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Identification of bacterial multidrug resistance in wounds of pets

(Identificação de cepas bacterianas multirresistentes isoladas de feridas de cães e gatos)

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ABSTRACT

The objective of this study was to identify the bacteria present in wounds of dogs and cats, monitor the resistance profile of the main groups of antimicrobials and identify multidrug-resistant strains of interest in public health. 54 bacterial isolates from 38 wounds were evaluated in 48 dogs and 6 cats from the Veterinary Hospital (HV) of the State University of Maringá (UEM), Umuarama Regional Campus (CAU) from March 2012 until February 2014. The evaluation of the isolates, performed in the Laboratory of Veterinary Microbiology of HV-UEM-CAU, consisted of bacterial identification by morpho-tinturials and biochemical characteristics, antimicrobial susceptibility by disk diffusion method with 32 antimicrobial and phenotypic research of MRS, MRS-MLSB, ESBL and VRE strains. Multiple antimicrobial resistance (MAR) was calculated by the MAR index. The results were submitted to descriptive analysis in order to calculate the absolute and relative frequencies. 55.5% (30/54) of the strains tested were identified as gram-positive cocci, 35.2% (19/54) as fermenting gram-negative bacilli and 9.3% (5/54) as non-fermenters. The highest prevalence of strains identified was *Staphlococcus* spp. (48.1%), followed by *Escherichia coli* (11.1%), *Citrobacter* spp. (7.4%), *Pseudomonas* spp. (7.4%) and *Providencia* spp. (5.5%), others were found in small percentages. A total of 1433 reviews of antimicrobial drugs were conducted where the percentage of drug resistance *in vitro* considered by CLSI was 42.3 % (n=606) and with intermediate resistance was 7.4% (n=106), totaling 49.7% (n=712) rating with partial or total resistance. The average MAR index was 0.42, where 83.3% (45/54) of the isolates were multiresistant ($\geq$ 0.2). The drugs regarded as the most susceptible to resistance were penicillin (R=87.5%), ampicillin (R=79.2%), amoxicillin (R=71.7%), clindamycin (R=80%), oxacillin (R=63 %) (only tested in Gram-positives), cefoxitin (R=63.3%), tetracycline (R=61%), erythromycin (R=61%) and rifampin (R=52%). Drugs considered to be less susceptible to bacteria resistance were imipenem (R=0%), meropenem (R=4.5%), polymyxin (R=9.5%) (only tested in Gram-negative), gentamicin (R=17%), tobramycin (R=19%), amikacin (R=21%) and neomycin (R=26%). There were found 16 samples phenotypically characterized as MRS, 12 as MRS-MLSB, one as VRE and 5 as ESBL strains.
KEY-WORDS: animal, antimicrobial resistance, MRS, ESBL.

RESUMO

O objetivo deste estudo foi identificar as bactérias presentes em feridas de cães e gatos, monitorar o perfil de resistência destas aos principais grupos de antimicrobianos e identificar cepas multirresistentes de interesse em saúde pública em amostras clínicas de feridas de animais. Foram avaliados 54 isolados bacterianos provenientes de 38 feridas em 48 cães e 6 gatos atendidos no Hospital Veterinário (HV) da Universidade Estadual de Maringá (UEM), Campus Regional de Umuarama (CAU) entre março de 2012 até fevereiro de 2014. A avaliação dos isolados, realizada no Laboratório de Microbiologia Veterinária da HV-UEM-CAU, consistiu da identificação bacteriana por características morfo-tinturiais e bioquímicas, susceptibilidade antimicrobiana pelo método de disco-difusão com 32 antimicrobianos e pesquisa

fenotípica das cepas MRS, MRS-MLSB, ESBL e VRE. A múltipla resistência aos antimicrobianos (MAR) foi calculada pelo índice MAR. Os resultados obtidos foram submetidos à análise descritiva para cálculo das frequências absoluta e relativa. 55,5% (30/54) das cepas avaliadas foram identificadas como cocos Gram-positivos, 35,2% (19/54) como bacilos Gram-negativos fermentadores e 9,3% (5/54) como não fermentadores. A maior prevalência de cepas identificadas foi de *Staphlococcus* spp. (48,1%), seguido de *Escherichia coli* (11,1%), *Citrobacter* spp. (7,4%), *Pseudomonas* spp. (7,4%) e *Providencia* spp. (5,5%), os demais foram identificados em pequenas porcentagens. Um total de 1433 avaliações de drogas antimicrobianas foram realizadas, onde o percentual de drogas consideradas suscetíveis a resistência *in vitro* segundo CLSI foi de 42,3% (n=606) e com resistência intermediária, 7,4% (n=106), totalizando 49,7% (n=712) das avaliações com resistência parcial ou total. O índice MAR médio foi de 0.42, onde 83,3% (45/54) das cepas isoladas foram consideradas multirresistentes (≥0.2). As drogas consideradas mais suscetíveis à resistência foram: penicilina (R=87,5%), ampicilina (R=79,2%), amoxacilina (R=71,7%), clindamicina (R=80%), oxacilina (R=63%) avaliada apenas em gram positivos, cefoxitina (R=63,3%), tetraciclina (R=61%), eritromicina (R=61%) e rifampicina (R=52%). As drogas consideradas menos suscetíveis a resistência foram: imipenem (R=0%), meropenem (R=4,5%), polimixina (R=9,5%) avaliada apenas em gram negativos, gentamicina (R=17%), tobramicina (R=19%), amicacina (R=21%) e neomicina (R=26%). Foram encontrados 16 amostras caracterizadas fenotipicamente como MRS, 12 amostras MRS-MLSB, uma amostra VRE e 5 amostras ESBL.
PALAVRAS-CHAVE: animal, resistência antimicrobiana, MRS, ESBL.

INTRODUCTION

The wounds, either from accidental or surgical injury, are characterized by disruption of the normal continuity of the skin and / or deeper tissue layers (HOSGOOD, 2003; MOORE et al., 2003; PAVLETIC, 2003) may be classified in various ways, taking into account five basic features: presence of skin breakdown, mechanism of injury, tissue injury extent, degree of contamination and color (ANDRADE et al., 2006; TAZIMA et al., 2008). With respect to the degree of contamination, the wounds can be classified as clean (aseptically created in surgery without respiratory, gastrointestinal or urogenital involment); clean-contaminated, which have minimal contamination and can be surgically resolved to within six hours of surgery involving abuse or traumatic wounds in their initial presentations; contaminated (accidental wounds with exposure times between six and twelve hours or presenting cell debris or foreign bodies, but still have no exudate); and infected or dirty, which occurred more than twelve hours, with clear signs of infection or contamination and may present exudate, devitalized tissue or foreign bodies or with opening septic wells (BELLAH et al., 1999; MOORE et al., 2003; FORD et al., 2007; TAZIMA et al., 2008; GARZOTTO, 2009).

A wound infection interferes with the repair phase. Infected tissues become infected if invasive bacteria multiply to 10^5 microorganisms per gram of tissue. The development of infection in wounds depends on the degree of tissue trauma, presence of a foreign body and competence of the defense mechanisms of animal. Bacterial toxins and associated inflammatory infiltrates cause cell necrosis and vascular thrombosis (HOSGOOD, 2003; ANDRADE et al., 2006; FOSSUM, 2007).

The wounds in dogs and cats are common in the routine of veterinary clinics and mostly result from surgical wounds, bites from other animals or running over (ARIAS et al., 2008).

The inappropriate use of antibiotics to treat bacterial infections in animals is always much discussed, as it can contribute to the development of bacterial resistance, making the treatment more difficult (ISHII et al., 2011). There is also the potential risk of continuing the resistance to certain drugs between humans and animals (COELHO et al., 2007; SOARES et al., 2008; UMBER and BENDER, 2009). According to Wannmacher (2004) antimicrobials are the only drugs that influence not only the patient being treated, but throughout the ecosystem where it is inserted, with deep potential repercussions.

