http://dx.doi.org/10.1590/1678-4162-10978

C.M.M. Corsini
https://orcid.org/0000-0002-6109-1466
V.O. Silva
https://orcid.org/0000-0001-9958-8251
O.V. Carvalho
https://orcid.org/0000-0003-1282-0084
R.V. Sepúlveda
https://orcid.org/0000-0002-9084-1879
F.L. Valente
https://orcid.org/0000-0001-5037-1887
E.C.C. Reis
https://orcid.org/0000-0002-9794-6972
M.A.S. Moreira
https://orcid.org/0000-0002-5380-4913
A. Silva Júnior
https://orcid.org/0000-0002-0614-7527
A.P.B. Borges
https://orcid.org/0000-0002-7489-3847

Arq. Bras. Med. Vet. Zootec., **v.72, n.4, p.1213-1220, 2020**

Emergence of multidrug-resistant bacteria isolated from surgical site infection in dogs and cats

[*Emergência de bactérias multirresistentes isoladas da infecção no sítio cirúrgico em cães e gatos*]

C.M.M. *Corsini*, V.O. *Silva*, O.V. *Carvalho*, R.V. *Sepúlveda*, F.L. *Valente*, E.C.C. *Reis*, M.A.S. *Moreira*, A. *Silva Júnior*, A.P.B. *Borges**

Universidade Federal de Viçosa - Viçosa, MG

ABSTRACT

Surgical site infections (SSIs) and antimicrobial resistance among pathogens causing SSI are a growing concern in veterinary hospitals. One major reason, the widespread use of antimicrobials, has led to increased incidence of SSIs. This study identified bacteria and resistance profiles to antimicrobials in the SSI cases diagnosed at the Surgical Clinic of Small Animals in the Veterinary Hospital, Federal University of Viçosa, Brazil. The main genus identified was *Staphylococcus*, followed by *Escherichia*, *Enterococcus*, *Bacillus*, *Shigella*, *Citrobacter*, *Proteus*, *Morganella*, *Serratia*, *Enterobacter*, *Pseudomonas* and *Klebsiella* were also found, but in small number. The results indicated the predominance of Gram-negative bacteria among the collected samples. Most of isolates identified were resistant to more than one of the following antimicrobials: ampicillin, tetracycline, enrofloxacin, amoxicillin/clavulanic acid and cephalotin. Of the 17 *Staphylococcus* sp. isolates, two (11.8%) were methicillin-resistant *Staphylococcus aureus* (MRSA) and 11 (64.7%) of them were methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). There were bacterial genera identified with resistance to all tested antimicrobials in different proportions. This should alert veterinary hospitals to the emergence of multidrug-resistant bacteria and to the requirement for the revision of surgical protocols with regard to antimicrobial prophylaxis and therapy.

Keywords: nosocomial infection, minimal inhibitory concentration, resistant pathogens

RESUMO

As infecções em sítio cirúrgico (ISCs) e a resistência bacteriana entre os patógenos relacionados constituem uma preocupação crescente nos hospitais veterinários. O aumento na incidência de ISCs possui forte relação com o uso amplo e disseminado de antibióticos. O presente estudo identificou bactérias e perfis de resistência a antibióticos nos casos de ISCs diagnosticados na Clínica Cirúrgica de Pequenos Animais do Hospital Veterinário da Universidade Federal de Viçosa, Brasil. O principal gênero identificado foi Staphylococcus, *seguido pelos gêneros* Escherichia, Enterococcus, Bacillus, Shigella, Citrobacter, Proteus, Morganella, Serratia, Enterobacter, Pseudomonas e Klebsiella, *porém, em menor quantidade. Os resultados demonstraram a predominância de bactérias Gram-negativas entre as amostras coletadas. A maioria dos isolados identificados eram resistentes a um ou a mais de um dos seguintes antibióticos: ampicilina, tetraciclina, enrofloxacina, amoxicilina/ácido clavulânico e cefalotina. Entre os 17 isolados de* Staphylococcus *sp., dois (11,8%) eram* Staphylococcus aureus *resistentes à meticilina (SARM) e 11 (64,7%) eram* Staphylococcus pseudintermedius *resistentes à meticilina (SPRM). Houve identificação de gêneros bacterianos com diferentes proporções de resistência para todos os antibióticos avaliados. Esses achados devem alertar os hospitais veterinários para a emergência de bactérias multirresistentes e para a necessidade de revisar a profilaxia e a terapia antimicrobiana referente aos protocolos cirúrgicos.*

