Amikacin in 30% poloxamer 407 is a versatile local therapy with method of application and outcome documented in 29 dogs

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OBJECTIVE
To report the clinical use, adverse events, and outcomes after using amikacin in 30% poloxamer 407 (amikacin-P407) during open wound management or in a closed wound application in dogs.

ANIMALS
29 client-owned dogs.

METHODS
Medical records from January 2017 to August 2023 from a single hospital were reviewed for dogs that received amikacin-P407 in an open or closed wound application. Information reviewed included signalment, nature of wound and/or surgical site infection (SSI), bacterial cultures, amikacin dose, gel volume, route of administration, estimated wound surface area, biochemistry parameters, urine casts, wound progression, and general clinical outcome.

RESULTS
Amikacin-P407 was applied during open wound care (10 dogs), via injection (5 dogs), and at time of wound closure (13 dogs) and was used both in open and closed wound management (1 dog). Wounds were associated with SSIs in 18 of 30 sites. Multidrug resistance was noted in 21 of 30 preapplication cultures. Median amikacin dose was 14.5 mg/kg (range, 3 to 59.5 mg/kg), median total volume was 5.0 mL (range, 1 to 12 mL), and median tissue surface area was 6.6 cm² (range, 1.6 to 36 cm²), for a local wound dose of 62.5 mg/cm² (range, 6.9 to 214.3 mg/cm²). No short-term adverse local or systemic effects were noted in any wounds or dogs. No dehiscence was seen in 17 of 19 closed sites.

CLINICAL RELEVANCE
The results of this case series suggested that Amikacin-P407 can be applied in a variety of ways with no adverse effects. Amikacin-P407 may be considered in open wound management or in a closed setting for infected wounds and SSIs.

Keywords: wound, amikacin, surgical site infection, poloxamer 407, local delivery

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handleability in wound use. Amikacin eluting from pluronic 407 (P407) was shown to match or exceed mean inhibitory concentrations for strains of biofilm-producing methicillin-resistant *Staphylococcus pseudintermedius* in vitro. In vivo sustained release from P407 has been described and used clinically with vancomycin for complex implant infections as well as for different applications such as an osteosarcoma removal and nasal aspergillosis with cibroform plate lysis.

Clinical use of topical antibiotic delivery has been described in several veterinary studies, including coated implants, CSH beads, collagen sponges, and polymers. The use of antibiotic-impregnated CSH beads has been demonstrated to be largely successful in treating deep SSIs associated with various orthopedic procedures, with no major associated complications. Surgical implant removal combined with placement of an amikacin-infused collagen sponge was sufficient for long-term resolution of SSIs associated with tibial plateau leveling ostetomies (TPLOs) in 30 of 31 dogs, including 19 MDR infections. Placement of amikacin- and clindamycin-infused hydrogel at the time of implant removal in 20 dogs with TPLO site infections resulted in complete long-term infection control without evidence of concerning laboratory or tissue changes. The clinical use of vancomycin in P407 in complex orthopedic implant–related surgery complications has recently been reported with clearance of infection in 26 out of 34 dogs. While CSH beads can be premade and to date have been the most often used clinical delivery method for antibiotics in orthopedic surgery, some superficial wounds might not lend themselves well for CSH bead application. The efficacy, ease of use, and inexpensive nature of P407 warrant its further investigation as a topical delivery agent for antibiotics in both open and closed wound applications. In addition to this, P407 might have inherent antibacterial properties of its own as well, as shown in vitro against methicillin-resistant *S. pseudintermedius* and *Escherichia coli*.

The aim of this study was to describe the use, adverse events, and outcomes after use of amikacin in 30% P407 (amikacin-P407) at a single institution. We hypothesized that amikacin-P407 would be (1) well tolerated in dogs when used for open wounds and in closed wound applications, including for SSIs, and (2) would aid in successful wound healing and resolution of infection.

### Methods

#### Search strategy

The medical records database from a single university hospital was searched for *pluronic*, *poloxamer*, and *amikacin* and patient logs were searched for *wound management* and *wound closure* for dogs treated between January 1, 2017, and August 31, 2023. The inclusion criterion was at least 1 application of amikacin-P407. Exclusion criteria were lack of in-person follow-up of at least 1 week following initial amikacin-P407 application and incomplete information regarding the manner of application and dose of the amikacin-P407 (lack of procedural notes).

