Veterinary Dermatology

Vet Dermatol 2020; **31**: 187–e39 DOI: 10.1111/vde.12835

In vitro killing of canine strains of *Staphylococcus pseudintermedius* and *Escherichia coli* by cefazolin, cefovecin, doxycycline and pradofloxacin over a range of bacterial densities

Joseph M. Blondeau*,† and Shantelle D. Fitch*

*Division of Clinical Microbiology, Royal University Hospital and Saskatchewan Health Authority, 103 Hospital Drive, Saskatoon, Saskatchewan, Canada, S7N 0W8

†Departments of Microbiology and Immunology, Pathology and Ophthalmology, University of Saskatchewan, 103 Hospital Drive, Saskatoon, Saskatchewan, Canada, S7N 0W8

*Correspondence: J.M. Blondeau, Department of Microbiology, Royal University Hospital, 103 Hospital Drive, Saskatoon, Saskatchewan, Canada, S7N 0W8. Email: joseph.blondeau@saskhealthauthority.ca

Background – Bacterial densities likely fluctuate during infection and may exceed the bacterial density used in susceptibility testing. As such, investigation of bacterial killing by antibiotics over a range of varying bacterial densities may provide important differences between compounds and could impact drug selection for therapy.

Hypothesis/Objectives – To measure killing of clinical isolates of *Staphylococcus pseudintermedius* and *Escherichia coli* by cefazolin, cefovecin, doxycycline and pradofloxacin at clinically relevant (minimum inhibitory, mutant prevention, maximum serum and maximum tissue) drug concentrations against varying densities of bacteria.

Methods and materials – Bacterial strains collected from dogs with urinary tract infections were studied. High bacterial densities ranging from 10^6 to 10^9 colony forming units (cfu)/mL were exposed to minimum inhibitory, mutant prevention, blood and tissue drug concentrations, and the percentages ($\log_{10}$) of viable cells killed following 30 min, 1, 2, 4, 6, 12 and 24 h of drug exposure were quantified.

Results – Doxycycline exhibited bacteriostatic properties with less killing than the other three agents. For example, at a 10^7 cfu/mL density of *S. pseudintermedius*, more cells were killed by pradofloxacin ($P < 0.0001$) and cefovecin ($P = 0.0014$) but not cefazolin when compared to doxycycline at the maximum serum drug concentration following 12 h of drug exposure.

Conclusions and clinical importance – Differences were seen between some drugs in the speed and extent of bacterial killing; this could be clinically important and may impact drug selection and length of therapy.

Introduction

Bacterial eradication is considered important for clinical cure from an infectious disease, even though this concept may be more complex for skin infections. Numerous authors have debated on similarities and differences between bactericidal and bacteriostatic agents, and where they might be best used in clinical medicine and influence clinical outcome.[1–3] Noninferiority clinical trials in humans with mild to moderate community-acquired infections failed to show differences in outcome in patients treated with bactericidal versus bacteriostatic agents and such observations are not surprising as differences in outcome were not expected (nor were the trials powered to detect differences). In patients with life-threatening infections, bactericidal agents alone or in combination with bacteriostatic agents have been recommended for therapy.[4]

Differentiating bactericidal agents from each other and from bacteriostatic agents has involved *in vitro* kill studies where $\log_{10}$ reductions in viable cells are used to determine -cidal versus -static activity. A reduction in viable cells of >3 $\log_{10}$ or greater differentiates a bactericidal drug from a bacteriostatic agent where a <2 $\log_{10}$ reduction is seen. Log reduction values between <2 $\log_{10}$ and >3 $\log_{10}$ is considered a grey or indeterminate zone.[5,6]

We have previously argued that clinically relevant drug concentrations need to be used in *in vitro* kill assays and over a range of bacterial densities expected to occur during acute or chronic infections.[7,8] In particular, higher bacterial densities are known to occur in central nervous system,[9,10] respiratory[11] and urinary tract infections,[12] and

Accepted 20 November 2019

Source of Funding: This work was funded, in part, by an unrestricted research grant from Bayer Animal Health.

Conflicts of Interest: Joseph Blondeau has received funding from Bayer Animal Health for *in vitro* investigations on pradofloxacin and enrofloxacin and comparator antibiotics; funding from Bayer Pharma for *in vitro* investigations with amikacin and ciprofloxacin; and grants with Bausch and Lomb for *in vitro* investigations with ophthalmic antibiotics; and has consulted with Pfizer/Zoetis. Shantelle Fitch has not declared any conflicts of interest.

likely others. In a previous report,[7] we compared killing of canine isolates of *Staphylococcus pseudintermedius* and *Escherichia coli* by cefazolin, cefovecin, doxycycline and pradofloxacin using bacterial densities of 10^5 colony forming units (cfu)/mL – the bacterial density used for *in vitro* susceptibility testing. Bacterial killing was measured over 180 min and differences in killing by the drugs tested were seen for *S. pseudintermedius* and *E. coli* at some of the various time points. In the present report, we extended the design to compare bacterial killing over 24 h and at higher bacterial densities ranging from 10^6 to 10^9 cfu/mL[7].

Methods and materials

Bacterial strains

The same clinical isolates from dogs as used in a previous study (three each of *S. pseudintermedius* and *E. coli*) were tested.[7] They had been identified by Matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) (BioMerieux; St Laurent, Quebec, Canada) and each isolate had to be susceptible to each agent based on current recommended susceptibility minimum inhibitory concentration (MIC) breakpoints.[13]

Antimicrobial compounds

Sources of antimicrobial compounds and their preparation and storage were as described previously.[7]

MIC/mutant prevention concentration (MPC) testing

The MIC/MPC testing using a modified protocol was as summarized in detail in our earlier publication.[7,14,15] Measured MIC and MPC values for the six strains examined are as reported previously and summarized in Table 1.[7]

Kill studies

Kill studies were a modification to protocols published previously.[16,17] Bacterial isolates were incubated overnight on blood agar (BA) plates for 18–24 h at 35–37°C in O_2. Following overnight incubation, an inoculum was transferred to Mueller–Hinton Broth (MHB) and incubated for 2 h in O_2 at 35–37°C; then spectrophotometric readings (>0.3) verified cell densities >10^9 cfu/mL which were subsequently confirmed by colony counts.[14] Further adjustment of inocula to achieve cell densities ranging from 10^6 to 10^9 cfu/mL were done in MHB; to this, cefazolin, cefovecin, doxycycline or pradofloxacin was added based on the measured drug MIC or MPC values or the C_{max} or $Tissue_{max}$ drug concentration for each drug tested against each strain. *In vitro* measurements did not take into account protein binding. A summary of C_{max} and $Tissue_{max}$ concentrations is presented in Table 1 and is as reported previously.[7] For cefovecin, cefazolin and doxycycline, skin drug concentration was estimated from previous publications.[18,19] Measurements of killing (log_{10} reduction in viable cells and % kill) were recorded at 30 min, 1, 2, 4, 6, 12 and 24 h following drug exposure by culturing aliquots on drug-free blood agar plates and incubating overnight as described and counting colonies. Colony counts recorded at time 0 at the 10^6, 10^7, 10^8 and 10^9 cfu/mL densities (respectively) were as follows:

Three separate aliquots were sampled at each time point and results averaged; as such each data point represents nine independent measurements (i.e. three strains x three triplicate samplings). The log_{10} and percentage kill reduction of viable cells were calculated and recorded.

Statistical analysis

Statistical analysis of the data was as described previously.[7] Values of $P < 0.05$ were considered significant for all analyses.

Results

Exposure of 10^6 cfu/mL of *S. pseudintermedius* (Table 2) to the MIC drug concentration of four drugs tested did not show any statistically significant differences in bacterial killing between the drugs (refer to Table 2 for all log_{10} reduction comparisons).

When 10^6 cfu/mL were exposed to MPC drug concentrations of the four drugs, pradofloxacin ($P = 0.0001$) and cefovecin ($P = 0.0001$) killed more cells than doxycycline did following 24 h of drug exposure.

Exposure of 10^6 cfu/mL to the C_{max} drug concentration showed statistically significant differences in kill following 4 h of exposure to pradofloxacin versus doxycycline ($P < 0.0006$) or cefazolin ($P = 0.0042$), pradofloxacin versus doxycycline ($P < 0.0001$) or cefazolin ($P = 0.0007$). Following 12 h of drug exposure, more cells were killed by pradofloxacin than by doxycycline ($P < 0.0001$). Following 24 h of drug exposure, more cells were killed by pradofloxacin than by doxycycline ($P < 0.0001$) and by cefovecin ($P = 0.007$) and cefazolin ($P = 0.0005$) than doxycycline.

Exposure of 10^6 cfu/mL to the $Tissue_{max}$ drug concentration showed a statistically significant difference in killing by pradofloxacin versus doxycycline ($P = 0.0054$) following 6 h of drug exposure, pradofloxacin versus doxycycline ($P < 0.0001$) following 12 h, cefazolin versus doxycycline ($P = 0.0312$) following 12 h, pradofloxacin versus doxycycline ($P < 0.0001$) at 24 h and cefovecin versus doxycycline ($P = 0.0004$) and cefazolin versus doxycycline ($P = 0.0018$) following 24 h of drug exposure.

