Assessment of wound bio-burden and prevalence of multi-drug resistant bacteria during open wound management

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Objective: To describe the bacterial bio-burden of open-treated wounds and make comparisons with bite wounds.

Design: Retrospective multicentre study.

Sample: Microbial culture between 2011 and 2013 from open-treated wounds in dogs and cats (initiation of therapy n=88, follow-up n=52) were compared to those from bite wounds (n=184).

Procedures: Bacteria were identified and tested for antibiotic susceptibility by two accredited laboratories.

Results: In total, 77/88 (88%) of open-treated wounds yielded positive bacterial cultures at the beginning of treatment, decreasing to 27/52 (52%) during treatment. Upon initial evaluation, 42/88 (48 %) of open-treated wounds were considered infected with multi-drug-resistant bacteria, with a drop to 22/52 (41%) during therapy. Bite wounds yielded fewer positive cultures 88/184 (48%) with only 11/182 (6%) being affected by multi-drug-resistant bacteria. Bacteria found most commonly in open-treated wounds were *Enterococcus* subspecies, *Escherichia coli*, *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa*.

Conclusion: The bacterial populations of open-treated wounds differed markedly from the bite wounds. The high incidence of multi-drug-resistant strains in open wounds highlights the need for alternatives to antibiotics.

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INTRODUCTION

Open-wound treatment (OWT) can be helpful prior to reconstructive surgery or to manage infection. Surgical site infection or bacterial infection complicated by multi-drug-resistant bacteria (MDR) is among the most frequent indications for open-wound therapy in dogs (Nolff *et al.* 2015). In addition, open wounds in humans have been reported to have high bacterial populations and high risk of being affected by MDR (Malic *et al.* 2009, Kirketerp-Möller *et al.* 2008, Gjodsbol *et al.* 2006). Numerous studies report the "normal" microflora of open wounds in humans and, recently, similar bacteria have been reported in horses undergoing open wound therapy (Westgate *et al.* 2011). The "top four" wound pathogens identified in both species included *Enterobacteriaceae*, *Enterococcus* species, *Pseudomonas aeruginosa* and *Staphylococcus* species (Westgate *et al.* 2011, Malic *et al.* 2009, Kirketerp-Möller *et al.* 2008, Gjodsbol *et al.* 2006). By contrast, the bacterial population of canine and feline open wounds has not been reported in detail. Fahie & Shettko (2007) stated in their review of OWT in small animals that "*bacterial organisms anticipated to be present within a wound - or to become present within a wound during open wound management - include Staphylococcus aureus, ß-hemolytic Streptococcus, Staphylococcus epidermidis, hemolytic Streptococci, Escherichia coli and Proteus spp.*", but investigation of the prevalence of MDR bacteria in open wounds is lacking. Fahie *et al.*'s list differs markedly from the "top four" bacterial species identified in human and equine wounds. *P. aeruginosa*, which is regarded as one of the most important pathogens in open wounds in humans and horses (Westgate *et al.* 2011, Malic *et al.* 2009), is notably absent.

The large impact of *P. aeruginosa* arises because it can form stable biofilms, is associated with high incidence of fatal infections in hospitalised humans, and has a strong tendency to develop multi-drug resistance (European Centre for Disease Prevention and Control 2014). *S. aureus*, *Enterococcus* species, and bacteria belonging to the *Enterobacteriaceae* family are also known to be responsible for severe healthcare-associated infections in humans (European Centre for Disease Prevention and Control 2014).

Since there is currently no information available on the bacterial population of wounds during OWT in dogs and cats, this study aimed to evaluate the wound status and report bacterial culture results with special emphasis on the prevalence of MDR.

MATERIALS AND METHODS

The medical records between January 2011 and October 2013 of two clinics were searched for dogs and cats that underwent open wound therapy and had reported bacterial culture results. These results in these patients were compared to those of a group treated for bite wounds at one of the clinics during the same period. Bite wounds were surgically debrided and culture swabs were obtained after debridement and before lavage. All bite wounds were closed primarily after insertion of Penrose drains. Results were categorised by species, clinic, treatment modality in OWT (foam dressing or negative pressure wound therapy) and antibiotic pre-treatment, plus the number of isolated bacterial species and detection of MDR.

Multi-drug resistant organisms were defined by their resistance against three or more major antibiotic classes (Gandolfi de Christopherus *et al.* 2013). In addition, the frequency of individual bacterial isolates was compared between initiation of OWT and follow-up assessments (taken at any time during OWT), as well as between initiation of OWT and bite wounds.