#### Demographics and application data

Patient-specific data recorded included sex, age, body weight (BW), breed, presenting complaint and nature of clinical signs, and surgical procedures if performed. Treatment-specific data included open versus closed wound management, the dose of amikacin delivered, volume of amikacin-P407 used, use of concurrent systemic antibiotics at time of application, number of applications, and route of application. Surface area (SA, in cm²) of the wound was based on descriptions in the electronic medical record or an approximation in case of lack of direct information. The method of data retrieval (surgery report vs general electronic medical record notes vs approximation) was noted. If approximated, the method and calculations were described. If only incision length was available, the wound width was estimated as 1 cm, and if pocketing was present, the wound width was estimated as 3 cm. Amikacin (amikacin sulfate, injection, 500 mg/2 mL; Sagent Pharmaceuticals) was added to sterile pluronic 407 (PCCA pluronic 407 NF gel 30%; PCCA) by the on-site pharmacy. Amikacin-P407 was available as 22.5-mg/mL, 50-mg/mL, 125-mg/mL, and 250-mg/mL concentrations and was ordered in milliliters.

#### Laboratory tests

Data acquired included bacterial culture before or immediately prior to application, what was cultured (tissue, fluid, implants), bacterial isolates, susceptibility of the organisms versus presence of MDR, timing of repeat culture with indication, and results. Multidrug resistance was classified as being resistant to 1 or more classes of antimicrobial agents, according to CDC guidelines. Blood urea nitrogen, creatinine, and phosphorus were assessed on preapplication panels to look for preexisting disease and any postapplication panels (≤ 7 days; > 7 days but ≤ 1 month; > 1 month but ≤ 1 year) to assess any change in renal status for the dogs included. All values were characterized as within normal limits, low, or elevated based on institutional reference ranges (BUN, 7 to 32 mg/dL; creatinine, 0.50 to 1.50 mg/dL; phosphorus, 2.2 to 7.9 mg/dL).

#### Outcome

The outcome/status of the wound or incision was recorded. Any wound-related issues or complications were noted. Short-term follow-up data included any wound issues or other complications that developed within the time from application to incision evaluation (minimum follow-up of 7 days; range, 7 days to 1 year). The length of short-term follow-up (up to 1 year) was defined as the time between the day of first application and the day of follow-up, with both examination days included in the count. If another procedure was performed in the same location (wound closure or implant removal), the day of surgery was taken as the final follow-up.
date. Long-term follow-up was included if in-person evaluation was performed more than 1 year after the first application.

Data analysis

Data were reported as descriptive, either with all variables fully reported for individual dogs or as median and range for treatment groups, without further statistical analysis given the diversity of etiologies and treatments. A spreadsheet (Excel, Microsoft 365; Microsoft Corp.) was used to collect data and calculated median and range.

Results

Demographics and application data

Medical records of 74 dogs were retrieved, and 29 met the inclusion criteria. Six dogs met all inclusion criteria except were not assessed in person within 30 days following amikacin-P407 application. Otherwise, the most common reason for case exclusion was lack of sufficient procedural notes except for wound dimension data. The median BW of dogs was 34.9 kg (range, 4.2 to 57.9 kg), and median age was 6.1 years (range, 1.1 to 14.6 years). Median BW of dogs included in the open wound treatment group was 36.5 kg (range, 4.2 to 50.0 kg), and median age was 7.7 years (range, 2.0 to 14.6 years). For dogs in the closed application group, median BW was 35.4 kg (range, 7.4 to 57.9 kg) and median age was 5.8 years (range, 1.1 to 11.3 years). The most common breeds represented were German Shepherd Dog (n = 5), Golden Retriever (4), and Labrador Retriever (3). Sex and neuter status were as follows: 12 spayed female dogs, 9 castrated male dogs, 4 intact male dogs, and 4 intact female dogs.

