Comparative in vitro killing of canine strains of Staphylococcus pseudintermedius and Escherichia coli by cefovecin, cefazolin, doxycycline and pradofloxacin

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Background – Bacterial eradication is necessary for clinical cure of infections and antimicrobial agents are important adjunctive therapies for inhibiting the growth of or killing bacteria. Pre-existing skin diseases predispose animals to infection by Staphylococcus pseudintermedius and, more rarely, by Gram-negative bacilli. The property of rapid killing of bacteria may influence drug selection and duration of therapy in the setting of infection.

Objectives – To test the killing of canine isolates of S. pseudintermedius and Escherichia coli by cefazolin, cefovecin, doxycycline and pradofloxacin at the minimum inhibitory, mutant prevention, maximum serum and maximum tissue drug concentrations.

Methods – Under standard conditions, bacterial cells were exposed to clinically relevant drug concentrations in vitro and the log10 reduction (and % kill) of viable cells measured at 5, 10, 15, 20, 25, 30, 60, 120 and 180 min after drug exposure.

Results – Statistically significant differences were seen between killing efficiencies by pradofloxacin versus the other agents, whereby pradofloxacin killed cells more rapidly than the others. For example, against the S. pseudintermedius strains, significantly more cells were killed by pradofloxacin following 15 min of maximum tissue drug concentration exposure than for cefazolin (P = 0.0002), cefovecin (P = 0.0007) and doxycycline (P ≤ 0.0001).

Conclusion and clinical importance – The rank order of potency based on these kill experiments was pradofloxacin > cefazolin > cefovecin > doxycycline. Rapid killing of bacteria affects the speed of clinical resolution and may influence drug selection and duration of therapy for skin infections.

Introduction

Bacteria cause infection when they gain entry to or invade sterile sites or tissues. Tissue destruction by the presence of bacteria and/or their liberated toxins, along with the host’s immune responses, contribute to clinical signs and disease manifestations.1 Eradication of bacteria has been linked to clinical cure;2 signs resolve when bacteria are eliminated whereas failure to eradicate bacteria leads to persistence or recurrence of disease after a period of an initial favourable clinical response. Clinical deterioration and death may occur in some patients with serious infections that are treated incorrectly or inadequately.3 Antimicrobial agents are used to treat infections with the aim to prevent morbidity and mortality, to alleviate symptoms and, in some instances, to prevent further spread.

Staphylococcus pseudintermedius and Escherichia coli are well known bacterial pathogens of dogs and cats and S. pseudintermedius can be especially problematic in canine skin infections.4 The pathogenesis of canine skin infection by S. pseudintermedius is not well understood and may be less related to organism virulence factors than to predisposing factors within the host; however, proteins that mediate organism adherence have been described.5 Breakdown of the skin’s natural defences promotes colonization and infection.6 By contrast, E. coli is more commonly a cause of urinary tract infections7, but may be found in dermatological infections (i.e. deep pyoderma) where a Gram-positive pathogen is typically also present. As summarized previously, primary infection with S. intermedius (pseudintermedius) causes tissue conditions more conducive to secondary invasion by Gram-negative microorganisms.6 Companion animal treatment guidelines have attempted to provide expert advice (based on critical review of the literature) on...
therapy for urinary tract and some types of skin infections; however, they have, in many areas, fallen short due to limited published data – both in vitro and clinical. In many cases, antimicrobial agents used widely in human medicine may be used to treat infected animals without any studies to validate their suitability for such clinical uses.

In both human and veterinary dermatology, data are lacking on achievable and sustainable antimicrobial drug concentrations in normal and inflamed or infected skin. The suitability and relevance of susceptibility/resistance breakpoints that have been established for antimicrobial drug concentrations in the blood may not always apply to skin. These concentrations typically have been established for systemic (usually blood-borne) infections and the amount of drug that gets into skin may be unknown. This complicates interpretation of susceptibility results and, ultimately, drug selection.