Tissue sampling and bacterial isolation

All wounds were swabbed from the surface of the wounds after debridement, avoiding skin contamination (sterile transport swab, Sarstedt AG & Co, Sarstedt, Germany or Transystem®, Hain Lifescience GmbH, Nehren). Culturing and identification of the bacteria were performed by two accredited diagnostic laboratories (Institute of Microbiology, Ludwig-Maximilians-University, Munich and Institute of Microbiology, University of Veterinary Medicine Hannover, Foundation). Phenotypic antibiotic resistance was assessed according to the Clinical and Laboratory Standarts Institute (CLSI VET01 document) guidelines. All isolates were tested for susceptibility to the following: doxycycline, sulfonamid-trimethoprime, amoxicillin-clavulanic acid, cefalothin, cefovecin, nitrofurantoine, enrofloxacin, marbofloxacin, gentamicin, imipenem, ampicillin and amicacin.

RESULTS

Patient data

Bacterial culture results were available for 88 patients at the beginning of open-wound therapy; of these, 63 (71%) were dogs and 25 (28%) were cats. The reason for open wound management was infection in 62 (71%), trauma in 22 (25 %), ischaemic necrosis in 2 (2%) and unknown in 2 (2%). OWT was performed using foam dressing in 70 (79%) and negative pressure wound therapy in 18 (21%). Follow-up bacterial cultures were available in 52 patients (36 dogs and 16 cats) during therapy. In the group with bite injuries, bacterial culture results were available for all 184 patients that underwent surgery; of these, 159 (86%) were dogs and 25 (14%) were cats.

Culture results

Bacterial cultures were positive in 77/88 (88%) at the beginning of OWT and 27/52 (52%) during OWT. In total, 88/184 (48%) patients with bite wounds had positive cultures (Fig 1). Mean time to follow-up culture in the OWT group was 7 days (range 3 to 18).

Of the isolated bacteria, 42/88 (48%) were classified as MDR at the beginning of OWT, decreasing to 21/52 (41%) of those available for analysis during treatment. In the bite wound group, only 11/184 (6%) of bacteria were considered MDR (Fig 1). Thus, patients undergoing OWT were more frequently affected by MDR bacteria both at the beginning and during OWT than bite wounds.

A total of 44 (50%) were pre-treated with antibiotics at initiation of OWT, compared with 5 (2%) bite injuries. Antibiotic pre-treatment was correlated with MDR at initiation of OWT [31/44 (70%) of the pre-treated patients were affected by MDR bacteria] and in the bite wound group [4/5 (80%) of the

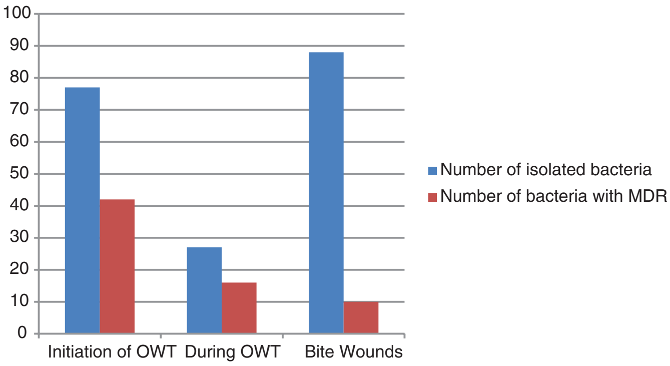

FIG 1. Graph showing the number of patients that were cultured positive at initiation of OWT, during OWT and in the bite wound group – and the number of patients affected by MDR bacteria within these positive cultures

Table 1. Relation between patients affected by MDR and antibiotic pre-treatment at initiation of OWT and in bite wounds

	Antibiotic pre-treatment	No antibiotic pre-treatment
	Initiation OWT (n=88)	
Isolated bacteria MDR*	31	11
Isolated bacteria non-MDR*	13	33
	Bite wounds (n=184)	
Isolated bacteria MDR*	4	7
Isolated bacteria non-MDR*	1	172

*Number of patients

pre-treated patients were affected by MDR bacteria]. No apparent differences were observed between the results in dogs and cats, nor an effect of OWT modality (Table 1).