Antimicrobial resistance, according to the World Health Organization (OMS / WHO), refers

to the resistance of a microorganism front an antimicrobial drug for which it was originally sensitive. According to Wannmacher (2004) resistance is defined by microbial strains able to multiply even with the application of therapeutic doses or high concentrations of antimicrobial. The evolution of resistant strains is a natural selection phenomenon which happens when microorganisms are exposed to antimicrobial drugs. The misuse of antimicrobial drugs accelerates this natural phenomenon. The resistance generates great concern to health professionals; it is a problem that involves many bacterial species, resistance mechanisms and transfers this resistance among certain types of bacteria (GUARDABASSI et al., 2004; MENDES et al., 2005).

Some bacterial strains are considered of great importance, especially in hospital settings, with references in the human line by the high rate of antimicrobial resistance, called MRSA (Methicillin-Resistant *Staphylococcus aureus*), VISA (*Staphylococcus aureus* with Intermediate Resistance to Vancomycin), VRSA (Vancomycin-Resistant *Staphylococcus aureus*), VRE (Vancomycin-Resistant *Enterococcus*), ESBL (Extended-Spectrum Betalactamase) and MBL (Metallo-β-Lactamase). In veterinary medicine, some of these are already known, but few studies have been conducted for their detection. However, these multidrug-resistant strains have great importance in public health and its research is of great importance for the prevention of the spread of resistance genes between animals and humans bacteria.

The objective of this study was to identify the bacteria present in wound of dogs and cats, monitor the resistance profile of the main groups of antimicrobial and identify multidrug-resistant strains of interest in public health.

MATERIAL AND METHODS

There were assessed 54 bacterial isolates from 38 wounds of pets (48 of dogs and 6 of cats) which have been met in Veterinary Hospital (HV), Umuarama Regional Campus (CAU) of the State University of Maringá (UEM) between March 2012 and February 2014. The evaluation of the isolates was performed in Veterinary Microbiology Laboratory, HV-CAU-UEM.

The samples were collected with sterile swabs in the Small Animal Medical and Surgical Sector, HV-CAU-UEM, and incubated in BHI broth (Brain Heart Infusion, OXOID®) at 36 ° C for 2-24 hours, according to turbidity of broth, being sequentially inoculated in Agar with 5% Sheep Blood (defibrinated) (OXOID®) and MacConkey (OXOID®) media. Bacterial identification was carried out through cultural, morphological, and biochemical characteristics, according to ANVISA (2012). Antimicrobial susceptibility was performed in Muller Hinton Agar (OXOID®) by disk diffusion method (BAUER et al., 1966). The inhibition zones were evaluated according to the CLSI M31-A3 standards (2008) (R=number of resistant).

The following 12 classes with 32 antimicrobial agents were tested: β-lactam penicillins: penicillin G (10U); β-lactam aminopenicillin: amoxicillin (10 μg) and ampicillin (10 μg); β-lactam/ β-lactamase inhibitors combinations: amoxacillin-clavulanic acid (30 μg) and ampicillin-sulbactan (20 μg); β-lactam penicillinase-stable penicillins: oxacillin (1 μg) (just for Gram-positive); β-lactam cephems cephalosporin: first generation - cephalexin (30 μg) and cephalothin (30 μg) and 3rd generation - ceftriaxone (30 μg); β-lactam cephems cephamycin: cefoxitin (30 μg); β-lactam carbapenems: imipenem (10 μg) and meropenem (10 μg); Glycopeptides: vancomycin (30 μg) (just for Gram-positive);

Polypeptides: polymyxin (300 µg) (just for Gram-negative); Aminoglycosides: gentamycin (10 µg), streptomycin (10 µg), amikacin (30 µg), neomycin (30 µg) and tobramycin (10 µg); Macrolides: 14-membered rings - erythromycin (15 µg) and 15-membered rings - azithromycin (15 µg); Lincosamides: clindamycin (2 µg); Ansamycin: rifampin (5 µg); Phenicols: chloranphenicol (30 µg); Nitrofurantoin: nitrofurantoin (10 µg); Fluoroquinolone: enrofloxacin (5 µg), norfloxacin (10 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg); Tetracyclines: tetracycline (30 µg) and doxycycline (30 µg); Folate pathway inhibitors: sulfamethoxazole-trimethoprim (25 µg) (NEWPROV®). Phenotypic detection of multidrug-resistant strains with public health significance was performed by disk diffusion using the following antimicrobials: oxacillin and cefoxitin to MRS (Methicillin-Resistant *Staphylococcus* spp.) according to CLSI (2008); erythromycin and clindamycin to MRS-MLSB (Macrolide-Lincosamide-Streptogamina B Resistant MRS) according to Kim et al. (2004); amoxacillin-clavulanic acid associated with aztreonam, ceftazidime, cefotaxime, ceftriaxone and cefepime to ESBL (Extended-Spectrum Betalactamase), according to Souza Júnior et al. (2004); and vancomycin to VRE (Vancomycin-Resistant *Enterococcus* spp.) according CLSI (2008).

The MAR index, calculated by the number of antimicrobial resistant divided by the number of antimicrobials, was evaluated according to Krumperman (1983).

The results were submitted to descriptive analysis to calculate absolute and relative frequencies (PETRIE and WATSON 2009; SAMPAIO, 2010).

RESULTS

Wounds of 38 animals were evaluated, totaling 39 samples (wound of one patient was evaluated in 2 different times of clinical evolution, both presenting *Staphylococcus* spp. with initial MAR index of 0.36 and later 0.7), comprising the total of 54 strains, where 30 (55.5%) were Gram-positive cocci and 24 (44.4%) Gram-negative cocci/bacilli (table 1).

Table 1. Frequency and percentage distribution and MAR index average in bacterial strains of wounds of pets from Veterinary Hospital of State University of Maringá

	Bacterial strain	Frequency	Percentage	MAR (average)	Frequency MAR ≥0.2
Gram-positive	*Staphylococcus* spp.	26	48,1	0.42	22
	Streptococcus spp.	2	3,7	0.28	1
	Enterococcus spp.	1	1,8	0.18	0
	Micrococcus spp.	1	1,8	0.22	1
	Total	**30**	**55,5**	**0.39**	**24**
Gram-negative	Fermenting strains				
	Escherichia coli	6	11,1	0.41	4
	Citrobacter spp.	4	7,4	0.49	4
	Providencia spp.	3	5,5	0.39	3
	Serratia spp.	2	3,7	0.54	2
	Salmonella spp.	1	1,8	0	0
	Proteus spp.	1	1,8	0.38	1
	Pantoea agllomerans	1	1,8	0.28	1
	Não identificado	1	1,8	0.48	1
	Total	**19**	**35,2**	**0.41**	**16**
	Non-fermenting strains				
	Pseudomonas spp.	4	7,4	0.57	4
	Burkholderia pseudomallei	1	1,8	0.82	1
	Total	**5**	**9,3**	**0.63**	**5**
TOTAL		**54**	**100**	**0.42**	**45**

The simple bacterial growth was found in 29 samples. Growth of more than one bacterial type was found in 14 samples, dual growth was identified in 8 of this samples and growth of three

different bacterial types in 3 of them. *Staphylococcus* spp. appeared in 64.3% (9/14) of multiple growths, followed by *Escherichia coli* with 21.4% (3/14). Gram positive growth associated with gram-negative occurred in 57.1% (8/14). A sample showed two strains of *Citrobacter* spp. with phenotypic characteristics of different resistance, than characterized by different strains.