Exposure of 10^7 cfu/mL of *S. pseudintermedius* (Table 3) to the MIC drug concentration of the four drugs tested showed a significant difference between cefovecin and cefazolin ($P = 0.0116$) following 12 h of drug exposure. At the MPC drug concentration, significantly more bacterial cells were killed by pradofloxacin than doxycycline ($P = 0.0123$) and by cefovecin ($P = 0.0118$) and cefazolin ($P = 0.0105$) than doxycycline following 12 h of drug exposure. Cefovecin killed more cells than doxycycline ($P = 0.0013$) following 24 h of drug exposure. At the C_{max} drug concentration, pradofloxacin killed

		x10^6	x10^7	x10^8	x 10^9
cefazolin	*Staphylococcus pseudintermedius*	1.4–8.7	1.6–9.7	1.9–8.8	2.1–8.7
	Escherichia coli	1.6–4.1	1.6–5.3x10^7	1.5–4.6	1.6–5.3
cefovecin	*Staphylococcus pseudintermedius*	1.6x10^6–7.0x10^7	1.5x10^7–1.1x10^8	1.2x10^8–1.3x10^9	1.1x10^9–1.1x10^{10}
	Escherichia coli	3.9–8.4	4.3–7.9	4.8–8.5	3.3x10^9–1x10^{12}
doxycycline	*Staphylococcus pseudintermedius*	3.1–8.4	4.8–7.1	4.7x10^8–1.2x10^9	2.9–8.7
	Escherichia coli	2.1–5.7	2.2–6.9	2.9–6.3	3.0–5.9
pradofloxacin	*Staphylococcus pseudintermedius*	5.7x10^6–4.3x10^7	6.7x10^7–1.3x108	4.7x10^8–9x10^9	7.3x10^9–1.7x10^{10}
	Escherichia coli	2.3–9.2	5.4–9.4	3.9–9.2	9.4x10^9–1.2x10^{10}

Table 1. Minimum inhibitory (MIC) and mutant prevention (MPC) concentration values for *Staphylococcus pseudintermedius* and *Escherichia coli* strains and pharmacological parameters for four antimicrobial agents.[7]

	Isolates RUH-CASP1		RUH-CASP2		RUH-CASP3			
Drug	MIC	MPC	MIC	MPC	MIC	MPC	Maximum Serum Concentration	Maximum Tissue (skin) Concentration*
S. pseudintermedius								
Cefazolin	0.125	2	0.063	4	0.063	0.25	74	18.5
Cefovecin	2	4	0.25	0.5	0.25	0.5	90	22.5
Doxycycline	0.063	16	2	64	0.063	8	5.7	2.8
Pradofloxacin	0.031	0.125	0.031	0.125	0.125	0.125	1.4	4.5
	RUH-CAEC1		RUH-CAEC2		RUH-CAEC3			
E. coli	MIC	MPC	MIC	MPC	MIC	MPC	Maximum serum concentration	Maximum tissue concentration
Cefazolin	1	64	2	128	1	32	74	18.5
Cefovecin	1	4	0.5	2	1	4	90	22.5
Doxycycline	0.5	64	1	64	1	64	5.7	2.8
Pradofloxacin	0.008	0.125	0.016	0.125	0.016	0.125	1.4	4.5

CASP companion animal *S. pseudintermedius*, CAEC companion animal *E. coli*, RUH Royal University Hospital.
*Maximum tissue (skin) concentration were estimated for cefovecin, cefazolin and doxycycline, from skin drug concentrations[18,19] and from serum drug concentrations.[37] For pradofloxacin, published data were used.[49,50]

more cells by 4 and 6 h than doxycycline did ($P = 0.0999$ and $P = 0.0081$, respectively) and showed a tendency toward more killing than cefazolin ($P = 0.06$) at 4 h. Following 12 h of drug exposure, pradofloxacin killed more cells than doxycycline did ($P < 0.0001$) as did cefovecin ($P = 0.0014$). At 24 h of drug exposure, more cells were killed by pradofloxacin ($P < 0.0001$), cefovecin ($P = 0.0002$) and cefazolin ($P = 0.0045$) than doxycycline. At the $Tissue_{max}$ drug concentration, more bacterial cells were killed by pradofloxacin than by doxycycline following 6 h ($P = 0.0001$) and 12 h ($P < 0.0001$) of drug exposure. Additionally, at 12 h, more cells were killed by cefovecin ($P < 0.0001$) and cefazolin ($P = 0.0017$) than by doxycycline (growth). Pradofloxacin ($P < 0.0001$), cefovecin ($P < 0.0001$) and cefazolin ($P = 0.0001$) killed more cells following 24 h of drug exposure than doxycycline did.

Exposure of 10^8 cfu/mL and 10^9 cfu/mL to the MIC, MPC, C_{max} and $Tissue_{max}$ drug concentrations of the four drugs tested did not yield any significant differences in organism killed by individual drugs (Tables 4 and 5).

Exposure of 10^6 cfu/mL of *E. coli* (Table 2) to the MIC drug concentration of the four drugs tested showed statistically significant differences in killing of bacterial cells by cefovecin versus pradofloxacin following 6 h ($P = 0.0288$), 12 h ($P = 0.0261$) and 24 h ($P < 0.0001$) of drug exposure. Statistically significant differences also were seen for cefovecin versus doxycycline following 12 h ($P = 0.0180$) and for cefovecin versus cefazolin following 12 h ($P < 0.0001$) and 24 h ($P < 0.0001$) of drug exposure. For MPC drug concentrations, statistically significant differences were not seen between the study drugs. Exposure to the C_{max} drug concentration yielded statistically significant differences in killing between the compounds: 1 h pradofloxacin versus doxycycline and cefovecin ($P < 0.0001$ for both comparisons), and cefovecin versus cefazolin ($P = 0.0386$); 2 h pradofloxacin versus cefovecin and doxycycline ($P = 0.0001$ for both comparisons), and cefovecin versus cefazolin ($P = 0.0002$); 4 h pradofloxacin versus cefovecin and doxycycline ($P < 0.001$ for both), and pradofloxacin versus cefazolin ($P = 0.0002$), doxycycline versus cefovecin ($P = 0.0066$) and doxycycline versus cefazolin ($P < 0.0001$); 6 h pradofloxacin versus doxycycline ($P < 0.0001$), cefovecin ($P < 0.0001$), and doxycycline and cefazolin ($P < 0.0001$) versus doxycycline; 12 h pradofloxacin versus doxycycline ($P < 0.0001$), and doxycycline versus cefovecin ($P < 0.0001$) and cefazolin ($P < 0.0001$); 24 h pradofloxacin versus doxycycline ($P < 0.0001$), and cefovecin ($P < 0.0001$) versus doxycycline and cefazolin ($P < 0.0001$) versus doxycycline. Exposure of 10^6 cfu/mL of *E. coli* to the $Tissue_{max}$ drug concentration showed more killing by pradofloxacin than cefovecin ($P = 0.0143$) following 30 min of drug exposure and more cells killed by pradofloxacin than cefovecin or doxycycline (*P*-values from <0.0001 to 0.02) following 1, 2, 4 and 6 h of drug exposure. Cefovecin killed more cells than doxycycline ($P = 0.0015$) following 6 h of drug exposure. Statistically significant differences in kill were seen between pradofloxacin and doxycycline ($P < 0.0001$), cefovecin and doxycycline ($P < 0.0001$), and cefazolin and doxycycline ($P = 0.02$) following 12 h of drug exposure. Finally, more cells were killed by pradofloxacin ($P < 0.0001$) than doxycycline and cefovecin killed more cells than doxycycline ($P = 0.0045$) following 24 h of drug exposure.

Exposure of 10^7 cfu/mL of *E. coli* (Table 3) to the MIC drug concentrations of the four drugs tested did not show statistically significant differences in kill by the four agents. At the MPC drug concentration, an overall effect was seen between pradofloxacin and doxycycline at all time points ($P = 0.0139$) and with cefovecin ($P = 0.0543$). At the C_{max} drug concentration statistically significant more cells were killed by pradofloxacin than by doxycycline or cefovecin following 30 min, 1, 2, 4, 6, 12 and 24 h after drug exposure (*P-value*s ranged from <0.0001 to 0.0441). Cefazolin killed more cells than did doxycycline following 1, 2, 4, 6, 12 and 24 h of drug exposure ($P < 0.0001$–0.0071). Cefazolin killed more cells than cefovecin did following 1 and 12 h of drug exposure (*P*-values 0.001 and 0.0057, respectively) and cefovecin killed more cells than doxycycline following 4, 6, 12 and 24 h of drug exposure ($P < 0.0001$ for all exposures).

Table 2. Log_{10} reduction in viable cells (percentage of cells killed) over time for suspensions of *Staphylococcus pseudintermedius and Escherichia coli* (10^6 cfu/mL) exposed to various concentrations of cefazolin, cefovecin, doxycycline and pradofloxacin