Amikacin-P407 was used in open wound management in 10 dogs (Supplementary Table S1) and used in a closed wound setting in 18 dogs (Supplementary Table S2). One dog (listed in Supplementary Tables S1 and S2 as dog D and dog 10, respectively) received both and was included once for reporting of the total population (29 dogs) but listed and counted in both separate groups for data on open versus closed wound management (a total of 30 separate treatments). Fourteen of 19 dogs treated in a closed wound setting had amikacin-P407 applied during surgery prior to wound closure, 3 had it injected in the tissues, and 2 had intra-articular injections. When applied at time of wound closure, the amikacin-P407 was placed in the deepest area of the wound: in dogs with otitis undergoing a total ear canal ablation and bulla ostectomy (ie, TECA-BO), this included starting at the bulla and filling the deep part of the wound. For other cases, amikacin-P407 was placed around implants, in the deepest area of the wound, in pockets underneath the skin in wound closures, and over the entire wound surface during fusion podoplasty. Intra-articular injection was performed during arthrocentesis without ultrasound guidance, and tissue injection was performed based on palpation: around the site of the total ankle replacement after draining a fluid pocket, along the plate postoperatively in the radius/ulna fracture revision, and through gaps of the draining incision in the dog with the hemilaminectomy. Three dogs with a wound managed open were not on systemic antibiotics, and 3 dogs with amikacin-P407 applied in a closed setting only received cefazolin (22 mg/kg, IV, at approx 90-minute intervals; cefazolin for injection, 1 gram/vial, 330 mg/mL; Apotex Corp) during the procedure. All other dogs were on concurrent systemic antibiotics, based on culture and sensitivity, with amoxicillin-clavulanic acid (range, 9.78 to 17.3 mg/kg, PO, twice daily as concurrent therapy; Clavamox; Zoetis) being the most frequently used (13 dogs). In total, 13 dogs did not receive IV antibiotics during the procedure/application: 9 out of 11 dogs during open treatment of wounds or incisions, 1 dog that received an intra-articular injection, 2 dogs that had amikacin-P407 injected into tissues, and 1 dog that had it injected via the incision into the surgery site. The 2 dogs with open wound management with periprocedural antibiotics received 30 mg/kg of ampicillin and sulbactam (ampicillin and sulbactam, 1.5 g/vial; Hikma Pharmaceuticals). Periprocedural antibiotics for the remainder of the dogs were cefazolin in 10 dogs (22 mg/kg, IV, at 90-minute intervals) or ampicillin and sulbactam in 6 dogs (22 to 30 mg/kg, IV, at 90-minute intervals).

Wound etiology was an SSI in 18 of 29 dogs (deep, n = 15; superficial, 3) and a non-SSI in 11 of 29 dogs. Deep SSIs developed most often following orthopedic implant placement (12/15), with the most frequently associated procedures being fracture repair (n = 5), arthrodesis (2), TPLO (1), and total hip arthroplasty (1). Amikacin-P407 delivered during surgery was used at time of implant removal (n = 2) or revision surgery (3), with 3 of the remaining 4 receiving a postoperative local injection and 1 undergoing open wound management. Superficial SSIs (3/18) were associated with a hind limb amputation in 1 dog, incomplete excision of a grade 1 soft tissue sarcoma in 1 dog, and primary closure of a traumatic tarsal wound in 1 dog. The remaining 11 infections were associated with chronic, nonhealing wounds and ulcers of various etiology (n = 3), otitis externa and/or media (3 [2 unilateral, 1 bilateral]), pododermatitis (2), traumatic wounds (2), and septic arthritis (1). Wound SA or incision length was available in the records for 8 wounds treated open, and 3 wounds treated with closed application (with the dog with application to the open wound and the surgery site included in both). An estimate was made for the remaining 19 sites (3 open and 16 with application in closed setting; Supplementary Table S3). The median SA of all affected tissues to which amikacin-P407 was applied was 6.6 cm² (range, 1.6 to 36 cm²). The median SA treated in open wound care was 6 cm² (range, 1.8 to 36 cm²), and the median SA treated in closed wound care was 7 cm² (range, 1.6 to 27 cm²).