Considerable debate has occurred regarding the clinical impact of drugs classified as bactericidal versus bacteriostatic and in which setting each may be most appropriate or acceptable. In vitro killing measurements remain important for differentiating bacteriostatic from bactericidal antimicrobial agents and have helped to inform expert opinion on how such data influence recommendations for clinical cure and positioning of these drugs within therapeuetic guidelines (either human or animal). Rapid and complete killing of bacteria have important clinical implications including use in patients with mild to moderate to severe infections, shorter duration of therapy, reduced likelihood for relapse or recurrent infection and the potential to minimize selection of resistance. The latter is often not considered when selecting an antimicrobial agent; however, it may be more important in an environment of increasing antimicrobial resistance. Standard in vitro measurements such as the minimum inhibitory concentration (MIC) determine the minimum drug concentration blocking growth of \(10^5\) colony forming units (CFU) per millilitre (ml), whereas the mutant prevention concentration (MPC) measures inhibition of growth of \(\geq 10^9\) CFUs. Neither MIC or MPC measure killing of bacteria, but rather inhibition of growth.

Antimicrobials used to treat dermatological infections in dogs and cats include penicillins, cephalosporins, lincomamides, fluoroquinolones, tetracyclines and others. Indeed, published therapeutic guidelines recommend 1st and 3rd generation cephalosporins as first- or second-tier choices; with doxycycline (or minocycline) and fluoroquinolone agents as second-tier drugs. For refractory cases not responding to first- or second-tier drugs, third-tier agents including linezolid, teicoplanin and vancomycin may be contemplated if supported by culture and susceptibility results; these drugs are usually reserved for human patients.

As very limited or no information is available on killing of these key veterinary pathogens by antimicrobials at clinically relevant drug concentrations, we compared killing of canine isolates of \(S.\) pseudintemdeus and \(E.\) coli by cefazolin, cefovecin, doxycycline and pradofloxacin for both speed and completeness of viable cell reduction by each compound, through use of clinically relevant (or estimated) drug concentrations.

Materials and methods

Bacterial strains

Three isolates each of \(S.\) pseudintermedius and \(E.\) coli from canine sources were generously provided by Heinz Wetzstein, Bayer Animal Health GmbH, Leverkusen, Germany. Organism identification was confirmed by reference methods. All strains were classified as susceptible to the studied drugs because MICs were below the susceptibility breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI).

Antimicrobial compounds

Pradofloxacin was obtained from Bayer Animal Health (Monheim, Germany) and the remaining compounds were purchased commercially (cefovecin – Zoetis; Kirkland, QC, Canada; cefazolin – Novo-pharma; Scarborough, ON, Canada; doxycycline – Sigma Aldrich; Oakville, ON, Canada). Each compound was dissolved/prepared according to the manufacturer’s instructions. Stock solutions were used as fresh preparations or from samples stored for <1 month at \(-70^\circ\)C.

Minimum inhibitory concentration testing

Susceptibility testing to determine the MIC was carried out in accordance with the guidelines established by the CLSI. Isolates were stored frozen in skimmed milk at \(-70^\circ\)C. Prior to testing, isolates were thawed and subcultured twice on tryptic soy agar containing 5% sheep red blood cells. Isolates were tested by microbroth dilution in Mueller–Hinton broth (MHB) (Difco Laboratories; Detroit, MI, USA). Briefly, cation-adjusted MHB containing two-fold concentration increments of antimicrobial agent was added to 96-well microdilution trays. Staphylococcus pseudintermedius and \(E.\) coli suspensions (from overnight cultures) equal to a 0.5 McFarland standard were further diluted to achieve a final inoculum of \(5 \times 10^7\) CFU/mL in trays. Cultures were incubated for 18–24 h in \(O_2\) and the MIC was taken as the lowest concentration that inhibited growth. MICs for the four drugs tested are summarized in Table 1. Drug concentration ranges tested against the \(E.\) coli and \(S.\) pseudintermedius strains were as follows: cefazolin 0.031–32 µg/mL, cefovecin 0.008–8 µg/mL, doxycycline 0.063–64 µg/mL. The following American Type Culture Collection (ATCC) control strains were also routinely tested: Staphylococcus aureus ATCC 29213, \(E.\) coli ATCC 25922, Enterococcus faecalis ATCC 29212 and Pseudomonas aeruginosa ATCC 27853.

