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TYPES OF WOUNDS AND THE PREVALENCE OF BACTERIAL CONTAMINATION OF WOUNDS IN THE CLINICAL PRACTICE OF SMALL ANIMALS

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ABSTRACT

Skin wounds are a common presentation in small animal practice. The successful management of wound healing in dogs and cats requires knowledge of the physiology of the wound healing process and the application of an appropriate therapeutic intervention. Many wounds are colonised by bacteria or show signs of clinical infection. Infections can delay wound healing, impair cosmetic outcome and increase healthcare costs. Because of a lack of papers giving an overall prevalence of bacteria in different types of wounds, 45 samples were taken from patients treated at the Small Animals Clinic, Section of Surgery, Orthopaedics, Roentgenology and Reproduction of the University of Veterinary Medicine and Pharmacy in Košice during the years 2017—2018 to determine the types of wounds and the prevalence of bacterial contamination of the wounds. Samples were obtained by using cotton-tipped swabs and then cultivated on Sabouraud's plates in the Institute of Microbiology and Gnotobiology of the University. All 45 animals used in this research were first subjected to an anatomical and clinical exam to determine the patient's health condition and the status of the wounds. Of these 45 samples, 9 were negative. Of the remaining 36 samples, 12 were cultivated and tested to give only the genera of the bacteria present, whilst 24 were tested more extensively for a specific diagnosis of the species. The most common wound was due to a bite from another animal; these made up 12 out of the 45 cases (26.67 %). There were 10 cases of dehiscence of old wounds (22.22 %), whereas there were only 2 cases of surgical wound complications (4.44 %). There were 5 puncture wounds or fistulas (11.11 %), 4 lacerations (8.88 %), 1 degloving injury (2.22 %), 1 seroma (2.22 %), 1 foreign body (2.22 %), 1 crushing injury (2.22 %), 1 case of contusion and necrosis (2.22 %), 1 cases of dermatitis with resulting pruritic lesions (2.22 %), and 1 cutting injury from a tight wire collar (2.22 %). Five cases (11.11 %) were wounds of unknown aetiology. The most commonly found bacteria was *Staphylococcus intermedius*, which was found in 14 out of the 45 wounds (31.11 %). From this study it appears that the first consideration for treatment of infected wounds should be a treatment plan which will have a high efficacy against

***Staphylococcus* spp. However, despite the high prevalence of *Staphylococcus* spp., our results revealed that they are not present all of the time.**

Key words: bacterial contamination; prevalence; skin; wound

INTRODUCTION

Bacterial contamination is a major cause of complications in wound healing. Wounds are usually polymicrobial, with many of these microbes being potentially pathogenic and caused by the invasion of pyogenic infections [16]. The role and significance of microorganisms in wound healing has been debated for many years. While some experts consider the microbial density to be critical in predicting wound healing and infection, others consider the types of microorganisms to be of greater importance. However, these and other factors such as microbial synergy, the host immune response, and the quality of tissue must be considered collectively in assessing the probability of infection [6].

Contamination often results in increased healing time and trauma [17], and therefore increased costs. Currently, the "go-to" treatment of wounds in veterinary medicine is usually the administration of broad spectrum antibiotics which can have adverse effects on the microbiota changes of the gastrointestinal tract and other systems of the body. Such microbiota changes can leave the patient susceptible to the colonisation of pathogenic levels of microbes, such as *Clostridium, Enterococcus* and *Candidiasis* [14]. However, a widespread opinion among wound care practitioners is that aerobic or facultative pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta-hemolytic Streptococci are the primary causes of delayed healing and infection in both acute and chronic wounds [6]. The continual use of these broad-spectrum antibiotics also aids the ever increasing resistance of certain bacteria to such drugs. Nosocomial infections in veterinary medicine caused by antibiotic resistant bacteria cause increased morbidity, dehiscence of wound, chronic wounds changes, higher cost, length of treatment and increased zoonotic risk because of the difficulty in the therapy [20].