Palavras-chave: infecção nosocomial, concentração mínima inibitória, patógenos resistentes

Recebido em 22 de agosto de 2018
Aceito em 10 de dezembro de 2019
*Autor de correspondência (*corresponding author*)
E-mail: andrea@ufv.br

INTRODUCTION

The surgical site infection (SSI) is a common and widespread nosocomial infection in human medicine (Humphreys, 2009) and is considered the most important cause of postoperative complications, contributing significantly to increased morbidity and mortality in these patients (Sitio…, 2009). In the past two decades, this type of infection has become an increasing concern in veterinary hospitals (Braga *et al*., 2012, Corsini *et al*., 2014, Murta *et al*., 2015).

SSI is the most frequently reported type of nosocomial infection in small animals, occurring in between 0.8% to 18.1% of all surgical wounds in dogs and cats (Nelson, 2011). Corsini *et al*. (2014) working in a veterinary hospital found an SSI infection rate of 5.24% and Murta *et al*. (2015) of 3.46%. Most are caused by multiple microorganisms, usually multidrug-resistant (MDR), and have many risk factors (Braga *et al*., 2012).

There is an increasing concern about the antibiotic resistance, which has several implications for human or animal health like higher costs, extended stay in the hospital, delayed recovery and patient death. Importantly, resistant bacteria can appear quickly after the application of antibiotics, but the process to eliminate them is slow, even in the absence of selective pressure. Incrementing resistance is a usual feature of microorganisms causing nosocomial infections, which present a wide variety of mechanisms for resistance (Nelson, 2011).

MDR pathogens have been reported in veterinary hospitals and are more common in animals that have undergone prior treatment with antimicrobials (Pellerin *et al*., 1998). Gibson *et al*. (2008) described 37 dogs with MDR *E. coli* and *Enterobacter* extraintestinal infection. Almost 90% received prior antimicrobial therapy and about 60% had a surgical procedure. A recent study presented 194 isolates from canine SSIs and multidrug resistance was uncommon, despite the fact that MDR microorganisms have been increasingly reported (Windahl *et al*., 2015).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in animals have been highlighted in recent years (Wieler *et al*. 2011). According to Kottler *et al*. (2010) methicillin-resistant strains, also called oxacillin-resistant, are resistant to all beta-lactam antibiotics, and are usually resistant to a variety of other classes of antibiotics as well. Although MRSA and MRSP share risk factors, particularly prior antibiotic use and hospitalization, they have probably emerged independently through adaptive process in their respective hosts (Huerta *et al*. 2011).

There are few published studies in veterinary medicine regarding the identification of nosocomial pathogens and assessment of control strategies for infection. Likewise, little information is available about the impact of multidrug and patterns of antimicrobial resistance related to isolated pathogens in small animals SSIs. Therefore, this study aimed to identify the bacteria causing SSI in dogs and cats and also evaluate antimicrobial resistance profiles of the isolates.

MATERIALS AND METHODS

The design of this study was approved by the Ethics Committee on Animal Use, Federal University of Viçosa (UFV-CEUA), under protocol number 60/2011.

The population used in this study was composed of dogs and cats submitted to surgery at HVT-UFV from August 2, 2010 to July 1, 2011. Animals undergoing dental procedures were excluded from the study, because these procedures are performed in a different room as other medical procedures. The basic requirements for patient inclusion in the study were: being submitted to pre-surgical evaluation, medical records completed with clinical examination and anamnesis of the responsible professional, not being a carrier of infection at the time of admission and having attended the ambulatory surgical post-return.