Isolated bacterial species

A total of 29 different bacterial species were detected at initiation of open-wound therapy. Of those, *Enterococcus* species, *Escherichia coli*, *Staphylococcus pseudintermedius*, *P. aeruginosa*, *Enterobacter* subspecies and *Pasteurella multocida* were most common. The median number of isolated bacterial species was two (range 1 to 5). There were no major differences in bacterial species isolated from dogs or cats, except for *S. aureus*, which was found more often in cats than in dogs in OWT [24% (n=6) of cats affected; 3% (n=2) of dogs affected, overall 9% (n=8) of isolated bacteria].

During OWT, a total of 16 different bacterial species were detected. Of these, *Enterococcus* species, *E. coli*, *S. pseudintermedius*, *P. aeruginosa* and *Enterobacter* species were most common (Table 2). There was an increase in proportion (but not number) of *P. aeruginosa* isolates from 16% (n=14) in the beginning to 27% (n=14) in the follow-up results during OWT (it must also be noted that these were not the same 14 patients at the two time points). No impact of treatment modality or clinic was apparent (Fig 2).

A total of 18 different bacterial species were detected in patients with bite wounds. Of those, *Streptococcus canis*, *Streptococcus* species, *Staphylococcus* species, *Pasteurella multocida*, *Enterobacter* species, *S. pseudintermedius*, *Enterococcus* species were most common. The median number of isolated bacteria per wound was one (range 1 to 5) (Fig 2). There were no major differences in detected species between dogs and cats in the bite wound group except for *Pasteurella multocida*, which was found more often in cats than dogs [19% (n=5) of cats affected, 4% (n=6) of dogs affected, overall 6% (n=11) of isolated bacteria].

Comparison of isolated bacteria in OWT and bite wounds

Enterococcus species, *E. coli*, *S. pseudintermedius* and *P. aeruginosa* were isolated more often from open wounds than bite wounds.

MDR bacteria in open wounds

While 19 (67%) *E. coli* isolates from open wounds at the beginning of OWT and 9 (83%) from open wounds during treatment were considered MDR, none of the *E. coli* isolates in bite wounds were considered MDR. Comparable results were found for *Enterococcus faecalis* [OWT initiation: 21 (72%), OWT follow-up 4 (66%), bite wounds 0 (0%)], *S. pseudintermedius* [OWT initiation 12 (57%), OWT follow-up 4 (100%), bite wounds 0 (0%)], *Enterobacter* species [OWT initiation 6 (66%), OWT follow-up 4 (80%), bite wounds 1 (8%)] and *P. aeruginosa* [OWT initiation 11 (78%), OWT follow-up 9 (82%), bite wounds 1 (12%)]. In summary, the proportion of MDR increased during OWT for all isolated bacteria except *Enterococcus faecalis*. Antibiotic treatment before OWT as well as before bite wound treatment was associated with presence of MDR (Table 1).

DISCUSSION

We found that *Enterococcus* species, *E. coli*, *S. pseudintermedius* and *P. aeruginosa* were the most common bacteria isolated from open wounds, which is similar to results reported during OWT in horses and humans (Westgate *et al.* 2011, Kirketerp-Möller *et al.* 2008, Malic *et al.* 2009, Gjodsbol *et al.* 2006). These bacteria are also frequently encountered in surgical site infections in small animals (Nelson 2011, Weese 2008), and the majority of patients with open-treated wounds represent complicated surgical site infections (Nolff *et al.* 2015).

All these isolates represent opportunistic ubiquitous pathogens, which reside in the environment (*Pseudomonas*), on the skin of the host (*Staphylococcus*) or within the gastrointestinal tract (Gandolfi de Christopherus *et al.* 2013, Garbacz *et al.* 2013, Osland *et al.* 2012, Damborg *et al.* 2009, Malic *et al.* 2009, Weese 2008, Hanssen & Ericson-Sollid 2006, Gjodsbol *et al.* 2006), and numerous reports have identified these ubiquitous pathogens as the main contaminants in open wounds (Malic *et al.* 2009). We also found a high prevalence of MDR in our open wounds compared to the bite wound group. Studies on hospitalised dogs have shown that MDR *Enterococcus* species isolates in the faeces of the patients increase to abnormally high levels (more than 50% of the total population) during hospitalisation and pre-treatment with antibiotics (Gosh *et al.* 2011). In

Table 2. Overview of patients in which the overrepresented bacteria were isolated at the beginning of OWT, during OWT and in the bite wounds (BW)