Most dogs with open wound management received multiple doses (median, 2 [range, 1 to 4]). All dogs with amikacin-P407 applied at wound closure had 1 application only. Three dogs had 1 dose of amikacin-P407 injected into the tissues, 1 dog had 2 doses injected into the elbow joint, and 1 dog had 3 doses injected into the shoulder joint. The median
amikacin dose per application for all 29 dogs was 14.6 mg/kg of BW (3 to 59.5 mg/kg of BW). The median daily dose of amikacin in open applications was 14.6 mg/kg of BW (7.2 to 59.5 mg/kg of BW). The median dose of amikacin applied in a closed setting or at wound closure was 14.6 mg/kg of BW (3 to 39.1 mg/kg of BW). The median volume of amikacin-P407 applied per dose was 5.0 mL (range, 1.0 to 12 mL) for all dogs, with a daily median volume for open wound management of 2.5 mL (range, 1.0 to 10 mL) and a median volume of amikacin-P407 instilled at wound closure or injected into a closed wound of 5.6 mL (range, 2.0 to 12 mL). These application volumes correlated with a median local tissue amikacin dose of 62.5 mg/cm² (range, 6.9 to 214.3 mg/cm²) per application for all dogs. The median daily dose of amikacin in open wounds was 53.8 mg/cm² (range, 6.9 to 208.3 mg/cm²), and the median amikacin dose used in a closed setting was 69.4 mg/cm² (range, 15.5 to 214.3 mg/cm²). The records reflected individual purchases for the desired amount per treatment in 1 dose.

**Laboratory tests**

Preapplication chemistry panels were available for 26 dogs (median, 2 days prior to application [range, 0 to 140 days]), with BUN and creatinine available for 26 dogs and phosphorus for 25 dogs. One dog had a low BUN (5 mg/dL); all other variables were within reference range. Median (range) was 14 mg/dL (5 to 29 mg/dL) for BUN, 0.8 (0.4 to 1.5 mg/dL) for creatinine, and 4.7 mg/dL (2.8 to 6.6 mg/dL) for phosphorus. Postapplication chemistry panels were available for 5 dogs (none ≤ 7 days; 3 ≤ 1 month; 1 ≤ 1 year), with creatinine, BUN, and phosphorus within reference range for all 5. Median (range) was 9 mg/dL (7 to 14 mg/dL) for BUN, 0.6 (0.5 to 1.2 mg/dL) for creatinine, and 5.6 mg/dL (3.1 to 6.0 mg/dL) for phosphorus. No urinalyses within 7 to 10 days after application were available.

All dogs had the wound or site cultured prior to or at the time of amikacin-P407 application with susceptibility testing performed. There were 21 susceptibility reports that were reflective of MDR bacteria and 9 that were universally susceptible. Of the 30 preapplication cultures (the dog with both open application and application at wound closure had 2 included), 15 were consistent with monomicrobial infections and 13 were consistent with polymicrobial infections, with the most common bacteria grown in both scenarios being *S. pseudintermedius* (n = 17). Three of the cultures obtained at the time of amikacin-P407 application grew 3 or more different microbes (2 dogs with externa otitis at the time of total ear canal ablation with lateral bulla osteotomy (TECA-LBO) and 1 dog with an infected decubital ulcer at the time of bandage placement).

Thirteen dogs had recheck cultures performed at various times following initial application (Supplementary Table S4). This included the dog (dog D) that had a second culture at the time of wound closure. In 1 dog, the incision had healed completely, and the recheck culture was of the skin surrounding the TECA-LBO incision (dog 1). Of the remaining 12 recultured wounds, 3 were negative and 5 had initially cultured an MDR organism with recheck cultures growing a non-MDR organism; in 2 recultured wounds, both cultures resulted in growth of MDR organisms. One dog (dog I) developed an MDR infection after 2 months of open wound management. One dog had a spinal fracture addressed after wound infection of a hemilaminectomy site, and the second culture during that revision surgery revealed an MDR organism (dog 18).

**Outcomes**

All dogs were reevaluated in person within 30 days of amikacin-P407 application. The full short-term follow-up time specific for the site where amikacin-P407 was used was a median of 30 days (range, 7 to 201 days) for all dogs. Follow-up for dogs with open wound management was 27 days (range, 7 to 201 days) and for dogs undergoing application in a closed setting was 32 days (range, 10 to 179 days). Two dogs had a longer short-term follow-up than was used in follow-up calculation (48 days [dog 4] and 165 days [dog E]), but these were for different concerns, and no specific note was made of the wound or surgery site. No local tissue reactions were noted following amikacin-P407 application in any of the records at time of follow-up. Of the 10 wounds treated open, 1 was fully surgically closed, 3 were partially surgically closed and then went on to heal fully, and 6 healed by second intention. No issues with application of amikacin-P407, such as lack of gelatination, were noted, and in several cases it was applied under skin edges in partially closed wounds.