A total of 1433 antimicrobial ratings were performed. According to CLSI (2008), 42.3% (n=606) of evaluations were resistant and 7.4% (n=106) had intermediate resistance, totaling 49.7% (n=712) with total or partial resistance.

The MAR index average was 0.42, according to Krumperman (1983), the value of this ratio greater than 0.2 is regarded as multidrug-resistant strain. Of the 54 strains evaluated, only 9 (16.7%) presented MAR below this value, considering thus 83.3% (45/54) of the strains isolated as multidrug-resistant, these averaging with 0.49 (table 1).

Drugs with greater resistance (above 70%) were β-lactam penicillins and aminopenicillins: penicillin (R=87.5%), ampicillin (R=79.2%) and amoxicillin (R=71.7%); and lincosamides: clindamycin (R=80%); with resistance between 50 and 70% were β-lactam penicillinase-stable penicillins: oxacillin (R=63%) evaluated only in gram positive strains; β-lactam cephems cephamycin: cefoxitin (R=63.3%); tetracyclines: tetracycline (R=61%); macrolides: erythromycin (R=61%); and ansamycin: rifampin (R=52%); and drugs considered to be less resistant were β-lactam carbapenems: imipenem (R=0%) and meropenem (R=4.5%); the polypeptides: polymyxin (R=9.5%), evaluated only in gram negative; and aminoglycosides: gentamycin (R=17%), tobramycin (R=19%), amikacin (R=21%) and neomycin (R=26%) (Table 2).

Table 2. Frequency and percentage distribution of resistance to antimicrobial drugs in bacterial strains of wounds of pets from Veterinary Hospital of State University of Maringá

Antimicrobial agents	**Gram-positive**		**Gram-negative**		**Total**	
	R (n)	**%**	**R (n)**	**%**	**R (n)**	**%**
Penicillin	25 (29)	86	10(11)	91	35 (40)	88
Amoxacillin	18 (29)	62	20 (24)	83	38 (53)	72
Amoxacillin-clavulanic acid	7 (29)	24	18 (24)	75	25 (53)	47
Ampicillin	21 (29)	72	21 (24)	88	42 (53)	79
Ampicillin-sulbactan	2 (22)	9	9 (18)	50	11 (40)	28
Oxacillin	17 (27)	63	-	-	17 (27)	63
Cefoxitin	13 (22)	59	6 (8)	75	19 (30)	63
Cephalexin	8 (27)	30	16 (22)	73	24 (49)	49
Cephalothin	6 (29)	21	16 (23)	70	22 (52)	42
Ceftriaxone	8 (29)	28	6 (24)	25	14 (53)	26
Meropenem	1 (25)	4	1 (19)	5	2 (44)	5
Imipenem	0 (18)	0	0 (15)	0	0 (33)	0
Vancomycin	16 (30)*	-	-	-	-	-
Polymyxin B	-	-	2 (21)	10	2 (21)	10
Gentamycin	3 (30)	10	6 (24)	25	9 (54)	17
Streptomycin	12 (23)	52	10 (24)	42	22 (47)	47
Amikacin	5 (30)	17	6 (23)	26	11 (53)	21
Neomycin	9 (26)	35	3 (21)	14	12 (47)	26
Tobramycin	5 (30)	17	5 (24)	21	10 (54)	19
Erythromycin	14 (29)	48	11 (12)	92	25 (41)	61
Azithromycin	11 (30)	37	11 (23)	48	22 (53)	42
Clindamycin	21 (29)	72	14 (15)	93	35 (44)	80
Rifampin	7 (25)	28	16 (19)	84	23 (44)	52
Chloranphenicol	5 (29)	17	5 (24)	21	10 (53)	19
Enrofloxacin	15 (30)	50	9 (23)	39	24 (53)	45
Norfloxacin	12 (29)	41	9 (24)	38	21 (53)	40
Ciprofloxacin	12 (26)	46	8 (19)	42	20 (45)	44
Levofloxacin	11 (30)	37	6 (23)	26	17 (53)	32
Tetracycline	20 (30)	67	13 (24)	54	33 (54)	61
Doxycycline	10 (30)	33	11 (24)	46	21 (54)	39
Sulfamethoxazole-trimethoprim	16 (30)	53	10 (23)	43	26 (53)	49
TOTAL	**328 (831)**	**39.5**	**278 (602)**	**46.2**	**606 (1433)**	**100**

* vancomycin was refered just by susceptible

There were found 16 samples phenotypically characterized as MRS, 12 as MRS-MLSB, one as VRE and 5 as ESBL (table 3).

Table 3. Phenotypic profile of resistance by public health bacterial strains of wounds of pets from Veterinary Hospital of State University of Maringá

Bacterial strain	Antimicrobial resistant	MAR
MRS 1	OXA, PEN, AMO, AMP, CFE, VAN, EST, NEO, ERI, AZI, CLI, ENO, NOR, LVX, DOX, SUT	0.67
MRS 2	OXA, PEN, CFO, AMP, EST, AMI, CLI, TET, DOX	0.36
MRS 3	OXA, PEN, CFO, AMO, AMP, CFE, CFL, CRO, VAN, CLI, RIF, ENO, NOR, CIP, LVX, TET, SUT	0.63
MRS 4	OXA, PEN, CFO, AMO, AMC, AMP, MER, VAN, EST, NEO	0.36
MRS 5	OXA, PEN, CFO, AMO, AMC, AMP, CFE, CRO, VAN, CLI,	0.36
MRS 6	OXA, PEN, AMO, AMP, VAN, EST, AMI, NEO, CLI, ENO, NOR, CIP, LVX, TET, DOX, SUT	0.55
MRS 7	PEN, CFO, AMO, AMP, EST, ERI, AZI, RIF, ENO, NOR, CIP, LVX, TET, DOX, SUT	0.50
MRS 8	OXA, PEN, CFO, AMO, AMP, CFL, MER, EST, TOB, ERI, AZI, CLI, CLO, ENO, NOR, CIP, LVX, TET, SUT	0.57
MRS 9	OXA, PEN, CFO, AMO, AMC, AMP, ASB, CFE, CFL, CRO, VAN, GEN, EST, AMI, TOB, ERI, CLI, RIF, ENO, CIP, LVX, TET, DOX, SUT	0.80
MRS 10	OXA, PEN, CFO, CFE, CFO, VAN, CLI, RIF, TET	0.30
MRS 11	OXA, PEN, CFO, AMO, AMP, CFE, CRO, GEN, EST, AMI, NEO, TOB, ERI, AZI, CLI, ENO, NOR, CIP, SUT	0.63
MRS 12	OXA, PEN, AMO, AMP, VAN, ERI, CLI, RIF	0.29
MRS 13	OXA, PEN, CFO, AMO, AMC, AMP, CFL, VAN, EST, ERI, CLI, RIF, TET	0.43
MRS 14	OXA, PEN, CFO, CRO, AMI, AZI, CLI, ENO, TET, SUT	0.37
MRS 15	OXA, PEN, CFO, AMO, AMP, CFE, CRO, RIF, TET, SUT	0.36
MRS 16	OXA, PEN, AMO, AMC, AMP, ASB, CFL, CRO, ERI, AZI, CLI, ENO, NOR, CIP, LVX, TET, SUT	0.63
VRE	PEN, AMO, CFO, VAN, CLI,	0.22
ESBL 1	AMO, AMP, GEN, EST, TOB, ERI, AZI, CLI, ENO, NOR, CIP, LVX, TET, DOX, SUT, CTX*, ATM*, COM*, CAZ*	0.64
ESBL 2	AMO, AMC, AMP, CFE, CFO, EST, AZI, CLI, RIF,	0.35
ESBL 3	PEN, AMO, AMP, ASB, CFE, CFL, CRO, EST, ERI, RIF, CLO, ENO, NOR, CIP, LVX, TET, DOX, SUT, CTX*, ATM*, CPM*	0.70
ESBL4	CEF, PEN, AMO, AMC, AMP, CFE, CFL, CRO, EST, AMI, ERI, AZI, RIF, ENO, NOR, CIP, LVX, CTX*	0.63
ESBL5	PEN, AMO, AMC, AMP, CFO, CFL, GEN, ERI, RIF, ENO, NOR, CIP, LVX, TET, DOX, SUT, CTX*, CRO, ATM*	