Antimicrobial medical device combination products provide a pathway for local delivery of antimicrobial therapeutics with the ability to achieve high local concentrations while minimizing systemic side effects [2]. Although appropriate systemic antibiotics are essential for the treatment of deteriorating, clinically infected wounds, debate exists regarding the relevance and use of antibiotics (systemic or topical) and antiseptics (topical) in the treatment of non-healing wounds that have no clinical signs of infection [6]. Topical antibiotics may reduce the microbial contaminant exposure following surgical procedures, with the aim of reducing surgical site infections which impair cosmetic outcome and increase healthcare costs [10]. The use of a topical antimicrobial is beneficial for infection control in wound healing care because wound infection is the major cause of delayed healing. The advantages of topical over systemic antimicrobials include a higher concentration at the target site, fewer systemic adverse effects, and a lower incidence of antimicrobial resistance [15].

Currently, a microbial diagnosis is usually carried out only if the broad spectrum antibiotics are unsuccessful. The differentiation of the microorganisms in different types of wounds during the first visit of the patient, allows the use a specific treatment in wound healing. This would result in quicker and more efficient treatment of wounds, and furthermore, have an impact in slowing down the spread of antibiotic-resistant bacteria. Evidence shows that a bacterial burden of 106 microorganisms or more per gram of tissue seriously impairs healing. Bacteria may stimulate a persisting inflammation leading to the production of inflammatory mediators and proteolytic enzymes. Among many other effects, this causes extracellular matrix degradation and inhibition of reepithelialisation [8].

Because a bacterial culturing of wound infection to determine the prevalence of bacterial strains is not a standard step in the examination process in veterinary management, and although it is evident that more research is being carried out into the prevalence of bacteria within wounds in dogs and cats, there is still a lot of work to be done in this area. The prevalence of resistant bacteria in animals may present a direct risk to public health and companion animals may act as reservoirs of antimicrobial resistant bacteria that can be transmitted directly to people [13]. In human medicine there has been extensive research into the aetiology of wounds and its link with the bacterial prevalence within the wound, however, less can be said at this moment for veterinary medicine.

Although research into bacterial prevalence in wounds has started to take off in the veterinary world, the majority of papers and reports appear to be focused on post-surgical infection, and bite wounds. At this moment, there is little published work on other forms of injuries, such as puncture wounds, lacerations, dehiscence or degloving (Figure 1), which is not related to either of the aforementioned topics. There is also a distinct lack of papers or reports giving an overall prevalence of bacteria in different wound types, or any form of comparison of bacterial prevalence in wounds of dogs and cats. [16]. In view of the above, the aim of our study was to determine the types of wounds and the prevalence of bacterial contamination of wounds in clinical practice of small animals.

MATERIALS AND METHODS

In this study, 45 patients from the Small Animals Clinic, Section of Surgery, Orthopaedics, Roentgenology and Reproduction at the University of Veterinary Medicine and Pharmacy in Košice were included. Of these, 36 were dogs and 9 were cats. Of the dogs, 14 were crossbreeds (Cross). There were also 3 Jack Russel Terriers, 3 German Shepherds, 2 Staffordshire Terriers, 2 Dobermans, 2 Labrador Retrievers, 2 German Shorthaired Pointers, 1 Pitbull, 1 Miniature Dachshund, 1 Dachshund, 1 Central Asia Shepherd Dog, 1 Bichon Frise, 1 Argentinean Mastiff, 1 Dalmatian and 1 Chihuahua. There were 17 males and 19 females. Of the cats, 8 were European short-haired cats. There was also 1 British Blue cat. There were 6 males and 3 females cats.

All animals used in this study were first subjected to an anatomical and clinical exam to determine the patient's health condition and the status of the wound healing during the year 2017—2018. Appropriate further treatment was then decided. Diagnosis and options for the future treatment of the patient were discussed with the clients before treatment was carried out. Clients were asked about the history and cause of the wound and a macroscopic view, sample collection and photos were taken before the treatments were carried out (Figure 2). The main aim of examining and treating patients was fast and optimal repair of the wound and recuperation of the patient.