Isolated bacteria	Affected initiation OWT	Affected during OWT	Affected BW
P. aeruginosa	14/88 (15·9%)	14/52 (26·9%)	8/184 (4·3%)
E. coli	28/88 (31·8%)	13/52 (25%)	7/184 (3·8%)
Enterococcus subspecies	29/88 (33%)	12/52 (23·1%)	11/184 (6%)
S. pseudintermedius	21/88 (23·9%)	6/52 (11·5%)	13/184 (7·1%)
Proteus vulgaris	2/88 (2·3%)	2/52 (3·8%)	0/184 (0%)

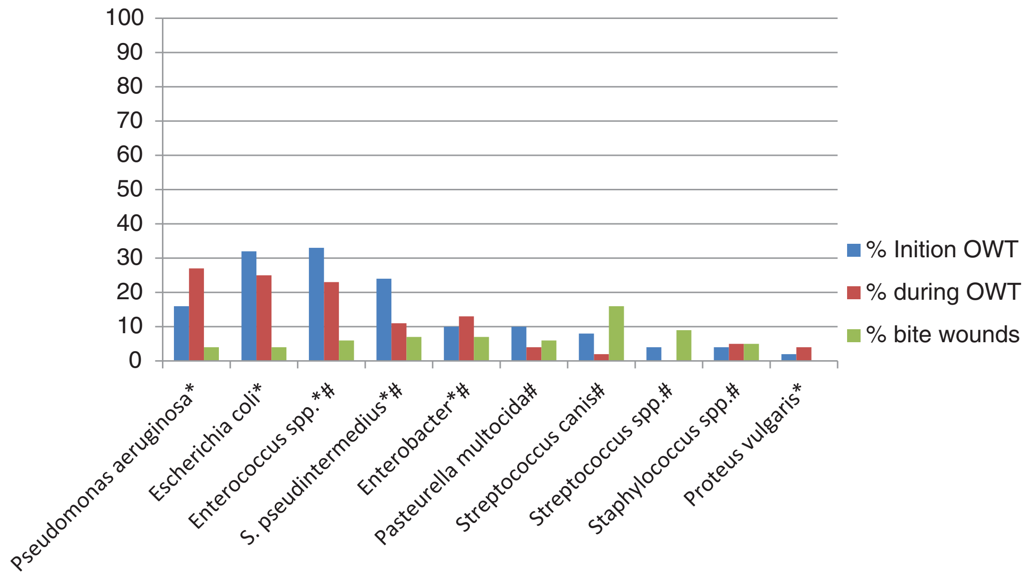

FIG 2. All isolates that were identified as overrepresented.*Isolates that were overrepresented in open wounds, # Isolates that were overrepresented in bite wounds

addition, the overall susceptibility of *Enterococcus* species isolates in diseased dogs treated with ß-lactam antibiotics is generally low (Damborg *et al.* 2009). Similar to the observations made for *Enterococcus* species, an increase of MDR during hospitalisation in dogs has been described for *E. coli* (Ogeer-Gyles *et al.* 2006) and *S. pseudintermedius* (Gandolfi de Christopherus *et al.* 2013). This may explain our observations on open wounds, because the majority of affected patients had previously been treated with antibiotics or hospitalised and, in some cases, patients had been receiving three or four different antibiotics prior to presentation for OWT and prior to any bacterial culture. In contrast, the majority of bite would patients (98%) was not hospitalised before wound treatment nor received antibiotics.

Similar to previous studies (Gandolfi de Christopherus *et al.* 2013, Ogeer-Gyles *et al.* 2006), we found that antibiotic pre-treatment appeared to be linked to isolation of MDR, which was detected at initiation of open-wound therapy. Antibiotic pre-treatment may have been effective in eradicating the "average bacteria," but selected for species we found most commonly. In contrast, patients that had sustained bite injuries were very rarely (2%) exposed to hospitalisation and antibiotic treatment. However, we were still able to detect a correlation between antibiotic treatment and MDR. This finding has to be interpreted with care, since only a very small number of patients (n=5) with bite wounds was affected by MDR at all.

All patients receiving OWT in this study also received at least one antibiotic during treatment but this was not associated with a change in MDR isolation. Increases in MDR strains of *E. coli*, *S. pseudintermedius*, *Enterobacter* species and *P. aeruginosa* were apparent. Among these, *P. aeruginosa* showed the largest increase in MDR during therapy.