Mutant prevention concentration testing

The procedure used to test \(S.\) pseudintermedius and \(E.\) coli isolates by MPC was as previously described. Briefly, starter cultures of two blood agar plates (tryptic soy agar containing 5% sheep red blood cells) (PML Microbiologicals; Richmond, BC, Canada) per isolate were inoculated to produce confluent growth and were incubated overnight (18–24 h) at 35–37°C in \(O_2\). The next day, the complete contents of the inoculated plates were removed from the plates with sterile swabs (Puritan; Guilford, ME, USA) and transferred to 100 mL of MHB and incubated overnight at 35–37°C in \(O_2\). Aliquots of 100 µL containing \(\geq 10^6\) CFU were applied to individual 100 mm blood agar plates. Bacterial densities were confirmed by spectrophotometric (Thermo Scientific GENESYS I0VIS; Mississauga, ON, Canada) readings (600 nm) \(\geq 0.3\) and colony counts. For each experiment, agar dilution plates were prepared by mixing tryptic soy agar containing 5% sheep red blood cells plus drug at desired concentrations. Plates were then poured and solidified at room temperature (21°C) following which they were stored at \(4^\circ\)C in the dark.

Antimicrobial agents were tested over a range of seven different concentrations (in doubling dilutions) in the blood agar plates. Plates were stored at \(4^\circ\)C and were used within 7 days of preparation. Drug concentration ranges tested were as follows: \(E.\) coli, cefazolin 0.25–128 µg/mL, cefovecin 0.25–32 µg/mL, doxycycline 0.25–64 µg/mL, pradofloxacin 0.004–8 µg/mL; \(S.\) pseudintermedius, cefazolin 0.125–32 µg/mL, cefovecin 0.063–16 µg/mL, doxycycline 0.25–64 µg/mL, pradofloxacin 0.004–8 µg/mL. Each experiment included the control strains (\(S.\) aureus ATCC 29213 or \(E.\) coli ATCC 25922). Incubated
plates were incubated for 24 h, examined and re-incubated for another 24 hours (48 h in total) at 35–37°C in O2 and were then screened for growth. To confirm MPC values, colonies were sub-cultured on plates containing the same drug concentration that they were isolated from, incubated as described and examined for growth. The MPC was recorded as the lowest drug concentration that prevented growth.

### Kill studies

The method used for the kill studies has been reported previously,14,19,20 *Staphylococcus pseudintermedius* and *E. coli* isolates were grown overnight on blood agar plates as described. The next day, an inoculum was transferred to MHB and incubated for 2 h at 35–37°C in O2 and were then screened for growth. To confirm MPC values, colonies were sub-cultured on plates containing the same drug concentration that they were isolated from, incubated as described and examined for growth. The MPC was recorded as the lowest drug concentration that prevented growth.

### Statistical analysis

Statistical analysis of the data was performed using a repeated measures ANCOVA for each drug dataset, with fixed effects consisting of drug and drug-by-time interaction. In each model, CFU count at time 0 was included as a covariate and a compound symmetric covariance structure was used. The transformed square root CFU counts were used to achieve a normal distribution. Bonferroni adjustments for multiple comparisons were made. Least-square means were back transformed and presented as log_{10} means. Values of \( P < 0.05 \) were considered significant for all analyses.

## Results

### Staphylococcus pseudintermedius

Killing using the measured MIC drug concentration is shown in Figure 1. For cefazolin, minimal killing or growth occurred over the 180 min of the study. Similar results were seen for cefovecin except at the 180 min measurement, a 0.44 log_{10} reduction (45% kill) in viable cells was seen. For doxycycline, growth occurred at most time points. Finally, for pradofloxacin a 0.12 log_{10} (27% kill) reduction in viable cells occurred at 180 min following drug exposure. Statistically significant differences were not seen between the four drugs at the MIC drug concentration.

Figure 2 shows the speed and extent of killing of *S. pseudintermedius* strains using the measured MPC drug concentrations. For cefazolin, a 0.28 log_{10} (41% kill) and 0.5 log_{10} (61% kill) reduction in viable cells was seen at 120 and 180 min, respectively, following drug exposure; a 0.11 log_{10} (12% kill) and 0.46 log_{10} (47% kill) reduction was seen for cefovecin at 120 and 180 min, respectively. For doxycycline, a 0.05 log_{10} (11% kill) and 0.11 log_{10} (21% kill) reduction in viable cells was seen following 120 and 180 min, respectively, of drug exposure. Significant differences in killing between drugs were seen at 60, 120 and 180 min of drug exposure for pradofloxacin compared to the other three drugs (P-values presented in Figure 2). Significant differences were not seen between the other drugs tested.