The diagnosis of SSI was conducted during outpatient postsurgical return, which occurred between seven to ten days after the procedure. In return, the following criteria were used to evaluate the surgical wound: the presence of hyperemia, increased local temperature and or systemic pain, dehiscence and the presence of purulent exudate at the incision site. As the gold

standard for diagnosis the presence of purulent exudate was assessed, which featured no reaction to the suture, according to the recommendations of the *Centers for Disease Control and Prevention* (CDC) through the *Guideline for Prevention of Surgical Site Infection* (Mangram *et al*., 1999).

The material used for microbiological analysis was collected through the edges, prior to cleaning of surgical wounds with a solution of polyvinylpyrrolidone (PVP), and 1% of the center thereof with 0.9% saline solution, to remove all of the purulent exudate present. The material to be analyzed was collected as deep as possible in the surgical wound with sterile swabs (Labor, Brazil). When the presence of scabs was observed, these were removed prior to cleaning the wound and sampling. Each swab containing different samples was inoculated with 1 ml of 0.85% NaCl and shaken vigorously. The contents of each were plated onto Petri dishes containing sheep blood agar (5% HiMedia Laboratories, India) and incubated at 37°C for 24 hours. After that time, the colonies that showed visually distinct morphology were streaked onto *Brain-Heart-Infusion* (BHI) agar (HiMedia Laboratories, India) to obtain pure cultures. Then, the cultures were stored in BHI broth supplemented with 20% glycerol and stored at -80°C.

After the end of the harvest period, the stored isolates were reactivated in BHI agar to perform the following screening tests: Gram staining, and analysis of catalase and oxidase (Newprov, Brazil). Additionally, the isolated Gram-negative species underwent the following tests: IMViC, oxidation-fermentation of Hugh and Leifson (HiMedia Laboratories, India) and inoculated agar-*Triple Sugar-Iron* (TSI) (HiMedia Laboratories, India). Bacteria identified as *Staphylococcus* sp. were submitted to the free and bound coagulase tests. All of the above analyses were performed according to the protocols described by Koneman *et al*. (2008). For the quality control of the adopted culture media, we used the standard strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 12600.

After triage, the isolates passed through genotypic characterization by genome sequencing. The isolates were subjected to a genomic DNA extraction kit using the *Wizard® Genomic DNA Purification* (Promega, USA). The coding region of the ribosomal 16S sequence was amplified by conventional PCR using the primers and amplification cycles described by Sterr *et al*. (2009). PCR reactions were performed using the kit *Go Taq® Green Master Mix* (Promega, USA).

The amplified fragments of approximately 1,513 bp were visualized by electrophoresis on 1% agarose gel (Invitrogen, USA) colored with GelRed™ Nucleic Acid Stain (1X) using a UV light image capture system in L-PIX HE (Loccus Biotechnology, Brazil).

The amplicons were purified by the *Wizard PCR clean up kit* (Promega, USA) and sequenced. The obtained sequences were edited using software *Sequencher™* Version 4.1.4 and compared with other sequences available in *GenBank* using the software *Basic Local Alignment Search Tool* (BLAST), which is available from the *National Center for Biotechnology Information* - NCBI (http://www.ncbi.nlm.nih.gov). The nucleotide sequences determined in this study have been deposited in GenBank [GenBank: JX482487 to JX482547]. Phylogenetic tree based on the 16S RNA nucleotide sequences was reconstructed using the neighbor-joining method with 10,000 replicates using MEGA program.

For the antimicrobial susceptibility test, the minimum inhibitory concentration (MIC) was determined by the Etest antimicrobial gradient strips (BioMerieux, France) according to the manufacturer's guidelines. For Gram-positive bacteria, the following antimicrobials were tested: amoxicillin/clavulanic acid, ampicillin, cephalothin, oxacillin and tetracycline, the following were assessed for Gram-negative bacteria: amoxicillin/clavulanic acid, ampicillin, tetracycline, and enrofloxacin. Staphylococci were tested for susceptibility to oxacillin as indicator for methicillin resistance. The antimicrobials were chosen based on the routine surgical use in the veterinary hospital. The quality control of the culture medium was the same as mentioned above for biochemical tests. The MIC reading was performed according to the manufacturer's guidelines. Subsequently, the samples were classified as sensitive, intermediate or resistant in accordance with the sensitivity limits determined by the *Standards Clinical*

Laboratory Institute, CLSI (Performance…, 2008).