Little is known about the role of *P. aeruginosa* infection in the wounds of domestic animals, despite the fact that it has been reported to cause surgical site infections (Weese 2008). As previously documented for strains of this bacterium isolated from human hosts, these isolates were also often MDR (Weese 2008). The increase during therapy may be associated with their propensity to form biofilm, the high potential for MDR development and their robust environmental persistence rate (Fazli *et al.* 2011, Kirketerp-Möller *et al.* 2008, Weese 2008).

In spite of the targeted use of antibiotics during OWT, we were unable to significantly reduce the number of colonised wounds relative to the initial presentation. Wounds, with their moist, protein rich environment, offer an ideal environment for biofilm formation (Malic *et al.* 2009), and the formation of mature biofilm in wounds has been described for *Staphylococcus pseudintermedus* in dogs and *Enterobacteriaceae*, *Enterococcus* species, *Staphylococcus* species, and *P. aeruginosa* in the horse (Singh *et al.* 2013, Westgate *et al.* 2011). The residual colonisation might result because the isolated bacteria are capable of biofilm formation (Oliveira *et al.* 2014, Garbacz *et al.* 2013, Gandolfi de Christopherus *et al.* 2013, Osland *et al.* 2012, Malic *et al.* 2009, Hanssen & Ericson-Sollid 2006). However, since we did not investigate biofilm formation in the wounds in our study this possibility is debatable. After the barrier function of the skin has been lost, these ubiquitous bacteria can colonise the wound surface and persist. While all other bacteria are eradicated by surgical intervention and antibiotic treatment, the few species that are well adapted to persist through biofilm formation and various resistance mechanisms remain. In contrast, the flora found in bite wounds resembles a mix of skin and environmental contaminants as well as oral flora of the animal that caused the bite. These bacteria differ from those found in chronic wounds since most of them are easily eradicated.

An important, but often neglected, question is whether eradication of bacteria within a wound should be the ultimate goal. All but one of the wounds included in this study healed well under OWT despite persistent positive bacterial culture. Nevertheless, it has been demonstrated that certain bacteria within a wound, even if it appears uninfected, delay healing (Park *et al.*

2014, Pastar *et al.* 2013, Westgate *et al.* 2011, Malic *et al.* 2009, Schierle *et al.* 2009, Percival & Rogers 2005, Madsen *et al.* 1997). Thus, healing might have been faster had we been able to achieve "clean" wounds. This, combined with the potential role of dogs and cats in the spread of MDR, suggests that perhaps eradication of bacteria should be encouraged. The usage of antibiotics in this context, however, seems questionable. There are reports in human medicine in which wounds colonised with MDR bacteria were referred to as "major challenges" that required new solutions apart from antibiotic treatment (Daeschlein 2013). Antiseptics including polyhexanide and octenidine have been advocated as the method of choice in such cases (Daeschlein 2013).

All the bacteria found commonly in OWT in this study, namely *Staphylococcus* species, *Enterobacteriaceae*, *Enterococcus* species and *P. aeruginosa*, have been classified as dangerous because of their high impact in human healthcare-associated infections (ECDC). The high prevalence of MDR bacteria in open wounds and its persistence despite antibiotic treatment is worrying, and underlines the need for microbial surveillance of wounds during OWT regardless of the clinical status of the wound.

There are some limitations of this study, mainly because of its retrospective nature. Besides investigating the number of detected MDR isolates, the impact on health status of the affected patients would be interesting to examine. However, because different treatment approaches were used, different wound types were included and different locations were wounded it is difficult to determine whether presence of MDR bacteria influenced the healing time in this study. Because only very few bite wounds were affected by MDR bacteria it was not possible to investigate their effects in any detail appears that large prospective studies would be required. A second limitation is that the time of repeated culturing was not standardised and we relied solely on superficial culture swabs. These do not necessarily give the full picture and other pathogenic bacteria, especially those residing in biofilms, may have been overlooked. In order to determine the true incidence of bacterial contamination, molecular identification of bacteria per gram tissue and correlation to clinically evident complications would have been ideal. This process is relatively expensive and not routinely performed in our clinics. Further studies including multiple centres at different locations and prospective, quantitative detection of bioburden as well as association of bioburden and clinical outcome would be needed to clarify the presented results.

CONCLUSION

We showed that *Enterococcus* species, *E. coli*, *S. Pseudintermedius* and *P. aeruginosa* were more common in open wounds than bite wounds and open wound therapy did not eradicate this bioburden despite wounds appearing uninfected. In addition, we detected a high proportion of MDR pathogens. Further studies are needed to determine the amount of biofilm formation.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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