Legend: 1: OXA = Oxacillin, PEN = Penicillin G, CFO = Cefoxitin, AMO = Amoxacillin, AMC = Amoxacillin-clavulanic acid, AMP = Ampicillin, ASB = Ampicillin-sulbactan, CFE = Cephalexin, CFL = Cephalothin, CRO = Ceftriaxone, MER = Meropenem, VAN = Vancomycin, GEN = Gentamycin, EST = Streptomycin, AMI = Amikacin, NEO = Neomycin, TOB -= Tobramycin, ERI = Erythromycin, AZI = Azithromycin, CLI = Clindamycin, RIF = Rifampin, CLO = Chloranphenicol, ENO = Enrofloxacin, NOR = Norfloxacin, CIP = Ciprofloxacin, LVX = Levofloxacin, TET = Tetracycline, DOX = Doxycycline, SUT = sulfamethoxazole-trimethoprim, CTX = Cefotaxim, ATM = Aztreonan, CPM = Cefepime, CAZ = Ceftazidim. *Antimicrobial agents tested just to ESBL identification and not computed in table values and in text.

DISCUSSION

Arias et al. (2008), studying 20 bacterial isolates from surgical wounds, found MAR≥0.2 in 19 (95%) of strains. The average of MAR index was 0.7, where three samples had MAR=1.0. Sfaciotte et al. (2014) found similar values, where 89.4% (17/19) were considered multidrug resistant, with average of MAR index was 0.65 and maximum was 1.0. Both studies found higher MAR values in Gram-negative. However, in both studies were tested few antimicrobial, 8 and 7 drugs per sample, respectively. With the increase in number of drugs tested, this index tends to have lower values, but with greater reliability, as occurred in the present study, evaluated an average of 26.5 antimicrobials by samples being tested at least one antimicrobial drug of each class. Mota et al. (2005) and Arias and Carrilho (2012) report a gradual increase in multidrug resistance to antimicrobial agents in Veterinary Medicine.

The β-lactam penicillins (penicillin with R=87.5%) and aminopenicillins (ampicillin with R=79.2% and amoxicillin with R=71.7%) showed high resistance rates, however, when associated

with beta-lactamase inhibitors (ampicillin-sulbactan with R=27.5% and amoxacillin-clavulanic acid with R=47.2%) it was possible to notice the improvement in sensitivity to drugs with 51.7% and 24.5% of cases respectively. This study thus recommends the association between aminopenicillins with beta-lactamase inhibitors. Arias et al. (2008) obtained a degree of sensitivity in samples of contaminated and infected wounds front amoxacillin-clavulanic acid (25%), being all Gram-negative samples resistant and approximately 80% of Gram-positive susceptible. The ampicillin alone, in the same study showed sensitivity less than 20%. Ishii et al. (2011) detected ampicillin-resistance in 63.6% of 22 samples. The resistance to amoxicillin was observed in 64% of 75 samples, however, associated with clavulanic acid, detected resistance in 68% of 25 samples, however, the study does not show parity between the association and the drugs in the studied strains, for correct evaluation.

Oxacillin and cefoxitin are, according to CLSI (2008), drugs to predict the *Staphylococcus* spp. resistance to all β-lactams. According to Kim et al. (2012) and Cartwright et al. (2013), oxacillin-resistance shows the presence of *mecA* gene of phenotypic form, since the cefoxitin-resistance with oxacillin-susceptible show the presence of *mecC* gene. These genes are responsible to production an additional penicillin-binding protein (PBP2a) which confers low binding affinity to β-lactam drugs. The *Staphylococcus* spp. which carrier these genes are called MRS. Several studies had shown the existence of MRS in Veterinary Medicine. In this study, of 26 samples of Staphylococcus spp., 15 were resistant to oxacillin and 12 to cefoxitin, demonstrating the presence of MRS in 16 (61.54%) strains of *Staphylococcus* (with prediction of both *mecA* and *mecC* genes). According to CLSI (2008), all should be reported as resistant to β-lactam drugs. Pereira et al. (2009) also reported the detection of MRS through oxacillin-resistance and detection of *mecA* gene in 15% of *Staphylococcus* samples obtained from dogs and cats.

The tested β-lactam cephalosporin drugs had intermediate resistance levels, ranging from 49% to cephalexin, 42 % to cephalothin and 26% to ceftriaxone. However, when compared bacterial types, Gram-negative showed greater resistance to cephalothin (73%) and cephalexin (70%). Associating the data cited above of β-lactam-resistance predicted by oxacillin and cefoxitin and cephalosporin-resistance themselves, in infections by 37 (66.7%) of 54 strains studied are not recommended the use of a 1st generation of cephalosporins (cephalexin and cephalothin) and in 25 (46.3%) the use of 3rd generation (ceftriaxone). Arias et al. (2008) related a degree of resistance in contaminated and infected wounds samples to cephalosporin of 75%, on the contrary, the bacteria were susceptible to cephalexin in almost 70% of Gram-positive and resistant to all Gram-negative as well as cephalothin. These authors also comment that cephalosporins are not effective in preventing infection *in vivo*, not being drugs of choice in treatment of infected wounds, especially those resulting from animal bites. Ishii et al. (2011) reported resistance to cephalothin in 61.5% (26), to cephalexin in 74.7%, (79) and to ceftiofur in 91.6% (12) of samples.

The β-lactam carbapenems have similar chemical structure of penicillin, with chemical characteristics which gives them greater affinity to PBPs, showing more power and expanded antibacterial spectrum. In 1979, imipenem emerged, 8 years later, meropenem, with greater activity against Gram-negative and decreased convulsing effects (POSSEBON and CAMARGO, 2003). The use of carbapenems in veterinary medicine in Brazil

was first reported by Montiani-Ferreira et al. (1999), although its spread is not wide in this area.

Carbapenems-resistance in *Enterobacteriaceae* is a serious world-wide public health problem, particularly by high mortality and small number of therapeutic options. After emergence and spread of ESBLs, carbapenems were considered the first option for treatment against these serious infections. In 2005, the first case of fatal infection due to *Klebsiella pneumoniae* resistant to carbapenems was reported in Brazil, followed by other reports not so infrequently (RIBEIRO, 2013). The detection of these cases points to an opportunity to control the spread of this type of resistance mechanism in Brazil, which can only be achieved with a large multidisciplinary effort, which includes, among other measures, early detection of colonized patients, implementation of contact precautions and appropriate treatment (NORDMANN and CORNAGLIA, 2012), as well as reduced use of these antimicrobial drugs.

This study identified five ESBL strains, two classified by *Escherichia coli* (MAR=0.7 e 0.64), two by *Citrobacter* spp. (MAR=0.59 e 0.35) and one by *Providencia* spp. (MAR=0.63), both carbapenems-susceptible. According to CLSI (2008), ESBL strains should be reported to resistant to all penicillins, cephalosporins and aztreonam.

Carbapenems, in this study, showed 4.5% of resistance (one MRS and one *Burkholderia pseudomallei*). Even though considering that their antimicrobial activity may be impaired in MRS, the use of carbapenems is not indicated in 31.5% (17/54) of the samples. Although there are no clear rules that prohibit the use of carbapenems in Veterinary Medicine, these drugs should be used with caution in order to avoid the pressure of selection of resistant clones and transmission of resistance to other bacteria, potentially contacts with human.