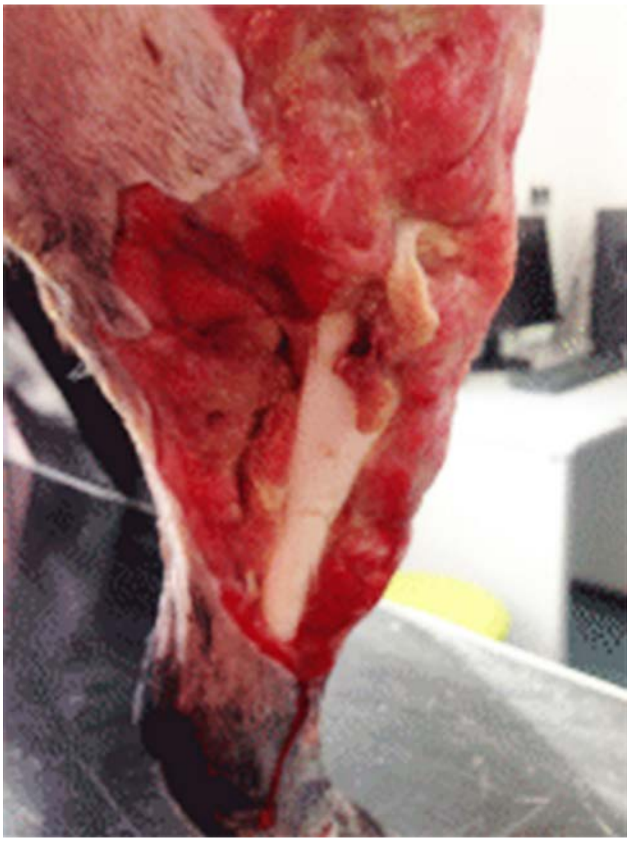

Fig. 1. Devastating injury with loss of muscles

Fig. 2. Secernation and necrotization of the skin and the surrounding tissue after trauma and bacterial contamination

Samples were obtained by using cotton-tipped swabs. This method of collection was chosen as it was the most practical method of collection in a clinical environment and was the least invasive to the patients. Samples were collected from patients on arrival into the clinic using Sarstedt swabs produced by Aktiengesellschaft & Co (Hamburg, Germany). These are held in a sterile tube which contained Amies transport medium without charcoal, which is suitable for collection, transport and preserving of bacteria [18].

Samples were taken for inoculation immediately after collection, or at latest within 24 hours. Inoculation was carried out using the streaking method. This is a quick and simple method which is used to dilute the bacterial concentration in the sample so that individual colonies can be isolated. This allows cultivation of a pure bacteriological culture. The swab was dragged in a zig-zag motion back and forth across the agar, then was rotated and a new streak was formed by use of a sterile inoculation loop. This step was repeated, so that there were four sections in total. The incubation of the plates at 37 °C for 24 hours allowed the rapid growth of the bacteria to develop. In the cases of chronic wounds where fungal infections may also be present, Sabouraud's plates required cultivation for approximately three days at room temperature to ensure optimal growth [12].

Although other methods of differentiation were also carried out, a definitive diagnosis was accomplished by a macroscopic view of colonies, the morphology and characteristics of a colony on different agar. The size of the colonies, their colour and whether they are rough, smooth or mucous can be enough to determine the type of bacteria. In determination we had used samples stained using the Gram method (Figure 4) to determine whether colonies were Gr^+ or Gr^-. In the case, when it was necessary to differentiate bacterial species, specific biochemical test were used.

RESULTS AND DISCUSSION

The most common wound was due to a bite from another animal; these made up 12 out of the 45 cases (26.67 %). There were 10 cases of dehiscence of old wounds (22.22 %), whereas only 2 cases of surgical wound complications (4.44 %). There were 5 puncture wounds or fistulas (11.11 %), 4 lacerations (8.88 %), 1 degloving injury (2.22 %), 1 seroma (2.22 %), 1 foreign body (2.22 %), 1 crushing injury (2.22 %), 1 case of contusion and necrosis (2.22 %), 1 cases of dermatitis with resulting pruritic lesions (2.22 %), and 1 cutting injury from a tight wire collar (2.22 %). Five cases (11.11 %) were wounds of unknown aetiology.