RESULTS AND DISCUSSION

In total, 401 animals (354 dogs and 47 cats) had a surgical intervention during the study period. Twenty-one animals (18 dogs and three cats) were diagnosed with SSI, for an overall rate of 5.24%. SSI rate at 5.24% identified in this study is similar to previous studies (Frey *et al*., 2010, Corsini *et al*., 2014), Already Murta *et al*. (2015) found an SSI rate of 3.46%. However, different surgery types are usually related to variations in SSI rates. Turk *et al*. (2015) reported an infection rate of 20% for dirty procedures and only 3.2% for clean surgical interventions. Overall, SSI rates of 0.8% to 18.1% have been described in small animal surgeries (Nelson, 2011).

From these animals, 61 strains were obtained among dogs and cats, with no standard number of isolates per sample. We identified 12 bacterial genera among them, with 63.93% characterized as Gram-negative and 36.07% as Gram-positive (Table 1 and Figure 1). Among the *Staphylococcus* samples, 80% were coagulase-negative and 20% coagulase-positive. Johnson and Murtaugh (1997) stated that the most common pathogens responsible for causing SSI in small animals are *Staphylococcus aureus*, *Staphylococcus spp*., *E. coli* and *Pasteurella spp*., most of them were identified in this work, except the genus *Pasteurella* (Table 1).

This study found 63.9% of species to be Gram-negative, confirming the previous results of Abdel-Fattah (2005), who conducted a study in a human hospital in relation to the identification of pathogens causing nosocomial infections, and found a higher rate of Gram-negative species. Also, in this work, the predominant genera were *Escherichia* and *Staphylococcus*, but this author did not stratify samples in his study, he simply related bacteria to SSI. However, our results agree with those found by Johnson and Murtaugh (1997) and suggest similarities between the bacteria found in the SSI of small animals and humans.

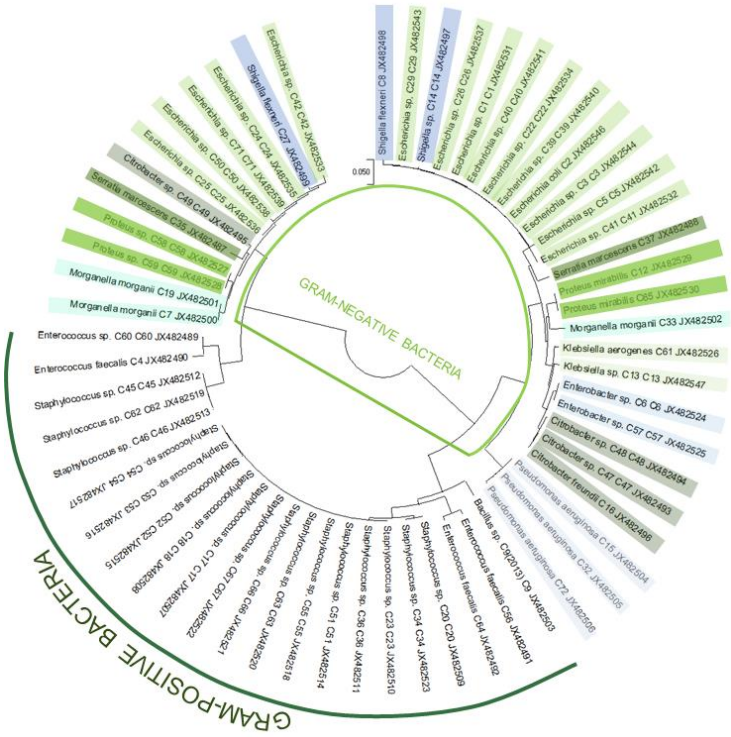

Figure 1. Phylogenetic clustering of 16S RNA partial sequence from bacteria of surgical site infections. Figure shows 61 bacteria isolates organized into gram-positive and gram-negative groups.