Figure 3 shows killing at the maximum serum concentration. For the *S. pseudintermedius* isolates, killing was seen by cefazolin over most time periods but was highest at 60, 120 and 180 min of drug exposure (0.12, 0.34 and 0.59 log_{10} reduction in viable cells – 24, 45 and 72% kill, respectively); 0.61 and 0.91 log_{10} (59 and 79% kill) following 120 and 180 min of exposure to cefovecin; 0.01 and 0.02 log_{10} (2.4 and 5.9% kill) reduction by doxycycline at

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**Table 1. Comparative minimum inhibitory concentration (MIC), mutant prevention concentration (MPC), maximum serum and tissue drug concentration values (in µg/mL) for four antimicrobial agents**

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<th>RUH-CASP2</th>
<th>RUH-CASP3</th>
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*For cefovecin, cefazolin and doxycycline, these were estimated from the peer-reviewed literature for skin drug concentrations;22,23 and from published serum drug concentrations.24 For pradofloxacin, published data were used.25,26*
120 and 180 min. For pradofloxacin, killing occurred at all measured time points with 90% of cells killed (0.74 log$_{10}$) following 20 min of drug exposure and this increased to 98% kill (1.95 log$_{10}$) by 60 min and >99% kill (2.68 log$_{10}$) following 120 min of drug exposure. Significant differences were seen in the killing by pradofloxacin versus doxycycline, cefovecin and cefazolin, respectively, and multiple time points (P-values are presented in Figure 3).

Figure 4 shows the kill data following the maximum tissue drug concentration. For cefazolin, 15, 40 and 64% killing of viable cells (0.1, 0.28 and 0.53 log$_{10}$) was seen following 60, 120 and 180 min of drug exposure as compared to 17, 64 and 84% kill (0.08, 0.58, 1.4 log$_{10}$) following exposure to cefovecin. Only 5% of viable cells (0.3 log$_{10}$) were killed following 120 min of exposure to doxycycline. For pradofloxacin, 66-87% of viable cells were killed (0.47 and 0.88 log$_{10}$) following 5 and 10 min of drug exposure; 92-97% of cells were killed following 15-60 min of drug exposure. Finally, >99% of viable cells were killed (2.9 log$_{10}$) following 120 min of drug exposure. Significant differences were seen in the killing of bacteria by pradofloxacin versus doxycycline, cefovecin and cefazolin, respectively, following 10, 15, 20, 25, 30 and 60 min of drug exposure time (P-values presented in Figure 4). Significant differences were also seen between other drug comparisons at other time points (Figure 4).

Escherichia coli

Figure 5 shows killing of the E. coli isolates at the MIC drug concentration. A 0.12 log$_{10}$ (68% kill) reduction in viable cells was seen following 180 min of exposure to cefazolin. By comparison, a 0.32 log$_{10}$ (15% kill) reduction was seen following 20 min of exposure to cefovecin which increased to a 0.78 log$_{10}$ (75% kill) reduction by 120 min of drug exposure. A 0.23 log$_{10}$ (19% kill) reduction in viable cells was seen following 10 min of exposure to doxycycline; however, regrowth occurred by the 120 and 180 min time point. For pradofloxacin, a 0.36 and 1.0 log$_{10}$ (10 and 78% kill) was seen following 120 and 180 min of drug exposure, respectively. Statistically significant differences are shown in Figure 5.

Exposure of the E. coli strains to the MPC (Figure 6) drug concentration yielded a 0.57 log$_{10}$ (72% kill) reduction by 20 min and a 2.56, 4.31 and 4.99 log$_{10}$ (>99% kill, respectively) were seen following 60, 120 and 180 min of exposure to cefazolin. For cefovecin, a 0.21 log$_{10}$ (33% kill) reduction was seen following 25 min of drug exposure and this increased to a 0.91 log$_{10}$ reduction (72% kill) by 180 min of drug exposure. Killing by doxycycline was minimal (growth to 28% kill) or absent. For pradofloxacin, a 0.29 log$_{10}$ reduction (46% kill) was seen following...
20 min of drug exposure, which increased to a 1.7, 3.6 and 4.5 \( \log_{10} \) reduction (84, 99.9 and 99.9% kill) following 60, 120 and 180 min of drug exposure, respectively. Statistically significant differences in kill times between drugs are shown in Figure 6.

