesionales sobre los riesgos biológicos a los que están expuestos, así como las precauciones que deben tomar.

Palabras clave: Alquilbencenosulfonato de sodio; antimicrobianos; hipoclorito; microbiología; patología; veterinaria (*Fuente: DeCS*).

INTRODUCTION

Autopsy is necessary to elucidate unnatural or suspicious circumstances deaths both in Human and Veterinary Medicine. The autopsy room has always been a potential source of infection and professionals who are directly or indirectly involved in conducting a post-mortem examination are at great risks of exposure to microorganisms (1). Thus, in order to lower the risk of contamination, sanitization products are used to disinfect these environments. The sodium alkylbenzene sulfonate is a surfactant used as a detergent; and the sodium hypochlorite or calcium hypochlorite, have wide, fast, and chip antimicrobial action against bacteria, virus, fungi, and it is generally used for disinfection non-critical materials (2).

Zoonotic infectious diseases are one of the main risks in animal necropsy, as many of their agents can be transmitted by the veterinarian's contact with the animal's secretions, such as saliva, blood, urine and feces. Among these agents, *Staphylococcus* genus and the Enterobacteriaceae family stand out as the most important (3). *Staphylococcus* spp. are Gram positive bacteria, widely distributed in the environment and normally present on the intact skin of humans and animals. In addition, to the oropharynx, gastrointestinal and urogenital tract; is implicated in important diseases (4). The *Enterobacteriaceae* family are Gram negative, aerobic or facultative anaerobic bacilli sugar fermenters they commonly inhabit the gastrointestinal tract of vertebrates and they are among the most common pathogens, especially E. coli and klebsiella spp., that infect humans and animals. These bacteria are indicators of surface hygiene and they are often associated with hospital infections. These microorganisms cause various infections in both hospitalized patients and the general population (5).

Therefore, due to the risks that the professionals who handle corpses are exposed to, the environment they frequent, and the importance of zoonoses related to the health of them associated with the concept of One Health, this study aimed to compare the effectiveness of two sanitizing products in the growth control of aerobic and anaerobic mesophilic bacteria and the characterization of the autopsy room environment, and their susceptibility to different antimicrobials.

MATERIALS AND METHODS

Environment characterization. Samples were collected from the autopsy room of the Pathobiology and Theriogenology Department of the Faculty of Agricultural and Veterinary Sciences (FCAV / Unesp), Jaboticabal Campus, which is used by an extensive team of professionals, such as teachers, veterinary undergraduate students and veterinary residents. The room has an extensive routine of activities due to the Health Residency and Professional Improvement Program (PRAPS – MVS).

Sampling. Three surfaces were selected to perform the four collections to obtain the samples. The choice was based on the places of greatest contact of the professionals who work in the autopsy room, as they are places where contact often occurs without the use of gloves or any other type of personal protective equipment (PPE). The selected locations were: (1) door handle of the autopsy room, (2) door handle of the cold chamber (storage of corpses), (3) door handle of the greenhouse where the instruments used in the necroscopic examination of the animals are. The collections were performed using a sterile swab, placed in a test tube containing 1% bacteriological peptone. In each selected area, four collections were made, two samples taken before and two after

use for each sanitizer, totalizing 24 samples. Samples were collected from the cold chamber door before and after necropsies. The samples were cultured at the Veterinary Microbiology Laboratory of the DPRSU (FCAV-UNESP). The sanitizing products used were concentrated sodium hypochlorite diluted 1:100 and sodium alkyl benzene sulfonate commercial formulation diluted 1:100. Each sanitizer was applied to the surfaces of the selected areas and the collections were carried out with a difference of 15 days between them.

Aerobic and anaerobic mesophilic counts. Quantification of aerobic and anaerobic bacteria was performed using the surface plating technique. Each sample was diluted up to eight times in peptone broth in a 1:10 ratio, with 0.1 ml of diluted samples plated into nutrient agar. The inoculum was spread with the aid of a Drigalski loop and then incubated at 37ºC for 24 hours in an aerobic environment and in an anaerobic jar. After this period, the Colony Forming Units (CFU) count was performed (6).