The 9 out of the 45 samples (20.00 %) were negative for any bacterial isolates. The 6 of these were samples taken from dogs (36), meaning that 16.67 % of swabs taken from wounds of dog's, were negative. In comparison, 3 (33.33 %) of all cat (9) wounds were negative for any bacterial contamination. The most commonly found bacteria was *S. intermedius* (Figure 3), which was found in 14 out of the 36 wounds (38.89 %). Of these, 11 were found in samples

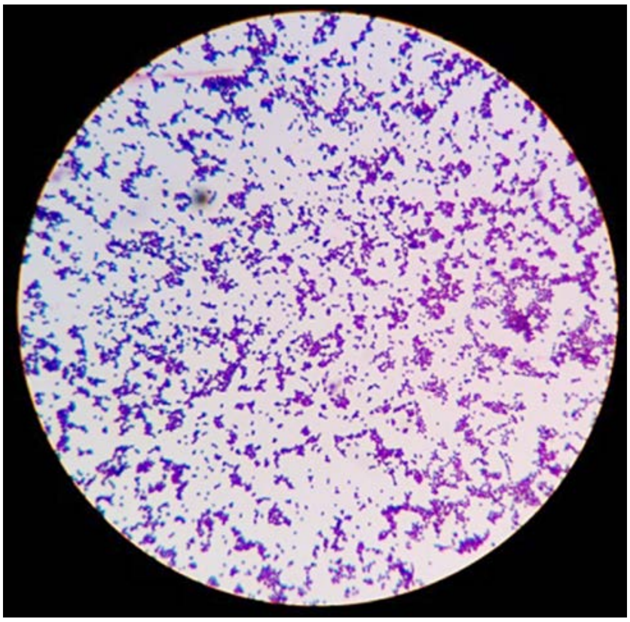

Fig. 3. Sample of *Staphylococcus* spp. from the wound due to a surgical complication. Blood Agar, *Staphylococcus intermedius*