	S. pseudintermedius				*E. coli*			
Variable	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin
Minimum inhibitory concentration (MIC)								
0.5 h	–0.03 (–7.55)	–0.05 (–0.66)	–0.01 (–3.06)	–0.17 (–17.81)	–0.09 (–18.13)	0.00 (1.92)	0.05 (19.04)	–0.03 (–1.02)
1 h	0.04 (12.78)	–0.01 (1.01)	0.07 (18.26)	–0.27 (–43.84)	–0.05 (–8.54)	0.00 (–1.10)	0.01 (2.86)	0.02 (5.60)
2 h	0.17 (51.05)	–0.04 (15.34)	0.06 (15.07)	–0.18 (–14.75)	–0.44 (–89.59)	–0.30 (–37.60)	0.09 (4.25)	0.05 (13.15)
4 h	–0.29 (209.45)	–0.46 (–24.17)	0.06 (15.46)	–0.53 (–69.13)	–0.03 (70.14)	–1.33 (–91.67)	0.02 (6.96)	–0.75 (–81.59)
6 h	–0.66 (591.99)	–0.65 (–30.50)	0.04 (10.99)	–0.99 (–88.04)	0.40 (1290.56)	–1.82 (–98.10)	0.09 (35.15)	–0.36 (17.40)
12 h	–0.33 (1324)	–1.37 (–94.76)	0.06 (182.08)	–1.65 (–87.08)	1.23 (2635.74)	–2.26 (–99.23)	0.18 (55.17)	–0.94 (–31.51)
24 h	0.95 (1427.62)	–1.44 (–60.87)	0.68 (772.31)	0.28 (–2.92)	2.24 (17438.95)	–1.37 (–87.32)	–1.79 (196.70)	1.27 (1981.05)
Mutant prevention concentration (MPC)								
0.5 h	–0.04 (–7.70)	0.01 (9.16)	–0.02 (–4.93)	0.09 (29.17)	–0.28 (–44.26)	–0.02 (–4.55)	–0.03 (–6.26)	–0.56 (–64.15)
1 h	0.04 (12.18)	–0.03 (–5.53)	–0.09 (–15.50)	–0.03 (–6.34)	–1.37 (–89.51)	–0.09 (–18.21)	–0.09 (–15.53)	–1.98 (–92.73)
2 h	–0.10 (–10.70)	–0.11 (–11.79)	–0.22 (–39.33)	–0.56 (–71.82)	–1.94 (–98.53)	–0.38 (–57.91)	–0.21 (–36.44)	–3.65 (–99.92)
4 h	–1.02 (–85.71)	–0.76 (–51.04)	–0.21 (–36.82)	–1.94 (–98.55)	–3.15 (–99.86)	–1.74 (–97.65)	–0.71 (–76.43)	–4.54 (–99.97)
6 h	–1.67 (96.52)	–1.68 (–91.16)	–0.43 (–47.46)	–2.31 (–99.47)	–4.04 (–99.97)	–2.62 (–99.71)	–1.01 (–86.97)	–5.12 (–99.98)
12 h	–2.23 (–98.76)	–3.12 (–99.85)	–0.72 (–77.86)	–3.20 (–99.89)	–4.89 (–99.99)	–2.90 (–99.70)	–1.09 (–92.27)	–5.94 (–100.00)
24 h	–2.54 (–99.05)	–3.87 (–99.98)	–0.93 (–79.70)	–3.69 (–99.94)	–5.21 (–99.99)	–4.14 (–99.99)	–2.15 (–99.19)	–5.40 (–99.99)
C_{max}								
0.5 h	0.11 (31.36)	0.05 (–19.35)	0.04 (10.04)	–1.18 (–87.16)	–0.49 (–67.08)	–0.14 (–27.68)	0.00 (6.07)	–1.53 (–87.07)
1 h	0.02 (6.20)	–0.14 (–40.29)	0.05 (11.67)	–1.46 (–95.32)	–1.22 (–98.29)	–0.33 (–45.14)	0.05 (13.61)	–3.38 (–99.84)
2 h	–0.02 (–1.98)	–0.27 (–48.70)	–0.03 (–7.73)	–2.32 (–98.95)	–2.56 (–99.70)	–0.74 (–74.71)	–0.03 (–5.78)	–4.51 (–99.99)
4 h	–0.20 (–28.82)	–0.86 (–83.03)	–0.04 (–3.40)	–3.45 (–99.86)	–3.31 (–99.95)	–2.76 (–99.81)	–0.10 (–17.50)	–5.77 (–99.99)
6 h	–1.15 (–88.35)	–1.85 (–96.64)	–0.15 (–24.95)	–3.85 (–99.95)	–4.06 (–99.99)	–3.53 (–99.97)	–0.01 (9.99)	–6.68 (–100.00)
12 h	–2.16 (–98.10)	–2.30 (–99.67)	–0.36 (–55.28)	–4.54 (–99.99)	–5.02 (–99.99)	–3.94 (–99.95)	–0.08 (–9.89)	–6.68 (–100.00)
24 h	–4.68 (–99.99)	–3.68 (–96.43)	–0.87 (–85.27)	–5.67 (–99.99)	–5.57 (–99.99)	–5.71 (–99.99)	–0.50 (–56.00)	–6.68 (–100.00)
$Tissue_{max}$								
0.5 h	0.08 (21.56)	0.09 (23.60)	–0.03 (–5.91)	–1.39 (–95.24)	–0.28 (–43.46)	–0.10 (–12.48)	–0.09 (–18.23)	–2.85 (–98.88)
1 h	–0.09 (–14.77)	0.11 (61.01)	0.03 (8.58)	–1.65 (–95.26)	–1.06 (–67.31)	–0.13 (–24.14)	–0.10 (–20.78)	–4.02 (–99.99)
2 h	–0.09 (–16.47)	–0.14 (–12.23)	0.02 (4.89)	–2.10 (–98.67)	–1.94 (–97.36)	–0.22 (–37.50)	–0.05 (–3.97)	–4.83 (–99.99)
4 h	–0.75 (–78.94)	–0.83 (–47.54)	–0.09 (–18.53)	–3.21 (–99.61)	–3.00 (–99.68)	–2.35 (–99.24)	–0.21 (–31.18)	–6.17 (–100.00)
6 h	–1.91 (–98.28)	–1.56 (–90.99)	–0.16 (–30.40)	–3.62 (–99.72)	–3.40 (–99.90)	–3.73 (–99.98)	–0.58 (–67.72)	–6.72 (–100.00)
12 h	–3.17 (–96.62)	–2.67 (–99.72)	–0.01 (14.44)	–4.44 (–99.90)	–3.75 (–99.98)	–4.42 (–99.82)	–0.52 (–47.71)	–6.72 (–100.00)
24 h	–4.07 (–99.94)	–4.31 (–99.99)	–0.74 (–81.19)	–5.26 (–99.99)	–4.47 (–99.99)	–4.59 (–99.95)	–1.24 (–91.03)	–6.72 (–100.00)

S. pseudintermedius

Negative values indicate a reduction in viable cells and positive values indicate growth.

MPC drug concentration pradofloxacin versus doxycycline at 24 h $P = 0.0009$, cefovecin versus doxycycline $P < 0.0001$.

C_{max} drug concentration at 4 h pradofloxacin versus doxycycline $P = 0.0006$, pradofloxacin versus cefazolin $P = 0$-.0007; 6 h pradofloxacin versus doxycycline $P < 0.0001$, pradofloxacin versus cefazolin $P = 0.0042$; 12 h pradofloxacin versus doxycycline $P < 0.0001$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P = 0.0007$, cefazolin versus doxycycline $P = 0.0005$.

$Tissue_{max}$ drug concentration at 6 h pradofloxacin versus doxycycline $P = 0.0054$; 12 h pradofloxacin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P = 0.0312$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P = 0.0004$, cefazolin versus doxycycline $P = 0.0018$.

E. coli

MIC drug concentration at 6 h pradofloxacin versus cefovecin $P = 0.0288$; 12 h pradofloxacin versus cefovecin $P = 0.0261$, cefovecin versus doxycycline $P = 0.0180$, cefovecin versus cefazolin $P < 0.0001$; 24 h pradofloxacin versus cefovecin $P < 0.0001$, cefovecin versus cefazolin $P < 0.0001$.

C_{max} drug concentration at 1 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$, cefovecin versus cefazolin $P = 0.0386$; 2 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$, cefazolin versus cefovecin $P = 0.0002$; 4 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P = 0.0002$, cefazolin versus doxycycline $P = 0.0001$, cefovecin versus doxycycline $P = 0.0066$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P = 0.0335$, cefazolin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$; 12 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P <$ 0.0001; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$.

$Tissue_{max}$ drug concentration at 0.5 h pradofloxacin versus cefovecin $P = 0.0143$; 1 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$; 2 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$; 4 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0007$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0219$, cefovecin versus doxycycline $P = 0.0015$; 12 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P = 0.0274$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P = 0.0045$.

Exposure of 10^8 cfu/mL of *E. coli* (Table 4) to the MIC drug concentration of the four drugs tested did not yield significant differences in killing between any of the compounds. At the MPC drug concentration, pradofloxacin killed more cells than cefovecin following 4, 6, 12 and 24 h of drug exposure ($P = 0.0004$–0.0441). Pradofloxacin killed more cells than doxycycline at 12 and 24 h of drug exposure ($P = 0.0083$ and 0.0087). Cefazolin killed more cells than doxycycline did at 6 h ($P = 0.0015$) and 12 h ($P = 0.0015$), and cefazolin killed more cells than cefovecin did at 6 h ($P = 0.0497$) and 12 h ($P = 0.0422$) following drug

Table 3. Log_{10} reduction in viable cells (percentage of cells killed) over time for suspensions of *Staphylococcus pseudintermedius and Escherichia coli* (10^7 cfu/mL) exposed to various concentrations of cefazolin, cefovecin, doxycycline and pradofloxacin.