Isolation and identification of *Staphylococcus* spp and Enterobacteriaceae. Sample were plated in MacConkey and Mannitol Salt Agar, selective and differential media for Enterobacteria and Staphylococcus genus respectively. After incubation at 37ºC for 24 hours, the colonies were evaluated for cell morphology, Gram stain and specific biochemical tests for each genus or species (7). The following standard biochemical tests: fermentation of glucose, lactose and sucrose, citrate, mannitol, malonate, phenylalanine, urea, motility and catalase were performed according to Koneman et al (8)

Antimicrobial susceptibility test. Antimicrobial susceptibility was assayed by Kirby-Bauer test or disk diffusion method. After Isolates were grown in 3 mL of Brain Heart Infusion (BHI) broth at 37ºC until they reached the 0.5 MacFarland standard. After incubation, the culture was sown into Mueller-Hinton agar and, after the surface have dried, the disks containing the antimicrobials were added. The antimicrobials tested were selected according to their use and importance in the routine of the veterinary hospital, namely: gentamicin (10µg) tetracycline (30µg) sulfamethoxazole + trimethoprim (25µg), cefazolin (30µg) and amoxicillin (5µg). The diameters will be compared with the CLSI guide (9).

The analysis of the results was carried out descriptively.

RESULTS

All the samples (twelve) that were collected before disinfection and were incubated in an aerobic atmosphere showed bacterial growth, while three samples that were collected before disinfection and were incubated in an anaerobic atmosphere did not show growth (Table 1). Therefore, an accentuated growth of aerobic bacteria in the autopsy room environment was observed. The hypochlorite was an effective disinfectant and in all 12 samples after it, was used for cleaning no bacterial growth was observed. In contrast, with the use of the detergent (sodium alkylbenzene sulfonate) among the samples that came from the handle of the cold room, one presented growth in both aerobic and anaerobic condition with no significant reduction (Table 1).

From 24 samples collected, 20 presented microorganisms growth and the isolates were classified according to morphological and tinting characteristics in: 15 samples (75%) classified as GRAM positive cocos (GPC) and 5 samples (25%) classified as Gram negative bacilli (GNB). The 15 GPC were catalase positive thus been suggested of the *Staphylococcus* spp. The microorganisms classified as GNB by Gram stain were plated on TSI medium for genus definition. From five samples, two showed an alkaline medium base (reddish color) and slightly acidic (yellowish color) TSI medium apex which shows the oxidative degradation of glucose. There was no visible gas formation (CO_2 and H_2) or iron salts precipitation that are the result of the production of sulphuric gas. Thus, it was concluded that they were *Pseudomonas* sp. The other three previously identified GNB presented both acidic TSI medium base and apex (yellowish in color) which is characteristic of oxidative degradation of glucose and the fermentation of lactose and/ or sucrose. Additionally, gas formation was identified in only one sample and iron salts precipitation was not observed in any of the samples, thus allowing to infer them as *E. coli* or *Klebsiella* sp. For the confirmation the citrate test was carried out, in which two were positive, thus been *Klebsiella* sp. and the negative reaction been *Escherichia coli*.

The 20 isolates were submitted to antimicrobial susceptibility test by the disk diffusion technique. The results against the 5 antibiotics tested are shown in the Table 2.

Table 1. Aerobic and anaerobic mesophyllic population count on PCA agar by surface.

Sampling / product	Samples	Total aerobes A * (UFC / mL)	Total aerobes D ** (UFC / mL)	Total anaerobes A * (CFU / mL)	Total anaerobes D ** (CFU / mL)
Sampling 1 (hypochlorite)	1	1.2×10^3	0	0	0
	2	1.1×10^2	0	0	0
	3	7.1×10^3	0	3.4×10^3	0
Sampling 2 (detergent)	1	2.8×10^3	0	2.3×10^3	0
	2	1.8×10^4	0	3.4×10^3	0
	3	4.0×10^2	0	0	0
Sampling 3 (hypochlorite)	1	1.3×10^3	0	1.0×10^3	0
	2	2.2×10^3	0	2.7×10^3	0
	3	4.0×10^2	0	2.0×10^2	0
Sampling 4 (detergent)	1	3.0×10^2	0	2.0×10^2	0
	2	7.0×10^2	5.0×10^2	5.0×10^2	3.0×10^2
	3	4.0×10^2	0	3.0×10^2	0

Legend: 1. Autopsy room handle; 2. Cold chamber door handle; 3. Sterilization oven handle. A*. Before disinfection; D **. After disinfection.

Table 2. Antimicrobial susceptibility profile of the 20 microorganisms isolated from manipulation sites of the autopsy room of the Veterinary Pathology Department of the FCAV-UNESP.