Exposure of the *E. coli* strains to the maximum serum drug concentration (Figure 7) for cefazolin showed a 0.27 \( \log_{10} \) (46% kill) reduction by 15 min, which increased to a 2.0, 3.2 and 3.2 \( \log_{10} \) reduction (99, 99.8 and 99.9% kill, respectively) following 60, 120 and 180 min of drug exposure; for cefovecin, a 0.13 \( \log_{10} \) (22% kill) following 20 min and drug exposure and a 0.37, 0.71 and 2.1 \( \log_{10} \) reduction (57, 76 and 99% kill) by 60, 120 and 180 min, respectively; and for doxycycline, a 0.24 \( \log_{10} \) (38% kill) reduction by 20 min with regrowth occurring at the 120 and 180 min time points. For pradofloxacin, a 0.51 \( \log_{10} \) reduction (48% kill) occurred following 10 min of drug exposure and this increased to a 1.8 \( \log_{10} \) reduction (88% kill) by 15 min and a >3 \( \log_{10} \) reduction (99.6% kill) by 25 min and the remaining time points thereafter (99.9% kill by 60 min; 100% kill by 120 min). Statistically significant differences in kill times between drugs are shown in Figure 7.

Exposure to the maximum tissue drug concentration (Figure 8) for cefazolin resulted in a 0.22 \( \log_{10} \) reduction (30%) by 25 min which increased to a 3.04 and 3.98 \( \log_{10} \) reduction (85.6 and 99.9% kill, respectively) by 120 and 180 min. For cefovecin, a 0.26 \( \log_{10} \) reduction (18% kill) was seen following 120 min of drug exposure which increased to a 0.6 (70% kill) at 120 min. A 0.33 \( \log_{10} \) reduction (30% kill) was seen following 20 min of exposure to doxycycline; however, growth occurred at the 180 minute time period. For pradofloxacin, a 0.9 \( \log_{10} \) reduction (83% kill) occurred following 5 min of drug exposure which increased to a 2.7 \( \log_{10} \) reduction (99% kill) at 10 min, a >3 \( \log_{10} \) (99.9% kill) reduction at 15 min and all time points thereafter (100% kill by 60 min). Statistically significant differences in kill times between drugs are shown in Figure 8.

**Discussion**

According to Pankey and Sabath, "Antimicrobial therapy... provides one of the only pharmacologic treatments
that cure disease.” 19 Batterby commented on responsible use of antimicrobial agents in companion animals and stated “clinicians understand the implications of the overuse of antibiotics and the principals of optimal prescribing.” 27 What then constitutes responsible use given that these drugs cure disease? Certainly, use of antimicrobials only when necessary is essential, and antimicrobial stewardship has evolved as an important strategy for responsible antimicrobial use.28 Antimicrobial stewardship arose from increasing antimicrobial resistance, the lack of new antimicrobials in development and the need to optimize therapy in infected patients. In essence, antimicrobial stewardship serves to optimize therapy by having the right antimicrobial, at the right dosage and for the correct duration of therapy. As stated previously, antimicrobial stewardship is less about saving money and more about optimizing therapy.29 Optimizing therapy is meant to ensure clinical and microbiological cure and reduce the likelihood for the selection of antimicrobial resistance selection and escalation. Advances in diagnostic laboratory technology will also impact upon clinical practice and antimicrobial therapy, both of which are important components of antimicrobial stewardship and, hence, responsible antimicrobial use.30

The study presented here has compared the killing of canine isolates of S. pseudintermedius and E. coli by cefazolin, cefovecin, doxycycline and pradofloxacin in three hour kill experiments, in order to determine the speed and extent of killing that occurs within the first few hours after drug exposure. The inoculum used was the same as that used to determine the MIC. The experiments used clinically relevant drug concentrations – MIC, MPC, maximum serum and maximum tissue drug concentrations. For cefazolin, cefovecin and doxycycline, tissue drug concentrations were estimated and serum drug concentrations for these three drugs were taken from a published source.24 Cefazolin was used as a surrogate for 1st generation cephalosporins (cefazolin, cephalothin, cefalexin) and the bactericidal and pharmacological proportion of this agent have been reported previously.31 For doxycycline, the maximum tissue drug concentration was taken as 50% of the peak serum drug concentration based on data summarized previously,22 and likely represents an overestimate of the actual concentration in skin. For cefazolin and cefovecin, the tissue concentration was taken as 25% of the maximum serum concentration.23 The cefazolin estimated skin drug concentration used in our study was consistent with the human and simulated organism are ongoing for the agents reported here.