After the discovery of multidrug-resistant Gram-positive bacteria, especially MRS, antimicrobial agents, including the glycopeptides vancomycin and teicoplanin, has been, for many years, the last alternative for the treatment against these microorganisms in Medicine. In the early 1990s, strains of *Staphylococcus aureus* resistant *in vivo* (failed therapy) to teicoplanin have been reported in the USA and Europe (KAATZ et al., 1990; MANQUAT et al., 1992). In 1997, in Japan, there were isolated the first strain of *Staphylococcus aureus* with reduced susceptibility to vancomycin (VISA) resulting in a significant concern about the future of the therapy of infections caused by these microorganisms (HIRAMATSU et al., 1997). In 2002, it was first isolated a sample of Vancomycin-Resistant *Staphylococcus aureus* (VRSA) (CHANG et al., 2003). This isolate was the first to have the *vanA* gene that causes resistance to vancomycin and teicoplanin in *Enterococcus faecalis* (SOUSA, 2006). The origin of the VRSA strain was explained by conjugal transfer of this gene from *Enterococcus* and *Staphylococcus* (WEESE, 2005). According to CLSI (2018), the detection of VISA and VRSA strains should be carried by Minimum Inhibitory Concentration (MIC) or screen agar screen tests for vancomycin. Strains with resistance by disk diffusion for vancomycin should be reassessed by MIC.

In Veterinary Medicine, studies concerning glycopeptides-resistance have been limited due to the little use of these drugs in animals (MONCHIQUE, 2013). In a study of Haenni et al. (2010) teicoplanin or vancomycin-resistant strains were not detectable in 60 *Staphylococcus* of horses. According to Monchique (2013), VISA and VRSA strains have not yet been reported in Veterinary Medicine, possibly due to lower incidence of

Enterococcus strains resistant to this antibiotic (WEESE, 2005).

Enterococcus spp. are intrinsically resistant to commonly used antibiotics including cephalosporins and some aminoglycosides (SHEPARD and GILMORE, 2002), and acquire high level resistance to penicillin/ampicillin, glycopeptides and others aminoglycosides, limiting the therapeutic practice (SOOD et al., 2008). Vancomycin is an important antibiotic in the treatment of these infections, but the efficiency of this drug has been limited by the emergence of Vancomycin-Resistant *Enterococcus* spp. (VRE). Thus, become one of the most important clinical bacteria with antimicrobial resistance throughout the world, with few therapeutic agents capable to treat infections caused by this microorganism and with ease transmission of resistance to other bacteria, especially *Staphylococcus* spp. (EISNER et al., 2005; FUJITA et al., 1998). In Brazil, the first isolated in human took place in Curitiba, in 1996, subsequently detected in hospitals in several cities around the country (DALLA et al., 1998; D'AZEVEDO et al., 2000; ZANELLA, 2003; CAMARGO et al., 2006; PALAZZO et al., 2011).

The results of the present study, using the disk diffusion, show that from 26 samples of *Staphylococcus* spp. found, 14 were *in vitro* susceptible to vancomycin and 12 require further evaluation by MIC for this drug. However, an *Enterococcus* spp. was considered resistant to vancomycin. Extensive monitoring of VRE as well as MRS, VISA and VRSA needs to be done in veterinary hospitals, reporting the cases and investigating their origin. Similar to carbapenems, glycopeptides should be evaluated with caution in veterinary medicine, in order to prevent the selection of microorganisms resistant to these two classes, which can result in cross-resistance between bacteria of animals and humans.

Aminoglycosides presented the highest susceptibility, with resistance of 17% for gentamicin (10% in Gram-positive and 25% in Gram-negative), 19% for tobramycin (17% in Gram-positive and 21% in Gram-negative), 21% for amikacin (17% in Gram-positive and 26% in Gram-negative), 26% for neomycin (34.6% in Gram-positive and 14% in Gram-negative) and 47% for streptomycin (52% in Gram-positive and 42% in Gram-negative). The gentamicin-resistance in MRS was observed in 2 (12.5%) strains and intermediate resistance in 3 (18.75%) strains. Arias et al. (2008) observed gentamycin-susceptibility of 45.45% in contaminated wounds and 64.28% in infected wounds. Ishi et al. (2008) observed a resistance of 36.6% (n=41) to gentamycin, 65.4% (n=26) to neomycin and 33.3% (n=27) to tobramycin. Sfaciotte et al. (2014) found resistance to gentamycin in only 12.5% of tested samples, similar to Smith et al. (2008), with 12.5% and 15.6% in samples of dogs and humans, respectively, Lilenbaum et al. (2000) found 15.9% of resistance. According to Farias (2002), aminoglycosides have been shown to be effective against staphylococcal infections, being appointed as drugs of choice in the treatment of these infections. Thus, in the micro region of Umuarama, the empirical choice of aminoglycosides, except streptomycin, is well accepted for the treatment of animals infections. However, they are not the best treatment for other locations, as described by Arias et al. (2008) and Ishii et al. (2011).

The MLSB group of antimicrobials is formed by Macrolides, Lincosamides and Streptogramin B, which have different formulas but with same mechanism of action, by inhibiting protein synthesis by binding to the 23S rRNA receptor, which in turn form part of the 50S subunit of bacterial ribosome. Since 1956, shortly after the introduction of erythromycin in clinic already had

MLSB-resistance in *Staphylococcus aureus* (ROSSI and ANDREAZZI, 2005; LECLERCQ, 2002). In Veterinary Medicine, clindamycin is widely used and also indicated for infections caused by staphylococci, especially MRSA (FIEBELKORN et al., 2003). However, Kim et al. (2004) studying the presence of MLSB-resistance in *Staphylococcus aureus* found that 97% of MRSA were resistant to at least one of the antibiotics of this group. Epidemiologically cross-resistance among these three classes of antimicrobial is very important (DIPERSIO and DIPERSIO, 2005), since they are widely used in veterinary medicine leading to an increase resistance in animals infections. In MRSA isolates from humans and animals, clindamycin-induced-resistance has been well documented (RUBIN et al., 2011). It is known that antimicrobial pressure causes the selection of resistant bacteria to MLSB group, but the horizontal transfer of resistance genes is also elucidated (PATTERSON et al., 2007).

The macrolides tested had intermediate resistance rates, with 61% of strains resistant to erythromycin (92% in Gram-negative and 48% in Gram-positive) and 42% to azithromycin (48% in Gram-negative and 37% in Gram-positive). Pereira et al. (2009) found 47.3% of azithromycin-resistance studying 151 samples, with high resistance in Gram-negative bacilli, and similar to the data obtained in this study. The clindamycin-resistance found here was 80% (100% in Gram-negative and 72% in Gram-positive), corroborating the data obtained by Ishii et al. (2011) with 82.3% and by Sfaciotte et al. (2014) with 100%, indicating that it is a drug that should not be used in the antimicrobial therapy whithout *in vitro* susceptibility.

In the present study, there were detected 50% (13/26) of *Staphylococcus* spp. resistant to MLS B group (two drugs tested), 5 (19.2%) were resistant to only one drug, 3 (11.5%) had intermediate resistance to drugs. 12 strains MLSB-resistant were phenotypically identified as MRS. Other 3 MRS identified were resistant to clindamycin or erythromycin.