	S. pseudintermedius				*E. coli*			
Variable	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin
Minimum inhibitory concentration (MIC)								
0.5 h	0.07 (19.57)	0.00 (1.00)	–0.02 (–4.39)	0.06 (14.49)	0.08 (4.68)	–0.01 (–2.52)	0.11 (39.07)	–0.05 (–12.15)
1 h	0.09 (25.67)	0.01 (10.52)	–0.03 (–0.89)	0.00 (0.41)	–0.05 (–10.85)	0.00 (–0.88)	0.01 (9.74)	–0.06 (–12.54)
2 h	0.19 (72.56)	–0.01 (2.05)	0.14 (39.74)	0.08 (21.90)	–0.27 (–43.94)	–0.21 (–30.93)	0.09 (22.58)	–0.05 (–9.29)
4 h	0.29 (124.33)	–0.22 (7.75)	–0.03 (–3.76)	–0.07 (–15.11)	–0.08 (76.36)	–0.73 (–62.94)	0.10 (24.58)	0.12 (36.02)
6 h	0.44 (355.49)	–0.12 (–8.43)	–0.06 (–12.01)	–0.20 (–30.13)	–0.16 (177.90)	–0.34 (–51.95)	0.18 (23.70)	0.08 (29.81)
12 h	0.45 (296.69)	–1.11 (–64.79)	0.12 (34.00)	–0.25 (–32.07)	0.71 (905.53)	–0.62 (61.40)	0.34 (127.55)	–0.02 (5.50)
24 h	0.61 (313.62)	–1.14 (–89.32)	0.60 (363.71)	0.00 (2.99)	1.22 (1704.31)	–0.22 (38.34)	–1.44 (149.08)	–0.15 (1.86)
Mutant prevention concentration (MPC)								
0.5 h	0.01 (9.37)	–0.02 (–4.88)	0.00 (0.66)	0.01 (14.77)	–0.48 (–64.62)	–0.07 (–12.58)	0.03 (13.16)	–0.55 (–55.40)
1 h	0.06 (17.33)	–0.10 (–19.07)	–0.04 (–6.68)	–0.10 (–19.26)	–1.57 (–95.04)	–0.05 (–9.96)	–0.04 (–8.11)	–2.17 (–95.07)
2 h	–0.09 (–12.89)	–0.15 (–24.27)	–0.06 (–9.52)	–0.48 (–61.47)	–2.21 (–99.34)	–0.45 (–62.43)	–0.10 (–20.71)	–3.32 (–99.65)
4 h	–0.55 (–63.21)	–0.57 (–33.46)	–0.11 (–10.80)	–1.18 (–87.57)	–3.20 (–99.90)	–1.30 (–92.57)	–0.45 (–63.45)	–3.91 (99.93)
6 h	–1.51 (–90.03)	–1.20 (–65.90)	–0.29 (–46.80)	–1.64 (–93.76)	–3.88 (–99.97)	–2.08 (–98.61)	–0.91 (–86.98)	–4.13 (–99.98)
12 h	–2.40 (–99.52)	–2.48 (–99.35)	–0.34 (–53.56)	–2.36 (–99.31)	–3.75 (–99.71)	–2.73 (–99.53)	–1.32 (–94.94)	–5.08 (–99.97)
24 h	–2.46 (–89.36)	–4.06 (–99.98)	–0.83 (–83.72)	–3.20 (–96.69)	–5.45 (–99.99)	–4.19 (–99.99)	–3.05 (–99.18)	–4.50 (–99.99)
C_{max}								
0.5 h	–0.03 (–2.00)	–0.03 (–13.40)	–0.03 (–18.28)	–0.79 (–82.66)	–0.36 (–55.00)	–0.16 (–29.05)	0.00 (1.16)	–2.30 (–97.04)
1 h	–0.05 (–6.91)	–0.09 (–17.16)	–0.04 (–6.72)	–1.34 (–93.51)	–1.68 (–97.81)	–0.01 (0.94)	–0.12 (–20.57)	–2.74 (–98.95)
2 h	–0.11 (–18.18)	–0.30 (–39.17)	–0.04 (–0.44)	–2.21 (–98.57)	–2.48 (–99.58)	–1.10 (–89.23)	–0.12 (–21.90)	–3.96 (–99.98)
4 h	–0.58 (–65.09)	–0.75 (–47.20)	–0.07 (–8.76)	–2.97 (–99.69)	–3.31 (–99.95)	–2.81 (–99.80)	–0.27 (–44.12)	–4.57 (–99.990
6 h	–1.20 (–91.10)	–1.57 (–90.15)	–0.18 (–31.68)	–3.40 (–99.88)	–3.73 (–99.99)	–3.25 (–99.94)	–0.16 (–41.24)	–5.61 (–99.99)
12 h	–3.11 (–99.91)	–3.49 (–99.91)	0.10 (218.22)	–4.19 (–99.96)	–4.92 (–99.99)	–3.52 (–99.97)	–0.41 (–55.87)	–5.97 (–99.99)
24 h	–4.69 (–99.99)	–4.80 (–99.99)	–1.09 (–91.84)	–5.09 (–99.99)	–5.52 (–99.99)	–5.08 (–99.99)	–0.54 (–70.56)	–7.39 (–100.00)
$Tissue_{max}$								
0.5 h	–0.01 (–24.80)	0.05 (13.45)	0.00 (4.68)	–1.34 (–91.04)	–0.30 (–47.280	–0.11 (–16.45)	–0.07 (–16.31)	–3.39 (–99.95)
1 h	–0.03 (–6.44)	–0.10 (–18.33)	–1.49 (16.01)	–1.67 (–93.69)	–1.44 (–76.60)	–0.12 (–24.52)	–0.09 (–18.65)	–3.81 (–99.98)
2 h	–0.11 (–22.90)	–0.18 (–26.34)	–1.27 (24.71)	–2.25 (–97.58)	–1.89 (–98.30)	–0.63 (–65.78)	–0.08 (–17.10)	–4.03 (–99.98)
4 h	–0.63 (–72.09)	–0.45 (–51.48)	6.40 (25.64)	–3.09 (–99.49)	–2.84 (–99.74)	–2.80 (–99.69)	–0.14 (–26.89)	–5.28 (–99.99)
6 h	–1.43 (–94.36)	–1.44 (–89.46)	0.10 (38.43)	–3.87 (–99.92)	–3.14 (–99.80)	–2.88 (–99.60)	–0.23 (–41.27)	–6.58 (–99.99)
12 h	–2.84 (–99.83)	–2.87 (–99.60	0.01 (4.82)	–4.72 (–99.97)	–2.82 (–99.77)	–3.54 (–99.96)	–0.13 (–25.42)	–7.30 (–100.00)
24 h	–4.42 (–99.99)	–4.23 (–99.99)	–0.70 (–65.65)	–5.78 (–99.99)	–3.03 (–85.93)	–4.61 (–99.93)	–0.45 (–62.30)	–7.79 (–100.00)

S. pseudintermedius

MIC drug concentration following 12 h of drug exposure cefazolin versus cefovecin $P = 0.0116$.

MPC drug concentration pradofloxacin versus doxycycline at 12 h $P = 0.0123$, cefazolin versus doxycycline $P = 0.0105$, cefovecin versus doxycycline $P = 0.0118$; 24 h cefovecin versus doxycycline $P = 0.0013$.

C_{max} drug concentration at 4 h pradofloxacin versus doxycycline $P = 0.0097$; 6 h pradofloxacin versus doxycycline $P = 0.0081$; 12 h pradofloxacin versus doxycycline $P < 0.0001$; cefovecin versus doxycycline $P = 0.0014$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P = 0.0002$, cefazolin versus doxycycline $P = 0.0045$.

$Tissue_{max}$ drug concentration at 6 h pradofloxacin versus doxycycline $P = 0.0001$; 12 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P = 0.0017$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$.

E. coli

C_{max} drug concentration at 0.5 h pradofloxacin versus doxycycline $P = 0.0089$ or cefovecin $P = 0.0051$; 1 h pradofloxacin versus doxycycline $P = 0.0006$ or cefovecin $P < 0.0001$, cefazolin versus doxycycline $P = 0.0007$, cefovecin versus cefazolin $P = 0.0010$; 2 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$, cefazolin versus doxycycline $P = 0.0003$; 4 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0441$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$; 12 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$, cefovecin versus cefazolin $P = 0.0057$; 24 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0008$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus doxycycline $P < 0.0001$.

$Tissue_{max}$ drug concentration at 1 h pradofloxacin versus doxycycline $P = 0.0167$ or cefovecin $P = 0.0172$; 2 h pradofloxacin versus doxycycline $P = 0.0021$; 4 h pradofloxacin versus doxycycline $P < 0.0001$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0264$; 12 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0$-.0056, cefovecin versus doxycycline $P = 0.0198$; 24 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P = 0.0002$.

exposure. At the C_{max} drug concentration, pradofloxacin killed more cells than doxycycline did following 1 h ($P = 0.0020$), 2 h ($P = 0.0002$), 4 h ($P < 0.0001$), 6 h ($P < 0.001$), 12 h ($P < 0.001$) and 24 h ($P < 0.0001$) of drug exposure. At the $Tissue_{max}$ drug concentration, pradofloxacin killed more cells than doxycycline did following 2, 4, 6, 12 and 24 h of drug exposure (*P*-values from <0.0001 to 0.0336). No other comparisons were statistically significant for differences in bacterial killing.

No significant differences in kill were seen between any of the investigated compounds when 10^9 cfu/mL were exposed to the MIC or MPC drug concentrations of the four agents tested (Table 5). At the C_{max} drug

Table 4. Log_{10} reduction in viable cells (percentage of cells killed) over time for suspensions of *Staphylococcus pseudintermedius* and *Escherichia coli* (10^8 cfu/mL) exposed to various concentrations of cefazolin, cefovecin, doxycycline and pradofloxacin.