Incisional or wound drainage was reported in 5 of 19 dogs following amikacin-P407 application in closed wounds: 4 had revision surgery for an infected orthopedic implant, of which 1 dog’s incision went on to dehiscence, and the fifth dog had incision-site drainage after a hemilaminectomy prior to instillation of amikacin-P407 into the wound. Drainage after application was initially still present. While the incision healed, the dog suffered a compression fracture due to discospondylitis, underwent spinal stabilization at 27 days after application, and had amikacin in CSH beads placed in the incision. A dog that had amikacin-P407 applied at the time of a fusion podoplasty experienced dehiscence that resolved after medical management with bandage care, and the wound healed by second intention.

Two dogs had long-term, in-person follow-up at our hospital or another hospital with complete medical records. Dog 1 had a follow-up dermatology appointment at 1,259 days, at which time no issues or draining tracts were noted associated with the TECA-LBO surgery sites. Dog 5 had an orthopedic-specific follow-up at 1,631 days after the first amikacin-P407 application and 1 further follow-up at the time of a cardiology referral (1,982 days after the first amikacin-P407 application). No complaints or issues pertaining to the left shoulder were noted at the orthopedic appointment or noted in the records of the cardiology appointment. In the remaining dogs, long-term follow-up was not reported, was incomplete, or was not conducted in person.
Discussion

The hypotheses for this study were that amikacin-P407 would be (1) well tolerated in dogs when used for open wounds and in closed wound applications including for SSIs and (2) would aid in successful wound healing and resolution of infection. In the 29 dogs reported in this study, amikacin-P407 was used in both open and closed applications, with no complications noted related to the gel, confirming our first hypothesis. However, we could not prove our second hypothesis, as drawing a firm conclusion about the effectiveness of amikacin-P407 in combating infections was difficult due to the retrospective nature and diversity in management and concurrent antibiotic use in the majority of dogs. The results do reflect the versatility and ease of use as well as good outcomes in cases where it was used, including in 6 dogs without concurrent systemic antibiotic use, both in open wound management and in closed applications.

Amikacin-P407 was easily applied in open wounds on the surface of the wound bed and gelatinate appropriately, over the wound bed surface as well as in partially closed areas. A ratio of 1:1 of liquid amikacin to sterilized 30% P407 was successfully used, which is similar to ratios of liquid carboplatin that will still gelatinate but is contrary to silver nanoparticle solution, which required a 1:2 to 1:3 ratio. If different solutions or different antibiotics are mixed, confirmation of appropriate gelatination prior to use might be appropriate. In our setting, single-dose purchase of the amount needed for a single treatment was possible, thereby avoiding attempts or need to save purchased drugs for future use or having to waste unused drugs in a barrier nursing setting. In the absence of an on-site pharmacy, a larger vial of P407 could be sterilized and kept in a designated pharmacy refrigerator to allow 1 dose of P407 to be drawn up and aseptically mixed with liquid amikacin on an as-needed basis.

The thermoreversible characteristics of P407 especially lend themselves well to instillation into closed wounds via a catheter or needle, and both intra-articular wound site installation as well as peri-implant injections were found. No complications related to the injection or progression of the wound or injection site to drainage or dehiscence were reported. One dog in which it was instilled in an already draining wound did have ongoing drainage, but the wound did not go on to dehiscence, although the single application did not prevent the development of discospondylitis. The ability to inject P407 might make drug-infused P407 especially attractive in cases where a culture taken at surgery indicates different organisms or a different resistance pattern than expected, as it would allow instillation of a high dose of antibiotics in the site without having to fully open and explore the healing incision. Prior reports of the clinical use of P407 have focused on applications in wounds at the time of surgery, with the noninvasive application in the current study highlighting other uses and the versatility of P407. Especially at higher concentrations (125 mg/mL) the volume of amikacin-P407 can be kept low, which is beneficial for injections and smaller wound sites. No dosages (total amount, mg/kg, or approx mg/cm²) were reported for a larger study using antibiotic-impregnated beads, although it was noted that the space was the limiting factor on number of beads used. Recommended manufacturer dosing and mixing instructions are 500 mg of amikacin (liquid) for a full set of CSH beads. A prior study used a commercially available combination of tobramycin with CSH beads where delivered total dose is 1.2 g, with an individual dose of approximately 20 mg/bead, based on a reported 60 beads kit. This, however, would rely on low intrabatch variety, while a variety of up to 23% in carboplatin content was reported between batches in a different study, and application of the full kit might therefore be preferable. The volume of a complete bead kit would most likely be larger than the equivalent 4 mL of high-concentration amikacin-P407, making amikacin-P407 a good alternative to antibiotic-impregnated beads. In addition, amikacin-P407 can be mixed and used immediately, rather than requiring a specified time to set (a recommended 50 minutes for commercial CSH beads for veterinary use).