Chloramphenicol was resistant in 19% of strains, with greater sensitivity to Gram-positive (17%) than for Gram-negative (21%). The chloramphenicol-resistance was detected in 12.5% (2/16) and intermediate resistance was detected in 6.25% (1/16) of MRS. Sfaciotte et al. (2014) and Mantilla and Franco (2012) found higher values in their studies, 56% and 59.2%, respectively. Resistance still higher to those was found by Mesquita et al. (2009) with 85.71%. However, Cruz et al. (2012) detected 35.19% of resistance in Gram-negative bacilli and 9.52% in *Staphylococcus*. These outliers in the literature show the unquestionable necessity of studies about antimicrobial resistance at different geographic regions.

The fluoroquinolones, according to CLSI (2008), should be reported together, where the resistance to a drug indicates the resistance of the entire class. The majority of samples showed similar values, with a few differences, with resistance of 45% for enrofloxacin (50% in Gram-positive and 39% in Gram-negative), 40% for norfloxacin (41% in Gram-positive and 38% in Gram-negative), 44% for ciprofloxacin (46% in Gram-positive and 42% in Gram-negative) and 32% for levofloxacin (37% in Gram-positive and 26.1% in Gram-negative). Of the 16 MRS phenotypically identified, 9 (56.25%) were resistant to fluoroquinolones and one (25%) of the 4 *Pseudomonas* spp. Sfaciotte et al. (2014) showed greater resistance in the samples studied, between 50 and 78% of resistant strains. Arias et al. (2008) obtained a degree of sensitivity in samples of contaminated and infected wounds against

norfloxacin 58.33%. In 2011, Ishii and colleagues also in Londrina - PR, detected resistance for enrofloxacin in 53.8% (n=117), ciprofloxacin in 42.8% (n=84) and orbifloxacin in 100% (n=6).

The sulfamethoxazole-trimethoprim, sulfonamide evaluated, showed resistance in 49% of isolates (43% in Gram-negative and 53% in Gram-positive). Resistance in 62.5% (10/16) of MRS was detected. Sfaciotte et al. (2014) found resistance of 100% in samples, Arias et al. (2008) of 92.3% (12/13) in samples of contaminated and infected wounds and Dal-Bo et al. (2013) of 75% to 50% by *Staphylococcus* and Gram-negative bacilli, respectively. Since Cruz et al. (2012) reported resistance of 35.19% and 52.38% in Gram-negative bacteria and Staphylococcus, respectively.

Rifampicin with 51% of resistance and tetracycline with 60%, are two other drugs not indicated for empirical antimicrobial therapy without an *in vitro* evaluation.

Several studies indicate bacterioscopic examination or culture at the beginning of animal care with infected traumatic wound, facilitating the choice of therapy to be introduced and prior determination of potentially infectious microorganisms (ARIAS and PEREIRA, 2002; PAVLETIC and TROUT, 2006). This procedure, as introduced in the beginning of the treatment, can quickly elucidate the causal agent, reducing the time and cost of treatment, improve the prognosis and decreasing potentially treatable chronic wounds in animals.

CONCLUSIONS

Bacterial genus/species found in wounds of pets are very heterogeneous, comprising mainly *Staphylococcus* spp. and Gram-negative fermenters, particularly *Escherichia coli*, varying according to etiology and progression of wound itself. Antimicrobial resistance in this study corroborate with others about the increasing of this resistance found both in human and veterinary medicine. This increase in resistance is an important natural event which occurs due to the selective pressure that indiscriminate the use of these drugs provides to the environment. Thus, the judicious use of these drugs is recommended always combined with *in vitro* tests, for evaluating the optimal therapy to be used, reducing resistance rates to certain drugs already very resistant and avoiding resistance to drugs still susceptible.

In this study, there were detected great importance bacterial strains as MRS, MRS-MLSB, VRE and ESBL in 40.74% (22/54) of isolates of wounds of pets. The early identification becomes an important step to minimize the transmission of bacterial resistance in animals.

Constant monitoring of bacterial resistance profile, which varies over years and differs from place to place, it is a reality that must be observed by the medical and veterinary professionals both clinicians and surgeons. The testing for bacterial identification and their susceptibility to assist in the proper selection of antimicrobial agent shown essential due to high bacterial resistance rates recorded in this and other studies, as well as the monitoring of local resistance to continued use of certain antimicrobial drugs . The prudent choice of adopted antibiotics reduces the use of antibiotics and consequently the development of bacterial resistance by selection, especially in hospital settings.

REFERENCES

ANDRADE I.; ANDRADE N.; OLIVEIRA B. A importância das actividades de autocuidado no atendimento ao paciente ambulatorial com lesão traumática: Um estudo de caso na enfermagem. **Cadernos de estudos e pesquisas,** v. 24, p. 43-54, 2006.

ANVISA. **Manual de microbiologia clínica para o controle de infecção relacionada à assistência à saúde – Módulo 6: detecção e identificação e bactérias de importância médica**. 2ª Ed. Agência Nacional de Vigilância Sanitária, Brasília: ANVISA, 2013.

ANVISA. NOTA TÉCNICA Nº 01/2013 - **MEDIDAS DE PREVENÇÃO E CONTROLE DE INFECÇÕES POR ENTEROBACTÉRIAS MULTIRESISTENTES**. Brasília, 17 de abril de 2013.

ARIAS, M.V.B.; CARRILHO, C.M.D.M. Resistência antimicrobiana nos animais e no ser humano. Há motivo para preocupação? **Semina: Ciências Agrárias**, v. 33, n. 2, p. 775-790, 2012

ARIAS, M.V.; PEREIRA, A.M. Manejo de feridas em cães e gatos- revisão. **Revista Clinica Veterinária**, v. 7, n. 38, p. 33-42, 2002.

ARIAS, M.V.B.; BATAGLLIA, L.A.; AIELLO, G.; CARVALHO, T.T.; FREITAS, J.C. Identificação da suscetibilidade antimicrobiana de bactérias isoladas de cães e gatos com feridas traumáticas contaminadas e infectadas. **Semina: Ciências Agrárias**, v. 29, p. 861-874, 2008.

BAUER, A.W.; KIRBY, W.M.M.; SHERRIS, J.C.; TURCK M. Antibiotic susceptibility testing by a standardized single disk method. **Am. J. Clin. Pathol**. 45:493-496, 1966.

BELLAH, J.; WILLIAMS J. Wound Closure Options and Decision Making, p.24-36. In: Williams, J. & Fowler, D. (Eds). **Manual of Canine and Feline Wound Management and Reconstruction**. BSAVA, British Small Animal Veterinary Association, United Kingdom. 161p, 1999.

CAMARGO, I.L.B.C.; GILMORE, M.S.; DARINI A.L.C. Multilocus sequence typing and analysis of putative virulence factors in vancomycin-resistant and vancomycin-sensitive *Enterococcus faecium* isolates from Brazil. **Clin Microbiol. Infect.**, v. 12, p. 1123–1130, 2006.

CARTWRIGHT, E.J.P.; PATERSON, G.K.; RAVEN, K.E.; HARRISON, E.M.; GOULIOURIS, T.; KEARNS, A.; PICHON, B.; EDWARDS, G.; SKOV, R.L.; LARSEN, A.R.; HOLMES, M.A.; PARKHILL, J.; PEACOCK, S.J.; TOROK, M.E. Use of Vitek 2 antimicrobial susceptibility profile to identify *mec*C in methicillin-resistant *Staphylococcus aureus*. **J. Clin. Microbiol.**, v. 51, p. 2732–2734, 2013.

CHANG, S.; SIEVERT, D.M.; HAGEMAN, J.C.; BOULTON, M.L.; TENOVER, F.C.; DOWNES, F.P.; SHAH, S.; RUDRIK, J.T.; PUPP, J.R.; BROWN, W.J.; CARDO, D.; FRIDKIN, S.K. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. **N. Engl. J. Med.**, v. 348, p. 1342-1347, 2003.