	S. pseudintermedius				*E. coli*			
Variable	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin
Minimum inhibitory concentration (MIC)								
0.5 h	0.00 (0.47)	0.01 (4.02)	0.04 (8.37)	–0.03 (–3.29)	–0.04 (–7.15)	–0.03 (5.79)	0.08 (34.16)	0.08 (21.68)
1 h	–0.01 (–1.30)	0.09 (25.82)	0.05 (11.210	0.01 (–4.83)	–0.17 (–27.56)	0.07 (23.33)	0.02 (13.14)	0.18 (54.39)
2 h	0.06 (15.51)	–0.01 (–0.16)	–0.01 (–0.66)	–0.05 (–8.30)	–0.14 (–25.14)	–0.06 (–8.31)	–0.0 (12.91)	0.09 (25.43)
4 h	0.13 (36.57)	0.07 (20.02)	0.07 (17.27)	0.07 (18.76)	–0.04 (23.27)	0.08 (26.12)	0.14 (43.25)	0.15 (43.49)
6 h	0.11 (32.13)	–0.08 (–12.33)	0.11 (32.85)	0.02 (3.68)	0.03 (24.48)	–0.15 (–27.88)	0.23 (80.77)	0.11 (32.32)
12 h	0.11 (27.75)	–0.07 (–27.92)	0.12 (34.16)	–0.02 (–3.56)	0.37 (176.68)	0.00 (3.16)	0.20 (63.02)	0.10 (31.13)
24 h	–0.09 (–15.89)	0.29 (424.16)	0.17 (50.35)	–0.12 (–16.37)	0.37 (140.46)	–0.18 (–32.09)	0.16 (57.97)	–0.01 (3.87)
Mutant prevention concentration (MPC)								
0.5 h	–0.01 (–1.73)	–0.02 (–5.08)	0.04 (10.62)	–0.05 (–11.27)	–0.45 (–60.34)	–0.13 (–24.48)	0.11 (32.19)	–0.53 (–64.26)
1 h	0.03 (6.13)	–0.14 (–25.10)	0.11 (31.94)	–0.01 (–1.37)	–1.33 (–90.58)	–0.01 (–0.30)	–0.01 (–0.06)	–1.25 (–80.57)
2 h	–0.02 (–3.84)	0.02 (13.22)	0.03 (9.61)	–0.11 (–21.50)	–2.01 (–98.60)	–0.05 (4.89)	0.09 (54.88)	–1.99 (–92.73)
4 h	–0.11 (–19.17)	0.01 (1.59)	0.06 (24.79)	–0.23 (–37.70)	–2.61 (–99.54)	–0.26 (–43,26)	–0.18 (–31.46)	–2.11 (–96.13)
6 h	–0.20 (–29.09)	0.11 (31.80)	0.07 (21.17)	–0.16 (–31.62)	–2.95 (–99.81)	–0.34 (–47.76)	–0.23 (–29.18)	–2.47 (–98.15)
12 h	–0.24 (–32.59)	–0.08 (–15.06)	0.06 (15/53)	–0.09 (–15.57)	–3.17 (–99.85)	0.00 (3.26)	–0.50 (–57.47)	–2.92 (–99.57)
24 h	–0.67 (–13.93)	–0.40 (–58.38)	–0.47 (–60.08)	–0.31 (–47.77)	–1.44 (–85.53)	–0.02 (–3.37)	–0.59 (–64.83)	–3.10 (–99.65)
C_{max}								
0.5 h	0.02 (7.93)	–0.02 (–3.27)	0.01 (0.74)	–0.50 (–66.46)	–0.31 (–45.13)	–0.14 (–22.37)	–0.12 (–23.56)	–2.07 (–98.45)
1 h	0.02 (4.84)	–0.06 (–12.95)	–0.03 (–7.48)	–0.82 (–83.62)	–1.12 (–92.19)	–0.17 (–30.03)	–0.09 (–17.05)	–3.10 (–99.88)
2 h	0.03 (10.71)	–0.14 (–30.35)	–0.13 (–24.48)	–1.39 (–94.44)	–2.20 (–99.20)	–0.64 (–74.22)	0.02 (6.40)	–3.59 (–99.97)
4 h	–0.10 (–9.71)	–0.10 (–19.20)	–0.01 (–2.76)	–1.72 (–96.11)	–2.51 (–99.64)	–0.83 (–78.70)	–0.09 (–13.50)	–3.87 (–99.98)
6 h	–0.12 (–20.47)	–0.07 (–41.19)	0.03 (–5.94)	–1.82 (–96.22)	–2.67 (–99.75)	–1.52 (–94.16)	–0.02 (10.78)	–4.09 (–99.97)
12 h	–0.40 (–51.29)	–0.16 (–24.48)	0.02 (7.07)	–1.61 (–92.52)	–2.46 (–99.11)	–2.56 (–99.52)	0.02 (11.00)	–4.81 (–99.99)
24 h	–1.45 (80.42)	–0.34 (–38.86)	0.15 (6.44)	–2.40 (–98.26)	–1.52 (–88.19)	–4.54 (–99.99)	–0.04 (7.54)	–5.51 (–99.99)
$Tissue_{max}$								
0.5 h	–0.06 (–16.35)	0.00 (4.47)	0.00 (–0.73)	–1.08 (–94.30)	–0.27 (–39.99)	–0.23 (–40.36)	0.03 (52.73)	–2.76 (–90.85)
1 h	–0.01 (–1.33)	–0.02 (–1.27)	0.03 (6.56)	–1.47 (–97.86)	–0.89 (–61.19)	–0.19 (–16.64)	–0.10 (8.07)	–3.20 (–99.94)
2 h	–0.09 (–14.91)	–0.04 (–6.15)	–0.12 (–23.94)	–1.79 (–98.82)	–1.93 (–97.42)	–0.61 (–62.96)	0.00 (6.38)	–3.65 (–99.99)
4 h	–0.15 (–27.75)	–0.39 (–030.35)	0.04 (15.59)	–2.12 (–99.33)	–2.34 (–99.15)	–1.36 (–84.62)	0.05 (38.98)	–3.98 (–99.96)
6 h	–0.26 (–37.79)	–0.17 (–27.77)	–0.01 (0.78)	–2.27 (–99.54)	–2.61 (–99.20)	–1.01 (–80.31)	–0.04 (53.83)	–4.44 (–99.98)
12 h	–0.90 (–57.97)	–0.18 (–30.02)	0.03 (9.40)	–2.06 (–99.29)	–2.21 (–98.27)	–2.34 (–95.97)	0.36 (229.89)	–4.81 (–99.99)
24 h	–1.72 (–57.91)	–1.51 (–85.59)	–0.02 (–3.47)	–1.96 (–99.12)	–1.25 (–35.85)	–3.74 (–96.02)	0.02 (56.97)	–6.54 (–99.99)

E. coli

MPC drug concentration at 4 h pradofloxacin versus cefovecin $P = 0.0441$; 6 h pradofloxacin versus cefovecin $P = 0.0392$, cefazolin versus doxycycline $P = 0.0015$, cefazolin versus cefovecin $P = 0.0497$; 12 h pradofloxacin versus doxycycline $P = 0.0087$ or cefovecin $P = 0.0039$, cefazolin versus doxycycline $P = 0.0015$, cefovecin versus cefazolin $P = 0.0422$; 24 h pradofloxacin versus doxycycline $P = 0.0083$ or cefovecin $P = 0.0004$.

C_{max} drug concentration at 1 h pradofloxacin versus doxycycline $P = 0.0020$ or cefovecin $P = 0.0012$; 2 h pradofloxacin versus doxycycline $P = 0.0002$ or cefovecin $P = 0.0011$, cefazolin versus doxycycline $P = 0.0347$; 4 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0010$, cefazolin versus doxycycline $P = 0.0045$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P = 0.0011$, cefazolin versus doxycycline $P = 0.0005$; 12 h pradofloxacin versus doxycycline $P < 0.0001$, cefovecin versus doxycycline $P < 0.017$; cefazolin versus doxycline $P = 0.0308$; 24 h pradofloxacin versus doxycycline $P < 0.001$ or cefazolin $P < 0.0001$, cefovecin versus doxycycline $P < 0.0001$, cefazolin versus cefovecin $P = 0.0016$.

$Tissue_{max}$ drug concentration at 2 h pradofloxacin versus doxycycline $P = 0.0336$; 4 h pradofloxacin versus doxycycline $P = 0.0086$; 6 h pradofloxacin versus doxycycline $P = 0.0074$; 12 h pradofloxacin versus doxycycline $P = 0.0001$; 24 h pradofloxacin versus doxycycline $P < 0.0001$.

concentration pradofloxacin killed more cells following 4 h of drug exposure than cefovecin did ($P = 0.0028$). At 6, 12 and 24 h following drug exposure, pradofloxacin killed more cells than doxycycline, cefovecin and cefazolin (*P*-values were at <0.0001 for all comparisons). At the $Tissue_{max}$ drug concentration, pradofloxacin killed more cells than doxycycline, cefovecin or cefazolin (growth) following 2, 4, 6, 12 and 24 h of drug exposure (*P*-values were <0.0001 for all comparisons).

Discussion

Previous reviews[20,21] included the use of 1st and 3rd generation cephalosporins, fluoroquinolones and tetracyclines (doxycycline) for the treatment of canine skin infections and, as such, were appropriate to investigate in this report and our previous study.[7] Cefovecin was shown to be as effective as amoxicillin/clavulanic acid for the treatment of skin infections in dogs,[22] and pradofloxacin was shown to be efficacious for the treatment of superficial and deep pyoderma in dogs.[23] A previous study reported on the bactericidal properties of pradofloxacin against veterinary pathogens including *S. pseudintermedius* and *E. coli* canine strains.[24] Another study reported on the *in vitro* activity of cefovecin against *S. pseudintermedius* and *E. coli* strains with MIC_{90} values of 0.25 μg/mL and 1 μg/mL, respectively.[25] The bactericidal activity of cefovecin also has been reported previously.[26] Previous studies[27] have reported on the bactericidal activity of cefazolin and commented on the

Table 5. Log_{10} reduction in viable cells (percentage of cells killed) over time for suspensions of *Staphylococcus pseudintermedius* and *Escherichia coli* (10^9 cfu/mL) exposed to various concentrations of cefazolin, cefovecin, doxycycline and pradofloxacin.