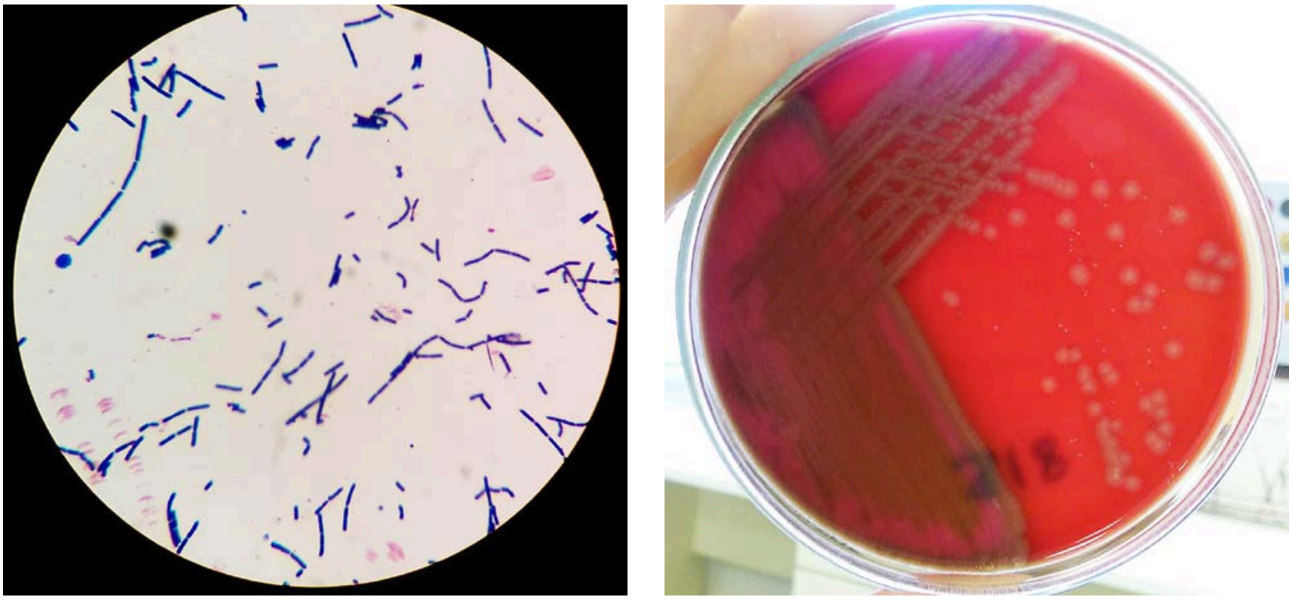

Fig. 4. Sample of *Bacillus* spp., Gram staining and blood agar

taken from dogs (36.67 % prevalence in dog wounds) and 3 were found in swab samples taken from cats (50 % prevalence in cat wounds). Other isolates of *Staphylococcus* spp. were *S. saprophyticus* (2 cases, 1 a laceration injury and 1 due to wound dehiscence, both in dogs), *S. haemolyticus* (2 cases, 1 laceration wound and 1 case of dermatitis, both in dogs), *S. warneri* (1 case, seen in surgical complication of open fracture in a cat) and *S. aureus* (1 case, seen in a cat puncture wound). Three cases of non-haemolytic *Staphylococcus* and 2 cases of β-haemolytic *Staphylococcus* were also found in the dog.

Streptococcus spp. also proved high in prevalence as it was isolated from 9 wounds (25.00 %). Of these, 7 were found in samples taken from dogs (23.33 %) and 2 were found in swab samples taken from cats (33.33 %). This was made up of: 5 cases of β-haemolytic *Streptococcus*, 3 cases of non-haemolytic *Streptococcus* and 1 case of α-haemolytic *Streptococcus*.

Bacillus spp. (Figure 4) was found in 7 (19.44 %) of all wounds. In 6 of the 7 cases these isolates were found in the wounds of dogs. *Bacillus* spp. made up 23.33 % of dog wounds and 16.66 % of cat wounds. The first isolate of *Bacillus* spp. was further tested for spp. determination and it was found to be *Bacillus cereus.* Five wounds (13.88 %) contained haemolytic *Escherichia coli*, 4 were found in dog wounds (13.33 %), whilst only 1 (16.66 %) was found in a cat wound. One case of non-haemolytic *E. coli* was also found in a swab taken from a dog (3.33 %).

Two cases of *Proteus mirabilis* (5.56 %) and 3 cases of *Pasteurella* spp. (8.33 %) were also isolated during this study. One of the *Pasteurella* isolates was further tested and found to be *P. multocida*. This was from a dog wound of unknown aetiology. Other species cultured included 1 case of *Pseudomonas aeruginosa* (3.33 %) which was cultured from a bite wound, and 1 case of *Morganella morganii*, subspecies morganii (3.33 %), which was cultured from a swab taken after a surgical complication. Both of these were found in swabs taken from the wounds of dogs. Other Gr-rods, which were not distinguished as any already noted were also found. There were four cases in total (8.33 %), 3 of which were found in wounds of dogs.

For the majority, *S. intermedius* is the main bacterial pathogen found in most wounds, however, this is not the case for puncture wounds and wound dehiscence. 40 % of all puncture wounds returned negative results upon culturing. This varied slightly between animal species, with 33,33 % prevalence in dogs and 50 % prevalence in cats. In the case of wound dehiscence, 30 % of wounds yielded negative results, while another 30 % were positive for *Bacillus* spp. As no cats with wound dehiscence were found in this study, this result was only based on the main bacteria spp. found in dogs.

Overall 100 % of contaminated cat wounds were found to contain Gr^+ bacteria, but only 50 % of dog wounds were found to contain Gr^- bacteria. The large difference between dogs and cats presented to the clinic seems quite surprising considering B o h l i n g et al. [5] reviews, which noted many more problems relating to wound healing in cats compared to dogs. In cats, there is some dispute between *Pasteurella* spp. and *Staphylococcus* spp. prevalence [21], however other research has shown the high prevalence of *Pasteurella* spp. to be mainly concentrated in subcutaneous abscesses and pyothorax in cats [11].