CLSI 2008. **Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals**. 3rd ed. Approved standard M31-A3, Clinical and Laboratory Standards Institute, Wayne, PA.

COELHO, S.M.O.; MORAES, R.A.M.; SOARES, L.C.; PEREIRA, I.A.; GOMES, L.P.; SOUZA, M.M.S. Mapeamento do perfil de resistência e detecção do gene *mec*A em *Staphylococcus intermedius* oxacilina-resistentes isolados de espécies humanas e animais. **Ciência Rural**, v. 37, n. 1, p. 195-200, 2007.

CRUZ, A.R.; PAES, A.C.; SIQUEIRA, A.K. Perfil de sensibilidade de bactérias patogênicas isoladas de cães frente a antimicrobianos. **Revista Veterinária e Zootecnia**, v. 19, n. 4, p. 601-610, 2012.

DAL-BÓ, I.S.; FERRIGNO, C.R.A.; FERREIRA, M.P.; CAQUIAS, D.F.I.; SOUZA, A.N.A.; RIZZO, M.F.C.I.; CAVALCANTI, R.A.O.; SANTOS J.F. Infecção óssea após osteotomia para tratamento da

ruptura de ligamento cruzado em cães. **Revista Acta ScientiaeVeterinariae**, v. 41, p. 1148, 2013.

DALLA COSTA, L.M.; SOUZA, D.C.; MARTINS, L.T.; ZANELLA, R.C.; BRANDILONE, M.C.; BOKERMANN, S.; SADER, H.S.; SOUZA, H.A. Vancomycin-Resistant *Enterococcus faecium*: First Case in Brazil. **Braz J Infect Dis**., v. 2, n. 3, p. 160-163, 1998.

D'AZEVEDO, P.A.; KACMAN, S.B.; SCHMALFUSS, T.; SILVA, A.; RODRIGUES, L.F. Primeiro caso de *Enterococcus* resistente a vancomicina isolado em Porto Alegre, RS. **J Bras Pat**., v. 36, p. 258, 2000.

DIPERSIO, J.R.; DIPERSIO, L.P. Update on the prevalence and spread of macrolide and lincosamide-resistant staphylococcal and streptococcal species. **Rev. Med. Microbiol**., v. 16, p. 117–123, 2005.

EISNER, A.; FEIERL, G.; GORKIEWICZ, G.; DIEBER, F.; KESSLER, H.H.; MARTH, E.; KÖFER, J. High prevalence of *van*A-type vancomycin-resistant enterococci in Austrian poultry. **Applied and Environmental Microbiology**, v. 71, p. 6407–6409, 2005.

FARIAS, M.F. Terapêutica otológica, p.198-204. In: Farias, M.F et al. **Manual de terapêutica veterinária**. 2.ed. Roca, São Paulo, 2002

FIEBELKORN, K.R.; CRAWFORD, S.A.; MCELMEEL, M.L.; JORGENSEN, J.H. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. **Journal of Clinical Microbiology**, v. 41, p. 4740-4744, 2003.

FORD, R.; MAZZAFERRO, E. Diagnóstico de urgência y procedimientos terapêuticos, p.6-66. In: Kirk & Bistner (Eds). **Urgencias en veterinária. Procedimientos y Terapéutica**. Elsevier Saunders, Phyladelphia, 2007.

FOSSUM, T. Surgery of the Integumentary System, p.159-175. In: Fossum, T. (Ed). **Small Animal Surgery**. Mosby Elsevier, St Louis, 2007.

FUJITA, N.; YOSHIMURA, M.; KOMORI, T.; TANIMOTO, K.; IKE, Y. First report of the isolation of high-level vancomycin-resistant *Enterococcus faecium* from a patient in Japan. **Antimicrobial Agents and Chemotherapy**, v. 42, p. 2150, 1998.

GARZOTTO, C. Wound Management, p.676-686. In: Silverstein D. & Hopper K. (Eds). **Small Animal Critical Care Medicine**. Saunders Elsevier, Phyladelphia, 2009.

GUARDABASSI, L.; SCHWARZ, S.; LLOYD, D. Pet animals as reservoirs of antimicrobial-resistant bacteria. **J. Antimicrob. Chemother**., v. 54, p. 321-332, 2004.

HAENNI, M.; TARGANT, H.; FOREST, K.; SÉVIN, C.; TAPPREST, J.; LAUGIER, C.; MADEC, J.Y. Retrospective study of necropsy-associated coagulase-positive staphylococci in horses. **Journal of Veterinary diagnostic Investigation**, v. 22, p. 953-956, 2010.

HIRAMATSU, K.; HANAKI, H.; INO, T.; YABUTA, K.; OGURI, T.; TENOVER, F.C. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. **J Antimicrob Chemother**, v. 40, p. 135–36, 1997.

HOSGOOD, G. Wound Repair and Tissue Response to Injury, 66-86. In: Slatter D. (Ed). **Textbook of Small Animal Surgery**. Saunders, Phyladelphia. 1286p, 2003.

ISHII, J.B.; FREITAS, J.C.; ARIAS, M.V.B. Resistência de bactérias isoladas de cães e gatos no Hospital Veterinário da Universidade Estadual de Londrina(2008-2009). **Pesquisa Veterinária Brasileira**, v. 31, n. 6, p. 533-537, 2011.

KAATZ, G.W.; SEO, S.M.; DORMAN, N.J.; LERNER, S.A. Emergence of teicoplanina resistance during therapy of *Staphylococcus aureus*

endocarditis. **J. Infect. Dis**., v. 162, p. 103–108, 1990.

KIM, H.B.; LEE, B.; JANG, H.C.; KIM, S.H.; KANG, C.I.; CHOI, Y.J.; PARK, S.W.; KIM, B.S.; KIM, E.C.; OH, M.D.; CHOE, K.W. A high frequency of macrolide-lincosamide-streptogramin resistance determinants in *Staphylococcus aureus* isolated in South Korea. **Microb Drug Resist**., v. 10, n. 3, p. 248-54, 2004.

KIM, C.; MILHEIRIÇO, C.; GARDETE, S.; HOLMES, M.A.; HOLDEN, M.T.G.; LENCASTRE, H.; TOMASZ, A. Properties of a novel PBP2A protein homolog from *Staphylococcus aureus* strain LGA251 and its contribution to the betalactam-resistant phenotype. **J. Biol. Chem**., v. 287, p. 36854–36863, 2012.

KRUMPERMAN, P.H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk source of fecal contamination of foods. **Applied Enrironmental Microbiology**, v. 46, n. 1, p. 165-170, 1983.

LECLERCQ, R. Mechanisms of resistance of macrolides and lincosamides: nature of the resistance elements and their clinical implications. **Clin Infect Dis**., v. 34n. 4, p. 482-92, 2002.

LILENBAUM, W.; VERAS, M.; BLUM, E.; SOUZA, G.N.. Antimicrobial susceptibility of Staphylococci isolated from otitis externa in dogs. **Letters Apllied Micology**, v. 31, p. 42-45, 2000.

MANQUAT, G.; CROIZE, J.; STAHL, J.P.; MEYRAN, M.; HIRTZ, P.; MICOUD, M. Failure of teicoplanin treatment associated with an increase in MIC during therapy of *Staphylococcus aureus* septicaemia. **J. Antimicrob. Chemother**., v. 29, p. 731–732, 1992.

MANTILLA, S.P.S.; FRANCO, R. M. Perfil de sensibilidade microbiana in vitro de linhagens patogênicas de *Escherichia coli* isoladas de carne bovina. **Colloquium Agrariae**, v. 8, n. 1, p. 10-17, 2012.

MENDES, C.; OPLUSTIL, C.; SAKAGAMI, E.; TURNER, P.; KIFFER, C. Antimicrobial susceptibility in Intensive Care Units: MYSTIC Program Brazil 2002. **Braz. J. Infect. Dis**., v. 9, n. 1, p. 44-51, 2005.