	S. pseudintermedius				*E. coli*			
Variable	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin	Cefazolin	Cefovecin	Doxycycline	Pradofloxacin
Minimum inhibitory concentration								
0.5 h	–0.01 (–1.30)	–0.04 (–0.74)	–0.05 (–11.41)	–0.05 (–5.55)	–0.11 (–20.93)	–0.11 (–21.92)	–0.06 (–7.10)	–0.07 (–13.40)
1 h	0.00 (–0.16)	–0.05 (–0.31)	–0.01 (–0.47)	–0.13 (–25.66)	–0.09 (–15.90)	0.04 (11.99)	0.06 (16.70)	–0.02 (–4.80)
2 h	0.00 (–0.49)	0.06 (27.44)	–0.03 (–3.11)	0.08 (1.68)	–0.05 (–11.88)	–0.05 (–10.27)	0.14 (39.75)	–0.03 (–5.86)
4 h	0.07 (17.49)	0.10 (48.24)	–0.09 (–17.05)	0.01 (3.15)	–0.09 (–10.16)	–0.25 (–40.82)	0.38 (166.05)	–0.01 (0.06)
6 h	0.04 (10.66)	–0.01 (11.90)	–0.07 (–12.51)	–0.15 (–24.79)	0.13 (34.86)	–0.15 (–12.64)	0.33 (179.36)	–0.03 (–4.00)
12 h	0.37 (211.77)	0.14 (1.34)	–0.30 (–33.03)	0.05 (13.65)	0.23 (73.20)	–0.10 (4.10)	0.13 (46.85)	–0.10 (–20.24)
24 h	–0.05 (–11.48)	–0.07 (–37.68)	–0.11 (15.89)	–0.13 (–24.28)	–0.04 (–8.02)	–0.18 (–30.73)	0.18 (63.01)	–0.13 (–10.99)
Mutant Prevention concentration								
0.5 h	–0.03 (–2.53)	0.14 (48.09)	–0.05 (–2.87)	0.00 (2.69)	–0.02 (–3.03)	–0.04 (–5.85)	0.16 (49.33)	0.18 (58.26)
1 h	–0.03 (–5.22)	0.22 (78.10)	–0.04 (–5.64)	–0.05 (–9.43)	–0.22 (–36.94)	0.13 (48.57)	–0.01 (–2.02)	0.16 (48.53)
2 h	0.00 (3.07)	0.15 (50.59)	–0.03 (0.28)	–0.03 (–3.47)	–0.08 (–13.48)	–0.09 (–18.80)	0.09 (37.83)	0.11 (32.76)
4 h	0.00 (–1.72)	0.20 (71.48)	–0.04 (–4.37)	–0.13 (–15.41)	–0.13 (–20.95)	–0.02 (–2.75)	0.05 (13.00)	–0.07 (–29.66)
6 h	–0.05 (–2.41)	0.18 (56.50)	–0.05 (–10.81)	0.03 (10.49)	0.21 (244.33)	–0.05 (0.55)	–0.07 (–12.38)	–0.14 (–12.01)
12 h	0.08 (–21.51)	0.15 (42.71)	–0.27 (–22.52)	–0.05 (–6.55)	0.09 (30.63)	0.14 (46.57)	–0.04 (1.39)	–0.16 (–21.60)
24 h	–0.15 (–19.57)	0.05 (33.26)	–0.08 (–13.82)	–0.15 (–22.26)	0.05 (15.37)	–0.06 (–12.17)	–0.08 (–15.63)	–0.44 (–59.93)
C_{max}								
0.5 h	0.05 (20.42)	0.01 (–0.17)	–0.02 (–0.27)	–0.09 (–16.70)	0.06 (14.45)	0.00 (4.30)	0.12 (37.69)	–0.48 (–61.12)
1 h	0.06 (15.06)	–0.14 (–21.34)	0.09 (27.08)	–0.15 (–26.11)	0.03 (10.07)	0.11 (49.33)	0.06 (18.09)	–0.63 (–67.51)
2 h	0.09 (24.65)	–0.01 (–4.07)	0.03 (10.62)	–0.20 (–33.97)	0.03 (11.66)	0.00 (6.64)	0.14 (51.59)	–1.26 (–93.04)
4 h	0.05 (20.74)	0.06 (20.06)	0.04 (11.97)	–0.40 (–56.04)	0.20 (91.82)	0.10 (26.28)	–0.05 (–9.90)	–1.97 (–98.48)
6 h	0.06 (14.76)	0.04 (9.16)	–0.09 (–11.99)	–0.60 (–65.82)	0.07 (19.63)	0.20 (62.89)	–0.04 (–5.76)	–2.83 (–99.80)
12 h	0.15 (41.35)	–0.03 (–4.26)	–0.01 (14.52)	–0.29 (–38.10)	0.15 (55.44)	0.26 (100.51)	0.14 (38.49)	–3.08 (–99.85)
24 h	0.09 (24.00)	–0.04 (4.92)	0.02 (7.55)	–1.04 (–88.54)	0.01 (23.95)	0.10 (57.90)	–0.15 (–11.64)	–3.58 (–99.93)
$Tissue_{max}$								
0.5 h	–0.04 (–8.21)	0.00 (18.55)	0.04 (14.59)	–0.30 (–47.54)	–0.10 (–19.87)	–0.08 (–24.16)	0.08 (20.01)	–0.87 (–85.73)
1 h	–0.02 (–3.91)	–0.05 (13.93)	0.04 (15.76)	–0.65 (–70.34)	–0.10 (–18.48)	–0.09 (–17.22)	0.00 (0.91)	–1.40 (–94.79)
2 h	–0.05 (–10.73)	–0.03 (9.38)	0.06 (22.88)	–0.91 (–85.87)	0.10 (28.61)	–0.06 (–12.95)	0.13 (36.43)	–2.20 (–99.66)
4 h	0.05 (13.21)	–0.16 (–2.88)	0.09 (29.31)	–0.86 (–77.41)	0.00 (1.51)	–0.11 (–21.61)	0.05 (13.05)	–2.98 (–99.94)
6 h	–0.08 (–17.02)	–0.11 (5.43)	0.11 (54.44)	–1.06 (–82.32)	0.11 (33.71)	–0.18 (–29.80)	0.02 (5.95)	–3.50 (–99.98)
12 h	0.06 (17.82)	–0.11 (3.82)	0.33 (101.77)	–0.74 (–64.43)	0.12 (49.27)	–0.12 (–23.42)	–0.08 (39.97)	–4.00 (–99.99)
24 h	–0.13 (–24.67)	–0.16 (0.57)	0.02 (6.03)	–1.30 (93.90)	0.04 (16.09)	–0.18 (–31.86)	0.06 (20.05)	–4.45 (–99.99)

E. coli

C_{max} drug concentration at 4 h pradofloxacin versus cefovecin $P = 0.0028$; 6 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 12 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 24 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$.

$Tissue_{max}$ drug concentration at 2 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 4 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 6 h pradofloxacin versus $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 12 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$; 24 h pradofloxacin versus doxycycline $P < 0.0001$ or cefovecin $P < 0.0001$ or cefazolin $P < 0.0001$.

tetracyclines being bacteriostatic.[28] The previous determination of bactericidal or bacteriostatic concentrations for the various drugs tested is consistent with the findings in this study. Doxycycline is recommended in humans for staphylococcal and streptococcal bacterial strains and skin structure infections,[29] yet randomized control trials in dogs are unavailable.[21] Likewise, although cefazolin (and cefalexin) have been investigated for treatment of skin and skin structure infections in humans[30,31], randomized controlled trials are unavailable in companion animals.[21] The pharmacokinetics of cefazolin for prophylactic administration in dogs has been studied.[32] Comparing antibiotics for bactericidal versus bacteriostatic activity as well as speed of kill has clinical relevance, as commented by others.[33,34]

In this report, we performed kill measurements using the same strains as in our previous report and the same clinically relevant drug concentrations. The major differences between this report and our previous publication were the time and intervals over which killing occurred (i.e. 3 h versus 24 h) and the densities of bacteria used in the assays. The varying bacterial densities are important to include as the densities of bacteria present during infection (6, 7, 8, 9, 10, 11) have been shown to exceed 10^5 cfu/mL, and testing drugs against the higher bacterial densities helps to effectively kill cells with reduced susceptibility, as has been shown to occur in bacterial densities $>10^7$ cfu/mL. These differences have been argued previously with MIC versus MPC testing.[7,8,35,36]

Cefazolin, cefovecin and pradofloxacin are all considered bactericidal agents, whereas doxycycline is considered bacteriostatic based on the classical definition.[5,6] The definition of bactericidal versus bacteriostatic is problematic as it is based on a standard bacterial inoculum of 10^5 cfu/mL and does not appear to have relevance when higher bacterial densities are tested. Additionally, the differentiation of bactericidal from bacteriostatic drugs based on a >3 log_{10} reduction in viable cells versus <2

log_{10} reduction (respectively) is arbitrary. In previous work from our laboratory, agents traditionally considered bacteriostatic showed bactericidal properties when tested against higher bacterial densities.[6] Having said that, in this study, doxycycline displayed bacteriostatic properties regardless of bacterial densities and/or drug concentrations tested; cefazolin, cefovecin and pradofloxacin were bactericidal over the densities tested and with killing more pronounced at MPC, C_{max} and $Tissue_{max}$ drug concentrations for all three drugs. For cefazolin and cefovecin, longer times of drug exposure were needed to achieve substantial reductions in viable cells and this is consistent with time-dependent drugs.

As reported previously, the drug concentration used in this study was from published reports or estimated from published reports.[18,19,26,37,38] Interestingly, for *E. coli* at the 10^6–10^8 cfu/mL densities and C_{max} drug concentrations, statistically more cells were killed by pradofloxacin than by doxycycline and cefovecin within the first 1–4 h of drug exposure and in most instances, these differences were seen over the 24 h of drug exposure. Statistically significant differences with any comparisons were not seen until 4 h of drug exposure at the 10^9 cfu/mL density and thereafter at the 6, 12 and 24 h samplings. Statistically significant differences also were seen between agents at the $Tissue_{max}$, MPC and MIC drug concentrations depending on the density of bacteria and the time after drug exposure sampling.

Overall, but not exclusively, statistically significant differences in kill were seen more often between doxycycline and cefazolin, cefovecin or pradofloxacin than between cefazolin, cefovecin and pradofloxacin for *S. pseudintermedius*. At the C_{max} and $Tissue_{max}$ drug concentrations, statistically significant differences were seen between pradofloxacin and doxycycline at earlier sampling times (i.e. 4–6 h) but by 12–24 h following drug exposure, differences also were seen for cefazolin and cefovecin compared to doxycycline at the 10^6 cfu/mL and 10^7 cfu/mL densities. At the MPC drug concentrations, statistically significant differences were not observed between any comparisons until 12–24 h following drug exposure at the 10^6–10^7 cfu/mL densities.

In this and our previous report,[7] we showed that killing of *S. pseudintermedius* and *E. coli* strains was different for the four drugs tested and did vary based on the bacterial density and time following drug exposure – generally being statistically different for pradofloxacin with short drug exposure times. A limitation of this and similar studies is that drug concentration remains constant over the duration of the measurements (i.e. 24 h in this report) and as such, does not truly reflect *in vivo* drug dynamics where drug elimination occurs; drug degradation over time was not measured. In addition, measurements were not corrected for protein binding; Dalhoff showed that high protein binding is associated with reduced antimicrobial activity,[39] but protein binding <80–85% appears to be of slight clinical importance.[40] Regardless, measurements as reported herein do allow for comparisons between drugs under controlled conditions which may be important clinically. Those observations are consistent with our previous report showing faster killing with pradofloxacin and also are consistent with cefazolin and cefovecin being time-dependent antibiotics and pradofloxacin being a concentration-dependent drug.

In human medicine, the overall trend has been toward shorter duration of antimicrobial therapy for uncomplicated infections, an approach not considered inferior to longer durations of therapy.[4,41–44] In four guideline publications for recommended therapies for companion animal infections,[21,45–47] longer durations of therapy are recommended with the acknowledgement that shorter durations of therapy might be possible but for data being limited or unavailable. Longer duration of therapy may be necessary for chronic infections where biofilm formation may be a contributing factor.[48]

Determining effective durations of therapy involves clinical investigation supplemented with *in vitro* data. As such, data as reported here and in our previous report add to the *in vitro* data showing differences and similarities between compounds for killing or inhibition of clinically important pathogens. Such data may contribute to decisions related to therapeutic choices and duration of therapy.

Acknowledgements

We thank Deb Hills for excellent clerical assistance and Terry Settje for performing the statistical analysis.