Gr^+ bacteria have been described as the major cause for pyogenic wound infections in several articles [1]. It is well known that *S. aureus* and Gr^- bacterial pathogens produce very potent virulence factors, responsible for maintaining the infection and delaying the process of wound healing [4]. Nevertheless, Gr^- bacteria have been described to be associated with nosocomial infections and intra-abdominal surgical procedures [19]. The most commonly found Gr^- bacteria in our study was *Escherichia coli*. Contaminated wounds included a surgical site complication, wound dehiscence, 2 bite wounds, a degloving injury and 1 wound of unknown aetiology. Although this seems like a low prevalence in comparison to *S. intermedius* in H a r i h a n a n et al. [9] study, however, it showed similar results with 2 out of 19 wounds (10.53 %) in cats proving positive for *E. coli* compared to 1 (11.11 %) of cats used in this study. Despite the high prevalence of *S. intermedius* found in this study, the type of wound sampled did appear to have some bearing on the type of bacteria isolated, as demonstrated by Table 1. Although *S. intermedius* was found in a large number of bite wounds and was the more common bacteria

Table 1. Occurrence of bacteria in wounds of dogs and cats

Sample No.	Species	Breed	Age	Sex	Wound description	Result
1	Cat	European short-haired cat	3 y	M	Shot/puncture wound	*Bacillus cereus* *Staphylococcus aureus*
2	Dog	Cross	3 y	F	Bite wound	Negative
3	Dog	Pitbull	5 y	M	3 day old bite wound	*Staphylococcus intermedius*
4	Dog	Labrador Retriever	8 m	M	Chronic dermatitis with open lesions	*Staphylococcus haemolyticus* *Pseudomonas aeruginosa*
5	Dog	Cross	2 y	F	Bite wound	*Staphylococcus intermedius* *Escherichia coli*
6	Dog	Cross	3 y	M	Bite wound	Negative
7	Dog	Cross	10 y	M	Bite wound	*Staphylococcus intermedius* *Escherichia coli*
8	Dog	Miniature Dachshund	2 y	M	Bite wound	*Staphylococcus intermedius*
9	Dog	Jack Russel Terrier	1 y	F	Unknown aetiology	*Staphylococcus intermedius* *Proteus mirabilis*
10	Dog	Dachshund	9 y	F	Foreign body in paw	*Staphylococcus intermedius*
11	Dog	German Shorthaired Pointer	1 y	M	Laceration wound	*Staphylococcus haemolyticus*
12	Dog	Cross	12 y	F	Dehiscence of wound	Negative
13	Cat	European short-haired cat	3 y	M	Laceration wound	*Staphylococcus intermedius*
14	Cat	European short-haired cat	4 y	F	Unknown aetiology	*Staphylococcus intermedius*
15	Dog	Cross	12 y	F	Bite wound	Gr- rods *Staphylococcus intermedius*
16	Dog	Doberman	4 m	F	Fistula after amputation	*Staphylococcus intermedius*
17	Dog	Cross	9 y	M	Bite wound	Negative
18	Dog	Staffordshire Terrier	6.5 y	F	Abscess/Seroma	*Staphylococcus intermedius*
19	Cat	British Blue	8 y	M	Surgical complication	*Staphylococcus warneri*
20	Dog	German Shorthaired Pointer	15 m	F	Puncture wound	Negative