Table 1. Bacteria isolated from surgical site infections in small animal surgery Bacterial strain

	n (%)		Access Number Genbank
Gram-negative	*Escherichia fergusonii*	14 (23.0)	JX482531-JX482544
Escherichia coli	2 (3.3)		JX482545, JX482546
Shigella flexneri	3 (4.9)		JX482497, JX482498, JX482499
Citrobacter freundii	3 (4.9)		JX482493, JX482494, JX482496
Citrobacter murliniae	1 (1.6)		JX482495
Proteus mirabilis	3 (4.9)		JX482527, JX482529, JX482530
Proteus vulgaris	1 (1.6)		JX482528
Morganella morganii	3 (4.9)		JX482500, JX482501, JX482502
Serratia marcescens	2 (3.3)		JX482487, JX482488
Enterobacter ludwigii	2 (3.3)		JX482523, JX482524
Enterobacter carcerogenus	1 (1.6)		JX482526
Klebsiella pneumoniae	1 (1.6)		JX482547
Pseudomonas aeruginosa	3 (4.9)		JX482504, JX482505, JX482506
Total		39 (64.0)	
Gram-positive	*Staphylococcus pseudintermedius*	14 (23.0)	JX482507, JX482508, JX482511, JX482512, JX482513, JX482515-JX482522
Staphylococcus aureus	2 (3.3)		JX482509, JX482523
Staphylococcus intermedius	1 (1.6)		JX482514
Enterococcus faecalis	3 (4.9)		JX482490, JX482491, JX482492
Enterococcus italicus	1 (1.6)		JX482489
Bacillus thuringiensis	1 (1.6)		JX482503
Total		22 (36.0)	

Orthopedic surgery showed high SSI rate (8.65%) compared to other surgeries. General, integumentary system, genitourinary, head and neck surgery showed SSI rate of 5.4%, 5%, 2.82% and 2.77%, respectively (Table 2). The two main genera present in SSI samples were Escherichia and Staphylococcus, which showed similar distributions in all of the cited locations/regions assessed. The detection percentage of bacterial isolates ranged from 1.64% (1/61) to 8.2% (5/61) (Table 2). The exceptions occurred in surgery around the head and neck, where only the presence of the genus *Staphylococcus* was identified, and in general surgery, with the same results for the genus *Escherichia*. *Staphylococcus* sp. and *Escherichia* sp. were detected in 33 (54.1%) of the positive cultures. However, *Staphylococcus* sp. was the most frequently SSI pathogen isolated (27.8%).

Most of bacterial species isolated in this study are usually found in the normal skin microbiota in healthy dogs and can be opportunistic pathogens in canine dermatitis and SSI. Some unusual bacteria isolates in small animals SSI might also be endogenous flora inoculation of surgical wounds or exogenous contaminants from environmental sources (Nelson, 2011).

The bacteria of the genera *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Enterococcus* identified in this study were also observed by Abdel-Fattah (2005) in human SSI. Van Eldere (2003) also noted the presence of *Pseudomonas* in human SSI, as well as other types of nosocomial infections. Boerlin *et al*. (2001) reported the presence of *Enterococcus faecium* and *Enterococcus faecalis* in SSI of dogs and cats. Also, in their papers, Abdel-Fattah (2005) observed the presence of the genera *Proteus* and *Serratia* in urinary tract infections in humans. There were no studies in veterinary medicine reporting the occurrence of the genus *Morganella* in SSI, which was another species identified in this study.