References

1. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. *Clin Infect Dis* 2004; 38: 864–870.
2. Nemeth J, Oesch G, Kuster SP. Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: systematic review and meta-analysis. *J Antimicrob Chemother* 2015; 70: 382–395.
3. Finberg RW, Moellering RC Jr, Tally FP, et al. The importance of bactericidal drugs: future directions in infectious disease. *Clin Infect Dis* 2004; 39: 1,314–1,320.
4. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Disease Society of America/American Thoracic Society Consensus Guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; 44 (Suppl 2): S27–S72.
5. Boswell FJ, Andrews JM, Wise R, et al. Bactericidal properties of moxifloxacin and post-antibiotic effect. *J Antimicrob Chemother* 1999; 43: 43–49.
6. Blondeau JM, Shebelski SD, Hesje CK. Killing of *Streptococcus pneumoniae* by azithromycin, clarithromycin, erythromycin, telithromycin and gemifloxacin using drug minimum inhibitory concentrations and mutant prevention concentrations. *Int J Antimicrob Agents* 2015; 45: 594–599.
7. Blondeau JM, Shebelski SD. Comparative *in vitro* killing of canine strains of *Staphylococcus pseudintermedius* and *Escherichia coli* by cefovecin, cefazolin, doxycycline and pradofloxacin. *Vet Dermatol* 2016; 27: 267–e63.
8. Blondeau JM, Hansen GT, Metzler KL, et al. The role of PK/PD parameters to avoid selection and increase of resistance: mutant prevention concentration. *J Chemother* 2004; 16: 1–19.
9. Feldman W. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. *J Pediatr* 1976; 88: 549–552.
10. Bingen E, Lambert-Zechovsky N, Mariani-Kurkdjian P, et al. Bacterial counts in cerebrospinal fluid of children with meningitis. *Eur J Clin Microbiol Infect Dis* 1990; 9: 278–281.
11. Frisch AW, Tripp JT, Barrett CD Jr, et al. The specific polysaccharide content of pneumonic lungs. *J Exp Med* 1942; 76: 505–510.

12. Coulthard MG, Kalra M, Lambert HJ, et al. Redefining urinary tract infections by bacterial colony counts. *Pediatrics* 2010; 125: 335–341.
13. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard – 10th ed. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
14. Blondeau JM, Zhao X, Hansen GT, et al. Mutant prevention concentrations (MPC) of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2001; 45: 433–438.
15. Blondeau JM, Borsos S, Blondeau LD, et al. Comparative minimum inhibitory and mutant prevention drug concentrations of enrofloxacin, ceftiofur, florfenicol, tilmicosin and tulathromycin against bovine isolates of *Mannheimia haemolytica*. *Vet Microbiol* 2012; 160: 85–90.
16. Blondeau JM, Hansen GT, Metzler KL, et al. Optimal killing of *Streptococcus pneumoniae* by gemifloxacin, levofloxacin and moxifloxacin. In: Gillespie SH, Tillotson GS eds. *Novel Perspectives in Antibacterial Action*. London: Royal Society of Medicine Press Ltd, 2002; 15–26.
17. Blondeau JM, Blondeau LD, Hesje C, et al. Application of two methods to determine killing of *Streptococcus pneumoniae* by various fluoroquinolones. *J Chemother* 2006; 18: 366–372.
18. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. *J Antimicrob Chemother* 2006; 58: 256–265.
19. Roberts JA, Lipman J. Tissue distribution of beta-lactam antibiotics: continuous versus bolus dosing. *J Pharm Pract Res* 2009; 39: 219–222.
20. Beco L, Guaguere E, Lorente Mendez C, et al. Suggested guidelines for using systemic antimicrobials in bacterial skin infections: part 2- antimicrobial choice, treatment regimens and compliance. *Vet Rec* 2013; 172: 156–160.
21. Hillier A, Lloyd DH, Weese JS, et al. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). *Vet Dermatol* 2014; 25: 163–e43.
22. Stegemann MR, Coati N, Passmore CA, et al. Clinical efficacy and safety of cefovecin in the treatment of canine pyoderma and wound infections. *J Small Anim Pract* 2007; 48: 378–386.
23. Restrepo C, Ihrke PJ, White SD, et al. Evaluation of the clinical efficacy of pradofloxacin tablets for the treatment of canine pyoderma. *J Am Anim Hosp Assoc* 2010; 46: 301–311.
24. Silley P, Stephan B, Greife HA, et al. Bactericidal properties of pradofloxacin against veterinary pathogens. *Vet Microbiol* 2012; 157: 106–111.
25. Stegemann MR, Passmore CA, Sherington J, et al. Antimicrobial activity and spectrum of cefovecin, a new extended- spectrum cephalosporin, against pathogens collected from dogs and cats in Europe and North America. *Antimicrob Agents Chemother* 2006; 50: 2,286–2,292.
26. Stegemann MR, Sherington J, Blanchflower S. Pharmacokinetics and pharmacodynamics of cefovecin in dogs. *J Vet Pharmacol Therap* 2006; 29: 501–511.
27. Bergeron MG, Brusch JL, Barza M, et al. Bactericidal activity and pharmacology of cefazolin. *Antimicrob Agents Chemother* 1973; 4: 396–401.
28. Maaland MG, Papich MG, Turnidge J, et al. Pharmacodynamics of doxycycline and tetracycline against *Staphylococcus pseudintermedius*: proposal of canine-specific breakpoints for doxycycline. *J Clin Microbiol* 2013; 51: 3,547–3,554.
29. Pulido-Cejudo A, Guzman-Gutierrez M, Jalife-Montano A, et al. Management of acute bacterial skin and skin structure infections with a focus on patients at high risk of treatment failure. *Ther Adv Infect Dis* 2017; 4: 143–161.
30. Chan JC. Ampicillin/sulbactam versus cefazolin or cefoxitin in the treatment of skin and skin-structure infections of bacterial etiology. *Adv Ther* 1995; 12: 139–146.
31. Giordano PA, Elston D, Akinlade BK, et al. Cefdinir vs. cephalexin for mild to moderate uncomplicated skin and skin structure infections in adolescents and adults. *Curr Med Res Opin* 2006; 22: 2,419–2,428.
32. Gonzalez OJ, Renberg WC, Roush JK, et al. Pharmacokinetics of cefazolin for prophylactic administration to dogs. *Am J Vet Res* 2017; 78: 695–701.
33. Johnson CC. *In vitro* testing: correlations of bacterial susceptibility, body fluid levels and effectiveness of antibacterial therapy. In: Lorian V ed. *Antibiotics in Laboratory Medicine*. 4th edition. Baltimore, MD: Williams & Wilkins, 1996; 813–834.
34. Craig WA. Qualitative susceptibility tests versus quantitative MIC tests. *Diagn Microbiol Infect Dis* 1993; 16: 231–236.
35. Blondeau JM. New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. *Vet Dermatol* 2009; 20: 383–396.
36. Blondeau JM, Shebelski SD, Hesje CK. Bactericidal effects of various concentrations of enrofloxacin, florfenicol, tilmicosin phosphate, and tulathromycin on clinical isolates of *Mannheimia haemolytica*. *Am J Vet Res* 2015; 76: 860–868.
37. Aucoin D. *TARGET: The Antimicrobial Reference Guide to Effective Treatment*. 5th edition. Port Huron, MI: North American Compendiums Inc., 2015; 68–71, 86–87.
38. Jain JG, Housman ST, Nicolau DP. Humanized tissue pharmacodynamics of cefazolin against commonly isolated pathogens in skin and skin structure infections. *J Antimicrob Chemother* 2014; 69: 2,443–2,447.
39. Dalhoff A. Seventy-five years of research on protein binding. *Antimicrob Agents Chemother* 2018; 62: e01663-17.
40. Scheife RT. Protein binding: what does it mean? *DICP* 1989; 23 (Suppl 7-8) : S27–31.
41. Vogel T, Verreault R, Gourdeau M, et al. Optimal duration of antibiotic therapy for uncomplicated urinary tract infection in older women: a double-blind randomized controlled trial. *Can Med Assoc J* 2004; 170: 469–473.
42. Gupta K, Hooton TM, Naber KG, et al. International Clinical Practice Guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis* 2011; 52: e103–e120.
43. el Moussaoui R, Opmeer BC, de Borgie CAJM, et al. Long-term symptom recovery and health-related quality of life in patients with mild-to-moderate-severe community-acquired pneumonia. *CHEST Journal* 2006; 130: 1,165–1,172.
44. Anzueto A, Miravitlles M. Short-course fluoroquinolone therapy in exacerbations of chronic bronchitis and COPD. *Respir Med* 2010; 104: 1,396–1,403.
45. Lappin MR, Blondeau JM, Booth D, et al. Antimicrobial use Guidelines for Treatment of Respiratory Tract Disease in Dogs and Cats: Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases. *J Vet Intern Med* 2017; 31: 163–e43.
46. Weese JS, Blondeau JM, Boothe DM, et al. Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. *Vet Med Int* 2011; 263768.
47. Weese JS, Blondeau J, Boothe D, et al. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. *Vet J* 2019; 247: 8–25.
48. Johani K, Malone M, Jensen SO, et al. Evaluation of short exposure times of antimicrobial wound solutions against microbial biofilms: from *in vitro* to *in vivo*. *J Antimicrob Chemother* 2018; 73: 494–502.
49. Restrepo C, Ihrke PJ, White SD, et al. The clinical efficacy and concentration time course of pradofloxacin in the skin and serum of dogs with and without pyoderma. *Vet Dermatol* 2008; 19: 111 (abstract).

50. Restrepo C. Tissue concentrations in canine pyoderma: does it reach high enough directly in skin? 2nd International Veraflox Symposium, Rome, Italy, 2012. Available at: https://www.veraflox.com/static/documents/Veraflox_2012_proceed_MediaAss_print_v2.pdf. Accessed Dec 3, 2019.

Résumé

Contexte – Les densités bactériennes fluctuent probablement au cours d'une infection et pourraient dépasser les densités bactériennes utilisées dans les tests de sensibilité. Ainsi, les recherches de bactéricidie par les antibiotiques sur une variété de densités bactériennes moyennes pourraient montrer d'importantes différences entre les composés et pourraient impacter le choix des molécules pour le traitement.

Hypothèses/Objectifs – Evaluer la bactéricidie sur des souches bactériennes de *Staphylococcus pseudintermedius* et *Escherichia coli* par la céfazoline, la céfovécine, la doxycycline et la pradofloxacine à des concentrations (minimales inhibitrices, de prévention des mutants, sériques maximales et tissulaires maximales) cliniquement importantes contre plusieurs densités bactériennes.

Méthodes et matériels – Les souches bactériennes prélevées de chiens avec infection urinaire ont été étudiées. Les densités bactériennes élevées allant de 10^6 à 10^9 cfu (colony forming units)/mL étaient exposées aux concentrations minimales inhibitrices, de prévention des mutant, tissulaires et sanguines des traitements et les pourcentages (log^{10}) de cellules viables après 30 min, 1, 2, 4, 6, 12 et 24 h d'exposition au traitement ont été quantifiés.