Isolate	Microorganism	Antimicrobials				
		cefazolin	gentamycin	Sulfamethoxazole + trimethoprim	Amoxicilin + clavulanate	tetracycline
1	*Staphylococcus spp.*	-	S	S	S	S
2	*Staphylococcus spp.*	-	R	R	S	I
3	*Staphylococcus spp.*	-	R	R	I	R
4	*Staphylococcus spp.*	-	I	R	R	R
5	*Staphylococcus spp.*	-	S	R	R	S
6	*Staphylococcus spp.*	-	S	S	R	S
7	*Staphylococcus spp.*	-	I	R	S	I
8	*Staphylococcus spp.*	-	S	R	S	I
9	*Staphylococcus spp.*	-	R	S	S	S
10	*Staphylococcus spp.*	-	S	S	I	S
11	*Staphylococcus spp.*	-	S	S	S	S
12	*Staphylococcus spp.*	-	S	S	S	S
13	*Staphylococcus spp.*	-	S	S	S	S
14	*Staphylococcus spp.*	-	S	R	S	I
15	*Staphylococcus spp.*	-	S	S	S	S
16	*Klebisiella spp.*	R	R	R	I	S
17	*Escherichia coli*	R	R	R	I	S
18	*Klebisiella spp.*	R	R	R	R	R
19	*Pseudomonas spp.*	R	I	R	S	R
20	*Pseudomonas spp.*	R	S	R	S	S

Legend: S: Sensitive; R: Resistant; I: Intermediate; (-): Not applicable

Gentamicin was the most effective antimicrobial against the 15 isolates of *Staphylococcus* spp. and the less effective was the combination sulfamethoxazole + trimethoprim. More than half of the *Staphylococcus* spp. isolates were susceptible to the chosen antimicrobials (61.6%;37/60); Twenty-five percent of the staphylococci were resistant to at least one antimicrobial (15/60) and 13.4% (8/60) had intermediate levels of resistance. The isolates of *Klebsiella* spp, *E. coli,* and *Pseudomonas* spp, were resistant to the antimicrobial agents chosen. Nevertheless, tetracycline was most effective antimicrobial tested and the lowest was cefazolin. It is noteworthy that one *Klebsiella* spp. isolate (18) was resistant to all antimicrobials chosen. A total of 64.0% (16/25) of the Gram-negative bacilli (BGN) showed resistance to at least one antimicrobial; intermediate resistance was observed in 12% (3/25) and susceptibility occurred in only 25% (6/25) of the isolates (Table 3).

Table 3. Degree of antimicrobial resistance of the isolates in the autopsy room of the FCAV-UNESP in Jaboticabal, SP.

Isolate	Microorganisms	Multi-drug antibiotic resistance			
		0	1	2	3+
1	*Staphylococcus sp.*	X			
2	*Staphylococcus sp.*				X
3	*Staphylococcus sp.*				X
4	*Staphylococcus sp.*				X
5	*Staphylococcus sp.*			X	
6	*Staphylococcus sp.*		X		
7	*Staphylococcus sp.*				X
8	*Staphylococcus sp.*			X	
9	*Staphylococcus sp.*		X		
10	*Staphylococcus sp.*		X		
11	*Staphylococcus sp.*	X			
12	*Staphylococcus sp.*	X			
13	*Staphylococcus sp.*	X			
14	*Staphylococcus sp.*			X	
15	*Staphylococcus sp.*	X			
16	*Klebsiella sp.*				X
17	*Escherichia coli*				X
18	*Klebsiella sp.*				X
19	*Pseudomonas sp.*				X
20	*Pseudomonas sp.*			X	

DISCUSSION

It was observed during the data collection step of this study that the literature regarding the infection control of the autopsy room is scarce and there was no sanitary parameters for the limit of microorganisms in this environment (1,10). Thus, it has been a neglected subject in teaching and research institutions and what can be used for autopsy rooms are the safety standards that were developed for clinical and research laboratories due to their specific degrees of risk (1).

Microorganism quantification is used to determine the quality of various products and environments (1). The difference observed between the results obtained by the hypochlorite and the detergent can be explained by their different mechanisms of action; hypochlorite has a broad spectrum against microbial activity and has an important role in sites decontamination (2) while the detergent does it by mechanical removal with the aid of water washing. Considering the environment of an autopsy room even a low microorganisms count must be taken into consideration (1) and for this reason, the use of disinfectant is necessary to avoid the spread of pathogens to other environments or professionals.