Staphylococci was the most prevalent infectious agent in our study and this finding was expected according to other studies, because *S. pseudintermedius* and *S. aureus* are opportunistic bacteria commonly related to SSI in animals (van Duijkeren *et al*., 2011). A recent study carried out by Windahl *et al*. (2015) have shown

Staphylococcus pseudintermedius as the most common SSI finding identified and nearly two-thirds of all isolates were staphylococci. Likewise, *S. pseudintermedius* was also the most prevalent pathogen according to another study (Turk *et al*., 2015).

Whittem *et al*. (1999) reported that bacteria of the genera *Staphylococcus* are usually the main cause of SSI in orthopedic procedures in small animals, as did Leonard *et al*. (2006), who suggested that the persistence of MRSA was the cause of most infections related to orthopedic devices. On the other hand, this was not observed in this work, since the main genera identified in orthopedic procedures with a positive diagnosis for SSI was *Escherichia*. Associations between deep wound infections and *Escherichia coli* isolates are more frequently observed than in superficial infections (Windahl *et al*., 2015).

Regarding antimicrobial resistance, the isolates showed high resistance to ampicillin. Among the Gram-positive species, tetracycline and cephalothin amoxicillin/clavulanic acid resistance was also observed, while the Gram-negative bacteria were resistant to tetracycline, enrofloxacin and amoxicillin/clavulanic acid, as outlined in Table 3. Out of the stored isolates identified as *Staphylococcus* sp., 76.47% showed resistance to oxacillin (see Table 3). Of the 17 *Staphylococcus* sp. isolates, two (11.8%) were methicillin-resistant *Staphylococcus aureus* (MRSA) and 11 (64.7%) of them were methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (Table 1 and Figure 1).

The observation of ampicillin resistance in both Gram-negative (51.28%) and Gram-positive bacteria (63.64%), suggests the improper use and/or abuse of this antimicrobial in routine surgery at the veterinary hospitals sampled, which may also be extrapolated to tetracycline and cephalothin. Nevertheless, this resistance can also be attributed to the widespread use of these antibiotics in other hospitals and veterinary clinics, and even outside the hospital.

The high rate of oxacillin resistance among isolates identified as *Staphylococcus* sp. in this work (76.47%) was consistent with the data found in human studies conducted by Arias *et al.* (2003). Of these methicillin-resistant staphylococcal species, all *Staphylococcus aureus* isolated (n=2) in the present study were MRSA. A previous study reported 11% of MRSA isolates among *S. aureus* strains recovered from dogs in seven teaching hospitals in the United States (Middleton *et al*., 2005).

There has been an increasing number of MRSA infections in pets in recent years, indicating that most of these infections are associated with SSI and non-surgical wound infections (Cain, 2013). The increasingly closer contact between cats and dogs and humans may serve as a contributing factor in the increasing number of cases of MRSA infection in these animals, as well as the emergence of strains of *S. aureus* in the community, outside the hospital, called cMRSA, which are resistant to fewer antibiotics than MRSA, but is still resistant to methicillin (oxacillin) (Leonard *et al*.,2006).

Most of methicillin-resistant strains isolated in the present study were MRSP. Methicillin-resistant *S. pseudintermedius* diagnosed in our study was also identified in other studies and this is of concern because MRSP had a notable increase in the prevalence of opportunistic infections in dogs and cats (Turk *et al*., 2015). Similar to the high rate of MRSP (11 of the 17 *Staphylococcus* isolates) observed in the present study, MRSP have been increasingly reported in Europe (Perreten *et al*., 2010). Unlike the MRSP incidence shown in this study and despite its expanding clinical impact, it was a rare finding in a recent study about SSI in dogs (Windahl *et al*., 2015).

According to Clarke (2006), antimicrobial resistance of bacteria affecting small animals varies considerably according to geographic location, history of exposure to antimicrobials, and the microorganisms involved, which could explain some variations found in this study. The small number of published studies in small animals SSIs may be the result of the absence of a database, which would be necessary for the development of an epidemiological study, as demonstrated by this work.

In Table 3 it can be observed that most bacteria identified in this study showed multidrug resistance, with isolates exhibiting resistance to more than one group of antimicrobials, reaching to a profile of resistance up to five

antimicrobials, the exceptions being the genera *Bacillus* and *Pseudomonas*.