The results from our study for doxycycline appear to be consistent with observations reported previously.37 In these experiments, killing of ATCC strains of S. aureus, Streptococcus pneumoniae, Pasteurella multocida and E. coli using ~10^6 cfu/mL of organism and drug concentrations of 2×, 4× and 16× MIC were compared over 24 h. Insignificant killing occurred within the first three hours after drug exposure for the S. aureus and E. coli strains. Staphylococcus pseudintermedius was not tested. Similar results were reported for one strain of S. pseudintermedius in a kill assay with doxycycline over 24 h and at the MIC, 2×, 4×, 8× and 16× MIC drug concentrations.35

The bactericidal properties of pradofloxacin have been reported previously for S. pseudintermedius and E. coli canine strains.38 The rapid killing seen in our study is similar to previous findings;38 however, the time to ≥3 log_{10} kill in our study was faster and likely related to the higher drug concentrations tested. The rate and extent of killing by cefovecin seen in this study appears consistent with colony count reduction data reported previously which indicated inhibition of bacterial growth in transudate and exudate following longer periods of drug exposure than those tested in our study (i.e. 2.97–3.61 log_{10} reduction following 48 hours of drug exposure).26,33 Further investigations to test this along with varying densities of test organism are ongoing for the agents reported here.

Cefazolin, cefovecin and pradofloxacin are classified as bactericidal agents whereas doxycycline is considered to be bacteriostatic. Differentiating antimicrobials based on bacteriostatic versus bactericidal status has been debated for years.39 and Ihrke40 suggested that skin drug concentrations are important in treating pyoderma and that bactericidal antibiotics should be recommended for deep pyoderma. Agents that are potentially useful for the treatment of pyoderma include 1st generation cephalosporins and fluoroquinolones amongst others.42 These drug classes also have been recommended in guideline documents for therapy of canine skin infections.37,41 A review of antimicrobial drug use patterns for canine and feline skin diseases in Europe reported use in cats to be 8% for 1st and 2nd generation cephalosporins, 8% for fluoroquinolones and 16% for 3rd and 4th generation cephalosporins.42 By comparison, use in dogs was 35% for 1st concentration reported in these two specimen types. Whether the transudate and exudate drug concentrations are an accurate representation of cefovecin drug concentration in dogs requires further investigation. Free peak drug concentration of cefovecin in transudate and exudate were 3.5 ± 3.83 µg/mL and 1.78 ± 0.88 µg/mL, respectively.33 Protein binding has been shown to impact antimicrobial activity: only the nonprotein bound fraction is microbiologically active. Indeed, drugs with higher protein binding appear to have higher modifications to antimicrobial action.34 For the antimicrobials tested, protein binding has been reported to be 81% for cefazolin in humans,31 96–98% for cefovecin in dogs,33 82–93% for doxycycline in dogs22,35 and <30% for pradofloxacin in dogs.36 Although protein binding was not measured in the present study, in vitro measurements which specifically address this factor and its impact on killing by these antimicrobials are ongoing.

The results from our study for doxycycline appear to be consistent with observations reported previously.37 In these experiments, killing of ATCC strains of S. aureus, Streptococcus pneumoniae, Pasteurella multocida and E. coli using ~10^6 cfu/mL of organism and drug concentrations of 2×, 4× and 16× MIC were compared over 24 h. Insignificant killing occurred within the first three hours after drug exposure for the S. aureus and E. coli strains. Staphylococcus pseudintermedius was not tested. Similar results were reported for one strain of S. pseudintermedius in a kill assay with doxycycline over 24 h and at the MIC, 2×, 4×, 8× and 16× MIC drug concentrations.35
and 2nd generation cephalosporins and 9% for fluoroquinolones. Although not specifically summarized for skin infections, use of tetracyclines in dogs and cats ranged from 4 to 21% depending on country (overall, 5% in dogs and 14% in cats).  

Cephalosporins, doxycycline (minocycline) and fluoroquinolones also are recommended for skin and soft tissue infections in humans.  

In this study, killing at the MIC drug concentration was slower and incomplete for all drugs tested. This observation has been previously reported. Statistically significant differences were seen between pradofloxacin and the other three antimicrobials for killing of the S. pseudintermedius strains. At the Cmax and maximum tissue drug concentration, statistically significant differences were seen in favour of pradofloxacin by 10–15 min after drug exposure and at multiple time points thereafter. Surprisingly, significant differences between doxycycline, cefazolin and cefovecin were not usually seen but might be different following longer drug exposure times. Similar results were seen for the killing of the E. coli strains. Cefazolin and pradofloxacin were never equal in their killing of the E. coli strains at the MPC drug concentration and statistically significant differences were seen for these two drugs versus cefovecin and doxycycline. Killing of E. coli also was more pronounced for cefazolin and pradofloxacin at the maximum serum and tissue drug concentrations but significantly faster and with more killing by pradofloxacin.