Table 1. Continued

Sample No.	Species	Breed	Age	Sex	Wound description	Result
21	Dog	Cross	9 m	F	Laceration wound	non-haem. *Escherichia coli*
22	Dog	Labrador Retriever	9.5 y	F	Surgical complication	*Escherichia coli* *Pasteurella* spp. β-haem. *Staphylococcus* spp.
23	Cat	European short-haired cat	2 y	M	Dog bite wound	Negative
24	Dog	Cross	3 y	M	Dehiscence of wound	*Bacillus* spp. *Staphylococcus* spp. β-haem. *Streptococcus* spp.
25	Dog	Jack Russel Terrier	6 y	F	Bite wound	*Bacillus* spp. *Pasteurella* spp.
26	Cat	European short-haired cat	4 m	F	Contusion and necrotization of muscle	non-haem. *Streptococcus* spp.
27	Dog	Central Asia Shepherd	3y	M	Old puncture wound	Gr-rods (β-haem.)
28	Dog	Cross	10 y	F	Bite wound	non-haem. *Streptococcus* spp. *Bacillus* spp. G-rods
29	Dog	Chihuahua	9 y	M	Dehiscence of surgical wound	*Escherichia coli*
30	Dog	Cross	8 y	F	Unknown aetiology	*Staphylococcus intermedius*
31	Cat	European short-haired cat	6 m	M	Old crushing injury	Negative
32	Dog	German Shepherd	5 y	M	Dehiscence of wound	*Staphylococcus saprophyticus*
33	Dog	Cross	5 y	M	Wire collar	β-haem. *Staphylococcus* spp. non-haem. *Streptococcus* spp.
34	Dog	Bichon Frise	13 y	M	Dehiscence of wound	*Proteus mirabalis* α-haem. *Streptococcus* spp.
35	Dog	Dalmatian	7 y	F	Dehiscence of wound	*Bacillus* spp.
36	Dog	Jack Russel Terrier	7 y	F	Bite wound	*Bacillus* spp. β-haem. *Streptococcus* spp.
37	Dog	Argentinean Mastiff	5 y	F	Dehiscence	Negative
38	Dog	Cross	4 y	M	Laceration	*Staphylococcus saprophyticus*
39	Dog	Doberman	2 y	M	Dehiscence	*Bacillus* spp. non-haem. *Staphylococcus* spp.
40	Dog	German Shepherd	6 y	M	Dehiscence	β-haem. *Streptococcus* spp.
41	Dog	Staffordshire Terrier	3 y	M	Unknown aetiology	*Staphylococcus intermedius* β-haem. *Streptococcus* spp.
42	Cat	European short-haired cat	7 m	F	Degloving injury	*Escherichia coli* *Staphylococcus intermedius* β-haem. *Streptococcus* spp.
43	Dog	German Shepherd	10 y	M	Unknown aetiology	haem. *Staphylococcus* spp. non-haem. *Staphylococcus* spp. *Pasteurella multocida*
44	Dog	Cross	13.5 y	F	Dehiscence of surgical wound	*Morganella morganii* subsp. *morganii*
45	Cat	European short-haired cat	4 y	M	Puncture wound	Negative

m — month; y — year; non-haem. — non-haemolytic; α-haem. — α-haemolytic; β-haem. — β-haemolytic in wounds of unknown aetiology, this was not the case for wound dehiscence or puncture wounds. These more often returned negative results, and in the case of wound dehiscence, the presence of *Bacillus* spp. This variation in wound contamination should be a consideration when treating wounds and dealing with wound infection. In order to maintain freedom in veterinary medicine to use antibiotics, culturing of wounds and sensitivity testing where possible before administration of antibiotics may in future be necessary in veterinary practices around the world, in order to try to stem antimicrobial resistance.

The findings of Rijal et al. [16] indicate the existence of high drug resistant bacteria in pyogenic wound infections. Particular attention has been paid, in recent years, to β-lactamase producing strains of *E. coli*, which have started showing resistance to amoxicillin-clavulanate (known as co-amoxiclav), one of the most commonly used antibiotic in veterinary medicine [3, 7]. The high use of β-lactam antibiotics and inappropriate infection control procedures in the hospitals might be the cause of rising rates of resistance among these bacteria. Moreover, longer duration of prophylactic antimicrobial exposure in surgical interventions may contribute to organisms for developing resistance. This highlights the need for understanding in the veterinary community that all wounds cannot be treated in the exact same manner, and consideration for the type of wound one is dealing with is necessary to ensure correct and optimum treatment is carried out.

CONCLUSIONS

In providing a detailed analysis of wound microbiology, together with current opinion and controversies regarding wound assessment and treatment, this review has attempted to capture and address microbiological aspects that are critical to the successful management of microorganisms in wounds. From this study it appears that the first consideration for treatment of infected wounds should be a treatment plan which will have a high efficacy against *Staphylococcus* spp. However, despite the high prevalence of *Staphylococcus* spp., our results reveal that they are not present all the time. This concern is further raised by the presence of Gr^- bacteria, which were isolated in this study, as although much lower than Gr^+ bacteria prevalence, their increased ability to develop resistance against today's antibiotics is of major concern in all areas of veterinary medicine.

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