CONCLUSIONS

The diagnosed SSI in the Surgical Clinic of Small Animal Veterinary Hospital of UFV, Brazil, showed the presence of bacteria from 12 genera, most of which were Gram-negative. The predominant genus was *Staphylococcus*, followed by *Escherichia*. A high percentage of MRSP was reported, but also had two MRSA isolates. Isolated pathogens were mostly resistant to more than one antimicrobial. Due to the profile of multidrug-resistant bacteria isolated from SSIs, this study suggests the necessity of establishing a Service of Hospital Infection Control, which will follow the evolutions of postoperative infections and coordinate the correct use of antibiotics in veterinary medicine.

ACKNOWLEDGEMENTS

This work was supported CNPq, CAPES and FAPEMIG. The authors thank the Veterinary Department, Federal University of Viçosa (UFV). Abelardo Silva-Júnior, Maria Aparecida Scatamburlo Moreira and Andréa Pacheco Batista Borges were supported by CNPq.

REFERENCES

ABDEL-FATTAH, M.M. Surveillance of nosocomial infections at a Saudi Arabian military hospital for a one-year period. *Ger. Med. Sci.*, v.3, p1-10, 2005.

ARIAS, C.A.; REYES, J.; ZÚÑIGA, M. *et al*. Multicentre surveillance of antimicrobial resistance in enterococci and staphylococci from Colombian hospitals, 2001–2002. *J. Antimicrob. Chemother.*, v.51, p.59-68, 2003

BOERLIN, P.; EUGSTER, S.; GASCHEN, F. *et al*. Transmission of opportunistic pathogens in a veterinary teaching hospital. *Vet. Microbiol*., v.82, p.347-359, 2001.

BRAGA, D.P.; BORGES, A.P.B.; CARVALHO, T.B. *et al*. Antibioticoprofilaxia em cirurgias de cães e gatos: necessidade e realidade. *Rev. Ceres*, v.59, p.758-764, 2012.

CAIN, C.L. Antimicrobial resistance in staphylococci in small animals. *Vet. Clin. N. Am. Small Anim. Pract*., v.43, p.19-40, 2013.

CLARKE, C.R. Antimicrobial resistance. *Vet. Clin. N. Am. Small Anim. Pract*., v.36, p.987-1001, 2006.

CORSINI, C.M.M.; BORGES, A.P.B.; ALBERTO, D.S.; SILVA, C.H.O. Incidência de infecção do sítio cirúrgico e fatores de risco associados na clínica cirúrgica de pequenos animais. *Arq. Bras. Med. Vet. Zootec*., v.66, p.737-744, 2014.

FREY, T.N.; HOELZLER, M.G.; SCAVELLI, T.D. *et al*. Risk factor for surgical site infection-inflammation in dogs undergoing surgery for rupture of the cranial cruciate ligament: 902 cases (2006-2006). *J. Am. Vet. Med. Assoc.*, v.236, p.88-94, 2010.

GIBSON, J.S.; MORTON, J.M.; COBBOLD, R.N. *et al*. Multidrug-resistant E. coli and enterobacter extraintestinal infection in 37 dogs. *J. Vet. Intern. Med*., v.22, p.844-850, 2008.

HUERTA, B.; MALDONADO, A.; GINEL, P.J. *et al*. Risk factors associated with the antimicrobial resistance of staphylococci in canine pyoderma. *Vet. Microbiol*., v.150, p.302-308, 2011.

HUMPHREYS, H. Preventing surgical site infection. Where now? *J. Hosp. Infect*., v.73, p.316-322, 2009.

JOHNSON, J.A.; MURTAUGH, R.J. Preventing and treating nosocomial infection. Part 2. Wound, blood and gastrointestinal infections. *Comp. Cont. Educ. Pract*., v.19, p.693-703, 1997.

KONEMAN, E.W.; ALLEN, J.; JANDA, W. *et al*. *Diagnóstico microbiológico*. 6.ed. Rio de Janeiro: Medsi, 2008. 1488p.