MESQUITA, A.M.R.C.; LIMA, N.L.; LIMA, A.A.M. Avaliação da susceptibilidade e resistência antimicrobiana de cepas de *Shigella* spp. isoladas de pacientes com diarreia nosocomial. **Revista de Ciências Médicas e Biológicas**, v. 8, n. 3, p. 292-300, 2009.

MONCHIQUE, C.R.O. **Evolução da resistência aos antibióticos em *Staphylococcus* spp. –1999 a 2006**. Dissertação de Mestrado Integrado em Medicina Veterinária, Faculdade de Medicina Veterinária da Universidade de Lisboa, Lisboa, Portugal. 67f, 2013.

MONTIANI-FERREIRA, F.; WARTH, J.F.; PACHALY, J.R.; REIFUR, L.; DOTTI, C.F. Contribuição ao estudo da ação da meropenema, *in vitro* e *in vivo*, em infecções diagnosticadas em pequenos animais. Arq. Ciên. Vet. Zool. UNIPAR, v. 2, p. 2, 1999.

MOORE, A.; GARDEN, C. Procesos quirúrgicos y su manejo, p.198-205. In: Moore M. & Simpson G. **Manual para Auxiliares Técnicos Veterinarios**. Colección BSAVA- British Small Animal Veterinary Association, United Kingdom, 2003.

MOTA, R.A.; FRETIAS, M.F.L.; PORTO, W.J.N.; SILVA, L.B.G. Utilização indiscriminada de antimicrobianos e sua contribuição a multirresistência bacteriana. **Brazilian Journal of Veterinary Research and Animal Science**, v. 42, n. 6, p. 465-470, 2005.

NORDMANN, P.; CORNAGLIA G. Carbapenemase-producing *Enterobacteriaceae*: a call for action! **Clin Microbiol Infect**, v. 18, n. 5, p. 411-2, 2012.

PALAZZO, I.C.V.; PITONDO-SILVA, A.; LEVY, C.E.; DARINI, A.L.C. Changes in vancomycin-

resistant *Enterococcus faecium* causing outbreaks in Brazil. **J Hosp Infect.**, v. 79, n. 1, p. 70-4, 2011.

PATTERSON, A.J.; COLANGELI, R.; SPIGAGLIA, P.; SCOTT, K.P. Distribution of specific tetracycline and erythromycin resistance genes in environmental amples assessed by macroarray detection. **Environ. Microbiol**., v. 9, p. 703–715, 2007.

PAVLETIC, M.M.; TROUT, N.J. Bullet, bile and burn wounds in dogs and cats. **The Veterinary Clinics of North America: Small Animal Practice**, v. 36, n. 4, p. 873-93, 2006.

PAVLETIC, M.M. Skin and Adnexa, 250-259. In: Slatter, D. (Ed). **Textbook of Small Animal Surgery**, Vol.1. Ed. Saunders, Philadelphia. 1286p, 2003.

PEREIRA, I.A.; SOARES, L.C.; COELHO, S.M.O.; PRIBUL, B.R.; SOUZA, M.M.S. Susceptibilidade á azitromicina de isolados bacterianos de processos infecciosos em cães e gatos. **Pesquisa veterinária Brasileira**., v. 29, n. 2, p. 153-156, 2009.

PETRIE, A.; WATSON P. **Estatística em Ciência Animal e Veterinária**. 2ed. São Paulo: Editora Roca. 248p, 2009.

POSSEBON, M.I.; CAMARGO, E.A. Resistência bacteriana aos carbapenêmicos. **RBM rev. bras. Med.**, v. 60, n. 6, p. 378-378, 2003.

RIBEIRO, V.B. **Detecção de resistência aos carbapenêmicos e avaliação da produção de *Klebsiella pnemoniae* carbapenemase (kpc) em isolados clínicos da família *Enterobacteriaceae***. Tese de Doutorado do Programa de Pós Graduação em Ciências Farmacêuticas da Universidade Federal do Rio Grande do Sul. 134f, 2013.

ROSSI, F.; ANDREAZZI, D.B. **Resistência bacteriana: interpretando o antibiograma**. Atheneu, São Paulo. 118p, 2005.

RUBIN, J.E.; BALL, K.R.; CHIRINO-TREJO, M. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. **Can Vet J**., v. 52, p. 153–7, 2011.

SAMPAIO, I.B.M. **Estatística Aplicada à Experimentação Animal**. 3ed. FEPMVZ-Editora, Belo Horizonte. 221p, 2010.

SFACIOTTE, R.A.P.; VIGNOTO, V.K.C.; WOSIACKI, S.R. Perfil de resistência antimicrobiana de isolados bacterianos de afecções clínicas do hospital veterinário da Universidade Estadual de Maringá. **Rev. Ci. Vet. Saúde Public**., v. 1, n. 1, p. 29-38, 2014.

SHEPARD, B.D.; GILMORE, M.S. Antibiotic-resistant enterococci: The mechanisms and dynamics of drug introduction and resistance. **Microbes and Infection**, v. 4, p. 215–224, 2002.

SOARES, L.C.; PEREIRA, I.A.; COELHO, S.M.O.; CUNHA, C.M.M.; OLIVEIRA, D.F.B.; MIRANDA, A.N.; SOUZA, M.M.S. Caracterização fenotípica da resistência a antimicrobianos e detecção do gene mecA em Staphylococcus spp. Coagulase-negativos isolados de amostras animais e humanas. **Ciência Rural**, v. 38, n. 5, p. 1346-50, 2008.

SOOD, S.; MALHOTRA, M.; DAS, B.K.; KAPIL, A. Enterococcal infections and antimicrobial resistance. **Indian Journal of Medical Research**, v. 128, p. 111–121, 2008.

SOUSA JUNIOR, M.A.; FERREIRA, E.V.; CONCEIÇÃO, G.C. Betalactamase de Espectro Estendido (ESBL): um importante mecanismo de resistência bacteriana e sua detecção no laboratório clínico. **NewsLab**, v. 63, p. 152-174, 2004.

SOUSA, J. C. **Manual de antibióticos antibacterianos** (2ª edição). Porto, Ed. Universidade Fernando Pessoa, 2006.

TAZIMA, M.F.G.S.; VICENTE, Y.A.M.V.A.; MORIYA, T. Biologia da ferida e cicatrização. **Medicina (Ribeirão Preto)**, v. 41, p. 259-64, 2008.

UMBER, J.K.; BENDER, J.B. Pets and antimicrobial resistance. **Vet. Clin. North America: Small Animal Pract.,** v. 39, n. 2, p. 279-92, 2009.

WANNAMACHER, L. Uso indiscriminado de antibióticos e resistência microbiana: Uma guerra perdida? **Uso racional de medicamentos**, v. 1, n. 4, p. 1-5, 2004.

WEESE, J.S. Methicillin resistant *Staphylococcus aureus*: an emerging pathogen in small animals. **Journal of the American Animal Hospital Association**, v. 41, p. 150-157, 2005.

WHO (2009): **WHO list of critically important antimicrobials for human medicine**. Geneva: World Health Organisation.

ZANELLA, R.C.; BRANDILEONE, M.C.C.; BOKERMANN, S.; ALMEIDA, S.C.G.; VALDETARO, F.; VITÓRIO, F.; MOREIRA, M.F.A.; VILLINS, M.; SALOMÃO, R.; PIGNATARI, A.C.C. Phenotypic and Genotypic Characterization of *van*A *Enterococcus* Isolated During the First Nosocomial Outbreak in Brazil. **Microbial Drug Resistance**, v. 9, n. 3, p. 283-291, 2003.