According to Mokracka et al (11), to be considered as multidrug-resistant a strain has to have low susceptibility to three or more antimicrobials classes simultaneously; based on this statement, it can be noted that 25% of the staphylococci showed multi-resistance and the gram-negative bacilli (enterobacteria and *Pseudomonas* sp.) demonstrated multi-drug resistance in 80% of the isolates (Table 3).

Da Silva et al (12) in a study in dental offices, concluded that the surfaces with the greatest contamination potential were the hands of professionals and spots they touched. This corroborates with the finding of this study since the isolates were obtained from places that are known to be often manipulated. When considering culture media multiplication, a greater growth was observed in mannitol salt agar (75%) followed by a 25% growth in Mac Conkey. Microorganisms isolation on MacConkey agar were higher when compared to those observed by Da Silva (12) and indicate faecal contamination which shows that there is a biological risk to users of this space.

The high level of *Staphylococcus* spp. contamination (75%) should be a reason for concern as they are etiological agents linked to abscesses, pyogenic infections, dermatitis and even fatal septicaemia (13). Although in this study the hypochlorite completely eliminated microorganisms after cleaning in places with the presence of organic material, Both et al (14) reports in their work that when using hypochlorite in the presence of organic material, 84.4% of Staphylococcus spp. isolates were active, whereas without the presence of organic matter, 100% of the isolates were inactivated. This is because disinfectants that release free chlorine have their action reduced because chlorine first oxidizes organic matter, thus reducing its availability for antimicrobial action (14).

Also, the research of Maujean et al (10) noted that the isolation of enterobacteria only occur during the necropsy procedure and that there is a close relationship between this type of bacteria and intestinal fluids. Therefore, the detections of isolates of *E. coli* and *Klebsiella* spp. at the spots evaluated in this work indicate that faecal contamination may come from poor hygiene and disinfection procedures errors.

Regarding antimicrobial resistance, it is known that it is the results of a natural phenomenon and has become one of the main public health concern in the world with many researchers correlating the appearance of resistant strains with the increase of the use of antimicrobials in animals (15).

In this regard, in this study a marked resistance, mainly from enterobacteria, against cefazolin, the association of sulfamethoxazole + trimethoprim and gentamicin were observed and it is corroborated in part with the findings of Bordignon (16) who observed low susceptibility of enterobacteria to cefazolin; however, this same study demonstrated a marked susceptibility of bacteria to gentamicin and sulfamethoxazole + trimethoprim. It is suggested that this variation in susceptibility is the result of previous contact of bacteria with all tested antimicrobials and the consequent development of resistance mechanisms.

A study (17) that isolated the Staphylococcus genus from dog otitis showed that trimethoprim-sulfamethoxazole and amoxicillin + clavulanate were among those with the highest percentages of resistance. In this study, tetracycline achieved satisfactory levels of antimicrobial susceptibility as well as the other tested antimicrobials. In a retrospective study that characterized the environmental bacteria on the clothing of health professionals, Oliveira et al (18) isolated bacteria from the same genus as in this study and reported a marked susceptibility to the combination of sulfamethoxazole + trimethoprim and cefazoline; results that are not in agreement with this research. The fact that there was a marked resistance to the antimicrobial agents tested in the isolates from the autopsy room in the veterinary hospital suggests the possibility of cross contamination and that the animals sent for necropsy can already carry these strains and can contaminate the environment of the autopsy room.

To date, there are still no parameters to indicate if the amount of bacterial found in the autopsy room is above or below what is acceptable and if this number is not accurate, since only 2.5% of the total bacterial species can be identified by culture methods, according to researchers from the University of Technology and the Medical University, both in Graz, Austria (19). Also, extremophilic bacteria may be present, but counting it may be difficult. These facts demonstrate the importance of decontamination and the correct use of PPE for the safety of the professionals.

In conclusion, it was possible to isolate *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. in the surfaces analysedand it is evident that sodium hypochlorite must be the first choice sanitizing agent for the control of pathogens on surfaces. In addition, the bacterial antimicrobial resistance profile observed in this study highlights the growing problem of antimicrobial resistance due to their isolation at the autopsy room which antimicrobials are not present or used. This data shows a constant need of surveillance especially in relation of the use of personal protective equipment (PPE) and the correct disinfection of hands and surfaces.

Conflict of interests

The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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