Applying statistical analysis to colony count reduction data between different antimicrobial agents has been reported. In that study and others, similar to our report here, killing was faster and more pronounced at the MPC, maximum serum and maximum tissue drug concentration for some agents. The rank order of potency based on speed and completeness of kill was pradofloxacin > cefazolin > cefovecin > doxycycline.

In moderate to severe infections (i.e. pneumonia, meningitis, endocarditis), bactericidal drugs – alone or in combination – are recommended, whereas in mild to moderate infections static or cidal drugs are often used. Clinical trials in humans for some infections have failed to show a clinical difference in patient outcome when treated with a cidal versus static agent; however, such studies are provided to show noninferiority and as such, a difference is unlikely. Kennis stated that empiric antibiotic therapy for canine superficial pyoedema should target S. pseudintermedius and that the duration of therapy should be a minimum of 3 weeks, or 2 weeks beyond clinical remission.

In human medicine, shorter durations of antimicrobial exposure have become standard in care for uncomplicated urinary tract infections, mild to moderate community-acquired respiratory tract infections and others without compromising patient care. We have shown statistically significant differences in the killing of S. pseudintermedius and E. coli strains by pradofloxacin versus cefazolin, cefovecin and doxycycline for drug-susceptible strains. Such findings might ultimately inform thinking on drug selection, dosing and duration of therapy; however, concomitant treatment of underlying skin disease clearly plays an important role.

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**References**


Blondeau and Shebelski


Killing of Staphylococcus pseudintermedius

Methods – Sous conditions standards, les cellules bactériennes ont été exposées à des concentrations médicamenteuses cliniquement significatives in vitro et la réduction log 10 (et % d’élimination) de cellules viables a été mesurée à 5, 10, 15, 20, 25, 30, 60, 120 et 180 min après exposition médicamenteuse.

Résultats – Des différences statistiquement significatives ont été observées entre l’efficacité d’élimination de la pradofloxacine et des autres agents, d’autant que la pradofloxacine tuait les cellules plus rapidement que les autres. Par exemple, contre les souches de S. pseudintermedius, significativement plus de cellules ont été tuées par la pradofloxacine après 15 min d’exposition à une concentration médicamenteuse tissulaire maximale que par la céfazoline ($P = 0.0002$), la céfovécine ($P = 0.0007$) et la doxycycline ($P = 0.0001$).

Conclusions et importance clinique – L’ordre de puissance basé sur ces expériences était : pradofloxacine > céfazoline > céfovécine > doxycycline. La vitesse d’élimination des bactéries affecte la vitesse de résolution clinique et peut influencer la sélection et la durée des traitements des infections cutanées.

Resumen
Introducción – La erradication bacteriana es necesaria para la cura clínica de infecciones y los agentes antimicrobianos son importantes terapias adyuvantes para inhibir el crecimiento o matar bacterias. La existencia de enfermedades de la piel anteriores puede predisponer a los animales a infecciones bacterianas. La propiedad de la rápida destrucción bacteriana puede influir la selección de fármacos y la duración de la terapia durante la infección.

Objetivos – probar la capacidad destructiva para aislados caninos de S. pseudintermedius y Escherichia coli mediante cefazolina, céfovécina, doxiciclina y pradofloxacina a la dosis mínima inhibitoria, prevención de mutaciones, y concentraciones máxima en suero y el tejido de los fármacos.

Métodos – bajo condiciones estándar, las bacterias fueron expuestas a concentraciones clínicamente relevantes de los fármacos in vitro y se midió la reducción logarítmica decimal (y % de destrucción) de las bacterias vivos midiendo a los 5, 10, 15, 20, 25, 30, 60, 120 y 180 minutos tras la exposición a los fármacos.

Resultados – se observaron diferencias estadísticamente significativas entre las eficiencias de destrución de pradofloxacina comparada con los otros agentes, ya que la pradofloxacina destruía las bacterias más rápidamente que los otros. Por ejemplo, frente a cepas de S. pseudintermedius, un número mayor de bacterias fueron destruidas por la pradofloxacina tras 15 minutos de exposición a la concentración máxima en tejidos de exposición de máxima en tejidos comparado con cefazolina ($P = 0.0002$), céfovécina ($P = 0.0007$) y doxiciclina ($P = 0.001$).