Résultats – La doxycycline a montré des propriétés bactériostatiques avec moins de mort que les trois autres agents. Par exemple, à la densité de 10^7 cfu/mL de *S. pseudintermedius*, plus de cellules étaient tuées par la pradofloxacine ($P < 0.0001$) et la céfovécine ($P = 0.0014$) mais pas par la céfazoline quand comparé à la doxycycline à la concentration sérique maximale de la doxycycline suivant 12h d'exposition au traitement.

Conclusions et importance clinique – Les différences étaient observées entres les molécules pour le temps et la durée de bactéricidie; ceci pourrait être cliniquement important et pourrait avoir un impact sur la sélection des molécules et la durée du traitement.

Resumen

Introducción – la densidad bacteriana probablemente fluctúa durante la infección y puede exceder la densidad bacteriana utilizada en las pruebas de susceptibilidad. Como tal, la investigación de la destrucción bacteriana por antibióticos en un rango de densidades bacterianas variables puede proporcionar diferencias importantes entre los compuestos y podría afectar la selección de fármacos para la terapia.

Hipótesis/Objetivos – valorar la destrucción de cepas clínicas de *Staphylococcus pseudintermedius* y *Escherichia coli* por cefazolina, cefovecina, doxiciclina y pradofloxacina a concentraciones de fármaco clínicamente relevantes (inhibición mínima, prevención de mutantes, máximo en suero y tejido) contra diferentes densidades de bacterias.

Métodos y materiales – Se estudiaron cepas bacterianas recolectadas de perros con infecciones del tracto urinario. se expusieron altas densidades bacterianas de 10^6 a 10^9 unidades formadoras de colonias (ufc/ml) a concentraciones mínimas inhibidoras, de prevención de mutantes, y concentración de sangre y tejidos, y se cuantificaron los porcentajes (log_{10}) de células viables destruidas después de 30 minutos, 1, 2, 4, 6, 12 y 24 h de exposición al fármaco.

Resultados – la doxiciclina exhibió propiedades bacteriostáticas con menos destrucción que los otros tres agentes. Por ejemplo, a una densidad de 10^7 ufc/ml de *S. pseudintermedius*, se destruyeron más células por pradofloxacina ($P < 0{,}0001$) y cefovecina ($P = 0{,}0014$) pero no cefazolina en comparación con doxiciclina a la concentración máxima de fármaco en suero que por doxiciclina después 12 h de exposición al fármaco.

Conclusiones e importancia clínica – se observaron diferencias entre algunos medicamentos en la velocidad y el porcentaje de la destrucción bacteriana; esto podría ser clínicamente importante y afectar a la selección de medicamentos y la duración de la terapia.

Zusammenfassung

Hintergrund – Die Bakteriendichte fluktuiert mit großer Wahrscheinlichkeit während einer Infektion und könnte die bakterielle Dichte, die für Empfindlichkeitstests verwendet wird, übertreffen. Aus diesem Grund könnte die Untersuchung vom Tod der Bakterien mittels Antibiotika über einen Bereich von variierender bakterieller Dichte wichtige Informationen über Unterschiede zwischen den Zusammensetzungen der Medikamente liefern und auf die Auswahl der Medikamente für eine Therapie einen Einfluss haben.

Hypothese/Ziele – Die Messung abgetöteter klinischer Isolate von *Staphylococcus pseudintermedius* und *Escherichia coli* mittels Cefazolin, Cefovecin, Doxycyclin und Pradofloxacin nach klinisch relevanten Wirkstoffkonzentrationen (minimale Hemmstoffkonzentration, Mutantenprävention, Maximum im Serum und Maximum im Gewebe) gegen variierende Bakteriendichten.

Methoden und Materialien – Die Bakterienstämme wurden von Hunden mit einer Harnwegsinfektion entnommen und untersucht. Hohe Bakteriendichten von 10^6 bis 10^9 Kolonie-bildenden Einheiten (cfu)/mL

waren einer minimalen Hemmstoffkonzentration, Mutantenprävention, Wirkstoffkonzentrationen in Blut- und Gewebe ausgesetzt und es wurden die Prozentanteile (log^{10}) der getöteten wachstumsfähigen Zellen 30 Minuten, 1, 2, 4, 6, 12 und 24h nach Wirkstoffexposition quantifiziert.
Ergebnisse – Doxycyclin zeigte bakteriostatische Eigenschaften, wobei weniger Bakterien abgetötet wurden als mit den anderen drei Wirkstoffen. Zum Beispiel wurden mehr Zellen bei einer Dichte von 10^7 cfu/mL an *S. pseudintermedius* von Pradofloxacin ($P < 0{,}0001$) und Cefovecin ($P = 0{,}0014$) abgetötet – nicht jedoch von Cefazolin, im Vergleich zu Doxycyclin bei maximaler Serumwirkstoffkonzentration als von Doxycyclin nach einer 12 stündigen Wirkstoffexponierung.
Schlussfolgerungen und klinische Bedeutung – Es wurden Unterschiede zwischen einigen Wirkstoffen in Bezug auf die Geschwindigkeit und das Ausmaß des Abtötens von Bakterien festgestellt; das könnte klinisch wichtig sein und die Auswahl des Wirkstoffes sowie die Dauer der Behandlung beeinflussen.

要約
背景 – 細菌密度は感染期間中に変動する可能性が高く、感受性試験で使用される細菌密度を超える可能性がある。そのため、様々な細菌密度の範囲にわたる抗生物質による殺菌調査は、化合物間の重要な違いを提供する可能性があり、治療としての薬物選択に影響を与える可能性がある。
仮説/目的 – 本研究の目的は、さまざまな密度の細菌に対する臨床的に関連する(最小発育阻止、耐性変異阻止、最大血清および最大組織)薬物濃度で、セファゾリン、セフォベシン、ドキシサイクリンおよびプラドフロキサシンによるブドウ球菌臨床分離株および大腸菌の死滅を測定することであった。
材料と方法 – 尿路感染症の犬から採取された細菌株を調査した。10^6～10^9コロニー形成単位(cfu)/ mLの範囲の高い細菌密度を最小発育阻止、耐性変異阻止、血中および組織薬物濃度の薬物に暴露し、薬物暴露30分後、2、4、6、12、および24時間の死滅生細胞の割合(log^{10})を定量化した。
結果 – ドキシサイクリンは、他の3薬剤よりも弱い殺菌力で静菌特性を示した。たとえば、S. pseudintermediusの10^7 cfu / mL密度で、12時間の薬物曝露後のドキシサイクリンよりも最大血清薬物濃度のドキシサイクリンと比較した場合、プラドフロキサシン($P < 0.0001$)およびセフォベシン($P = 0.0014$)により多くの細胞が死滅したが、セファゾリンではそうではなかった。
結論と臨床的重要性 – いくつかの薬物では、殺菌速度および程度に違いが見られた。これは臨床的に重要であり、薬剤の選択と治療期間に影響を与える可能性がある。

摘要
背景 — 细菌密度可能在感染期间发生波动,并可能超过药敏试验中使用的细菌密度。因此,在不同细菌密度范围内,研究抗生素杀菌能力可发现药物之间的重要差异,并影响治疗时的药物选择。
假设/目的 — 检测头孢唑啉、头孢维星、多西环素和普多沙星在临床相关(最小抑制、突变预防、最大血清和最大组织)药物浓度下,对不同细菌密度的假中间葡萄球菌和大肠杆菌临床分离株的杀灭作用。
方法和材料 — 对采集自尿路感染犬的细菌菌株进行研究。将 10^6 至 10^9 菌落形成单位 (cfu)/mL 的高密度细菌接触最低抑制、突变预防、血液和组织药物浓度,并定量测定接触药物30 min、1、2、4、6、12 和 24 h 后杀死的活细胞百分比 (log^{10})。
结果 — 多西环素表现出抑菌特性,与其他 3 种药物相比,杀菌效果较低。例如,当假中间葡萄球菌密度为 10^7 cfu/mL 时,在接触药物12h 后,与多西环素相比,在最大血清药物浓度下,普多沙星 ($P < 0.0001$) 和头孢维星 ($P = 0.0014$) 能杀死更多细胞,但头孢唑啉却不能。
结论和临床意义 — 一些药物在杀灭细菌的速度和程度方面存在差异;这可能具有临床意义,并可能影响药物选择和治疗持续时间。

Resumo
Contexto – A densidade bacteriana pode flutuar durante uma infecção e é possível que exceda aquela utilizada em testes de suscetibilidade. Sendo assim, exames que avaliam o extermínio de bactérias por antibióticos utilizando variadas densidades bacterianas pode demonstrar importantes diferenças entre compostos e assim influenciar na escolha de fármacos a serem utilizados no tratamento.
Hipótese/Objetivos – Mensurar a eliminação de *Staphylococcus pseudintermedius* e *Escherichia coli* por cefazolina, cefovecina, doxiciclina e pradofloxacino em concentrações clinicamente relevantes (inibitória mínima, prevenção de mutantes, sérica máxima e tecidual máxima) contra diversas densidades bacterianas.
Métodos e materiais – O estudo foi realizado com cepas bacterianas coletadas de cães com infecções do trato urinário. Altas densidades bacterianas variando de 10^6 a 10^9 unidades formadoras de colônia (ufc)/mL foram expostas a concentração inibitória mínima, de prevenção de mutantes, sérica e tecidual, e as porcentagens (log^{10}) de células viáveis eliminadas após 30 min, 1, 2, 4, 6, 12 e 24 h de exposição aos fármacos foram quantificadas.
Resultados – A doxiciclina demonstrou propriedades bacteriostáticas com propriedades bactericidas menores que os outros três agentes. Por exemplo, em uma densidade de 10^7 ufc/mL de *S. pseudintermedius*, mais células foram mortas por pradofloxacina ($P < 0.0001$) e cefovecina ($P = 0.0014$), mas não cefazolina quando comparado à doxicilina na concentração sérica máxima do fármaco e posteriormente à doxicilina após 12 horas de exposição à droga.

Conclusões e importância clínica – Observou-se diferenças entre alguns fármacos na velocidade e extensão da eliminação de bactérias; isto pode ser clinicamente relevante e pode impactar na seleção de drogas e na duração do tratamento.