KOTTLER, S.; MIDDLETON, J.R.; PERRY, J. *et al*. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in three populations. *J. Vet. Intern. Med*., v.24, p.132-139, 2010.

LEONARD, F.C.; ABBOTT, Y.; ROSSNEY, A. *et al*. Methicillin-resistant Staphylococcus aureus isolated from a veterinary surgeon and five dogs in one practice. *Vet. Rec.*, v.158, p.155-159, 2006.

MANGRAM, A.J.; HORAN, T.C.; PEARSON, M.L. *et al*. Guideline for prevention of surgical site infection, 1999. *Infect. Control Hosp. Edemiol*., v.20, p.250-278, 1999.

MIDDLETON, J.R.; FALES, W.H.; LUBY, C.D. *et al*. Surveillance of Staphylococcus aureus in veterinary teaching hospitals. *J. Clin. Microbiol*., v.43, p.2916-2919, 2005.

MURTA, A.R.; ABREU JR., N.B.; OLIVEIRA, L.S. *et al*. Perfil epidemiológico e análise microbiológica da infecção de sítio cirúrgico em pacientes humanos e animais de companhia. *Pesqui. Vet. Bras.*, v.35, p.652-658, 2015.

NELSON, L.L. Surgical site infections in small animal surgery. *Vet. Clin. N. Am. Small Anim. Pract*., v.41, p.1041-1056, 2011.

PELLERIN, J.L.; BOURDEAU, P.; SEBBAG, H.; PERSON J.M. Epidemiosurveillance of antimicrobial compound resistance of Staphylococcus intermedium clinical isolates from canine pyodermas. *Comp. Immunol. Microbiol. Infect. Dis*., v.21, p.115-133, 1998.

PERFORMANCE standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Document M31-A3. Approved standard. 3.ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2008. p.116.

PERRETEN, V.; KADLEC, K.; SCHWARZ, S. *et al*. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicenter study. *J. Antimicrob. Chemother*., v.65, p.1145-1154, 2010.

SÍTIO cirúrgico: critérios nacionais de infecções relacionadas à assistência à saúde, 2009. Brasília: ANVISA, 2009. Disponível em: <https://www.anvisa.gov.br/servicosaude/manuais/criterios_nacionais_ISC.pdf>. Acessado em: 1 ago. 2018.

STERR, Y.; WEISS, A.; SCHMIDT H. Evaluation of lactic acid bacteria for sourdough fermentation of amaranth. *Int. J. Food Microbiol*., v.136, p.75-82, 2009.

TURK, R.; SINGH, A.; WEESE J.S. Prospective surgical site infection surveillance in dogs. *Vet. Surg*., v.44, p.2-8, 2015.

VAN DUIJKEREN, E.; CATRY, B.; GREKO, C. *et al*. Review on methicillin-resistant *Staphylococcus pseudintermedius*. *J. Antimicrob. Chemother*., v.66, p.2705-2714, 2011.

VAN ELDERE, J. Multicentre surveillance of Pseudomonas aeruginosa susceptibility patterns in nosocomial infections. *J. Antimicrob. Chemother*., v.51, p.347-352, 2003.

WHITTEM, T.L.; JOHNSON, A.L.; SMITH, C.W. *et al*. Effect of perioperative prophylactic antimicrobial treatment in dogs undergoing elective orthopedic surgery. *J. Am. Vet. Med. Assoc*., v.215, p.212-216, 1999.

WIELER, L.H.; EWERS, C.; GUENTHER, S. *et al*. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing Enterobacteriaceae in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *Int. J. Med. Microbiol*., v.301, p.635-641, 2011.

WINDAHL, U.; BENGTSSON, B.; NYMAN, A.K.; HOLST B.S. The distribution of pathogens and their antimicrobial susceptibility patterns among canine surgical wounds infections in Sweden in relation to different risk factors. *Acta Vet. Scand.*, v.57, p.11, 2015.