Conclusión e importancia clínica – el rango de orden de potencia basado en estos experimentos destrucción fue pradofloxacina > céfovécina > doxiciclina. La rápida destrucción de bacterias afecta la velocidad de resolución clínica y puede influir la selección de fármacos y la duración de la terapia en infecciones de la piel.

Zusammenfassung

Methoden – Unter Standardbedingungen waren die Bakterienzellen in vitro klinisch relevanten Wirkstoffkonzentrationen ausgesetzt und die überlebenden Zellen wurden in einer log10 Reduzierung (und % Abtötung) bei 5, 10, 15, 20, 25, 30, 60, 120 und 180 Minuten nach Exponierung mit dem Wirkstoff gemessen.

Ergebnisse – Statistisch signifikante Unterschiede konnten zwischen der Effizienz des Abtötens durch Pradofloxacin im Gegensatz zu den anderen Wirkstoffen gesehen werden, wobei Pradofloxacin eine größere Anzahl an Zellen rascher abgetötet hatte als andere Wirkstoffe. Zum Beispiel wurden bei den S. pseudintermedius Stämmen signifikant mehr Zellen durch Pradofloxacin nach einer 15 Minuten dauernden maximalen Gewebekonzentration mit dem Wirkstoff als durch Cefazolin ($P = 0.0002$), Cefovecin ($P = 0.0002$) und Doxycycline ($P = 0.0001$) abgetötet.

Schlussfolgerungen und klinische Bedeutung – Die Rangordnung der Potenz basierend auf diesen Tötungsexperimenten war Pradofloxacin > Cefazolin > Cefovecin > Doxycyclin. Ein rasches Abtöten der Bakterien beeinflusst die Geschwindigkeit der klinischen Abheilung und kann die Auswahl der Wirkstoffselektion und die Dauer der Therapie von Hautinfektionen beeinflussen.

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要約
背景 — 感染症の臨床的治療のために細菌の除去は必要であり、抗菌薬は細菌の増殖抑制もしくは殺菌のために重要な補助療法である。既に皮膚疾患が存在すると、動物はStaphylococcus pseudintermediusや、まれではあるがグラム陰性桿菌に感染しやすくなる。感染の状況下において、殺菌に関する即効性は薬剤選択や治療期間に影響を与える可能性がある。
目的 — イヌからの分離菌であるS. pseudintermediusおよびEscherichia coliの死滅をセファゾリン、セフェペシン、ドキシサイクリン、プラドフロキサシンを用いて最小発育阻止濃度・耐性菌出現阻止濃度・最大血清薬剤濃度・最大組織薬剤濃度に関して検査すること。
方法 — 標準条件下、細菌をin vitroにおいて臨床的に適切な薬剤濃度に暴露した。薬剤暴露後5、10、15、20、25、30、60、120、180分後に生存細胞のlog10 reduction（および死滅した細胞の割合）を測定した。
結果 — 統計学的にプラドフロキサシンとその他の薬剤との殺菌効果に統計学的な有意差が認められたことから、プラドフロキサシンは他の薬剤より速やかに細菌を殺滅させた。例えば、S. pseudintermedius株で最大組織薬剤濃度に暴露した15分後にプラドフロキサシンにより死滅した細胞数はセファゾリン（P = 0.0002）、セフェペシン（P = 0.0007）、ドキシサイクリン（P < 0.0001）と比較し有意に高かった。
結論および臨床的重要性 — これらの実験結果に基づいた効力の順位は、プラドフロキサシン > セファゾリン > セフェペシン > ドキシサイクリンであった。殺菌の即効性は臨床的な治療速度に影響し、皮膚感染症に対する薬剤選択や治療期間に影響を与える可能性がある。

摘要
背景 — 皮膚科的治療感染症に対して、細菌の除去は必須で、また抗菌薬の使用は重要である。なかでもS. pseudintermediusが問題となる。この細菌の増殖は抗菌薬の使用により抑制されるが、殺菌効果は薬剤の選択に大きく依存する。
目的 — 各薬剤の殺菌効果を比較し、臨床効果を予測する。
方法 — 培養を用いて細菌の増殖を観察し、薬剤の影響を評価する。
結果 — セファゾリン、セフェペシン、ドキシサイクリンの順に殺菌効果が見られた。S. pseudintermediusに対する殺菌効果は、セファゾリンが最も優れていた。
結論および臨床的意義 — これらの結果は、臨床治療に有用な情報を提供する。