One concern might be that single dosing and failure to eradicate the entire microbial population could lead to an increase in resistance pattern. Amikacin is a dose-dependent antibiotic and reported is rapidly bactericidal even at single dosing, if the achieved concentration is high enough. This benefit might be more applicable for gram-negative microbes and less predictable for gram-positive cocci, such as the majority in our dogs. In 1 canine tibial Staphylococcus aureus osteomyelitis model, single-dose local delivery of gentamycin (100 mg/dog) either in polymethylmethacrylate (9 tibiae in 9 dogs) or in a polylactide/polyglycolide implant (9 tibiae in 9 dogs) was compared with 3 mg/kg of BW for 28 days (16 tibiae in 8 dogs). No infection was found at necropsy in 8 of 9 sites with polymethylmethacrylate delivery, in 9 of 9 sites with polylactide/polyglycolide delivery, and in 10 of 16 tibia with systemic antibiotics. For the dogs reported here with post-amikacin-P407 usage cultures of the site, similar or less resistant bacteria were found in all but 2 dogs. The 1 dog with worsening resistance pattern had 1 application and 2 months of open wound care prior to the second culture.

There are inherent limitations to a retrospective study with regard to availability of all desired data. In this study, those main limitations include unavailability of precise wound measurements, inability to verify whether the full dose was used, a lack of multiple repeat cultures in wounds treated open, and lack of short-term postapplication chemistry and urinalysis to assess renal values and look for the presence of casts. We used an approximation of the SA to which amikacin-P407 was applied in most of the closed applications but were able to retrieve data for 8 open applications. We felt that it was valuable to report the data, even if an approximation was used, to show local dosing together with outcomes. It is possible that the full dose was not administered and that
there was an overestimation of the volume needed. Our reported doses therefore would be the maximum possible delivered dose at each setting, but could be lower. Practically, if the exact volume that could be delivered per application is not yet known, choosing the maximum concentration of amikacin-P407 (250 mg/mL) and having a second syringe with a volume of plain P407 might be an option to allow versatility in high-dose amikacin delivery and full SA coverage.

While we did not have follow-up chemistry or urinalyses, the risk for renal toxicity after local delivery of aminoglycosides appears low. Systemic uptake after local delivery of 56 mg/kg of BW tobramycin in CSH beads has been reported as peaking at 1 hour and becoming undetectable after 24 hours.\(^{31}\) Local concentrations similarly peaked at 1 hour but maintained therapeutic levels for 7 days. No serum biochemistry changes were noted for any of the dogs that had follow-up analyses performed. Unfortunately, no urinalyses were performed. While this dose was lower than the doses delivered to the dogs in this study, the nephrotoxicity concerns secondary to aminoglycosides increase after prolonged treatment.\(^{1}\) Using a once-daily, but higher, dose has been suggested to decrease risk, as has prolonging the interval between dosing.\(^{31}\)

Especially in dogs with open wound management, the use was not consistent, and other topical products were used during the duration of care, which limits the ability to draw conclusions on efficacy. However, no adverse effects were seen, and application of amikacin-P407 is an option for topical management either as a solo therapy or in addition to systemic antibiotics. P407 itself is FDA approved for topical use (in addition to intratympanic, ophthalmic, oral, and periodontal use).\(^{34}\) It might find its place similar to topical use of commercially available gentamycin ointments, which the authors have used on open wounds or wounds prior to free skin grafting.

Despite the limitations inherent to a retrospective study and the variety of cases and applications included, results of this study showed the versatility and range of possible applications for antibiotic delivery in P407. In addition, no adverse events were noted in any of the dogs included in our study, and outcomes in all cases were good, with no wound dehiscence noted in 17 of 19 dogs where it was used in a closed setting.

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