**Systemic antimicrobials impact mucosal staphylococci in dogs**

**Abstract**

**Background:** Antimicrobial resistant bacteria are increasingly isolated from veterinary patients.

**Objectives:** To determine risk factors for antimicrobial resistance (AMR) among canine mucosal staphylococci following routine antimicrobial treatment with cefalexin, clavulanate-amoxicillin, cefovecin, clindamycin, or a fluoroquinolone.

**Animals:** Mucosal swab samples (n=463) were collected from 127 dogs pre-treatment, immediately-, one- and three-months post-treatment.

**Methods:** Staphylococci were identified phenotypically and biochemically as coagulase negative (CoNS) or coagulase positive (CoPS); CoPS were speciated by *nuc* gene PCR. Antimicrobial susceptibility was determined using disc diffusion and mecA gene carriage by PCR. Multilevel, multivariable models examined associations between risk factors and presence/absence of CoPS, meticillin resistance (MR), multidrug-resistance (MDR) and fluoroquinolone resistance (FQR).

**Results:** The percentage of samples with CoNS increased and with CoPS (including *S. pseudintermedius*) decreased immediately-post-treatment with cefalexin, cefovecin and clindamycin (P≤0.001) and one-month post-treatment with clindamycin (P=0.003). By three-months post-treatment, there was no significant difference compared to pre-treatment samples. Immediately-post-treatment with fluoroquinolones there was significantly increased risk of isolating MRS (P=0.002), MDR (P=0.002) or FQR (P=0.013) staphylococci and of MDR following cefalexin treatment (P=0.019).

The percentage of samples with AMR staphylococci declined from immediately- to three-months post-treatment and there was no significant difference between resistance prevalence at one- or three-months post-treatment for most AMR traits and treatment groups. Exceptions include increased MDR following fluoroquinolone (P=0.048) or cefalexin (P=0.021), at one- and three-months post-treatment, respectively.

**Conclusions**: Systemic antimicrobials impact mucosal staphylococci. Immediately after therapy, the mucosa may be a reservoir for AMR staphylococci that are a source of mobile genetic elements carrying AMR genes.

**Introduction**

Staphylococci are normal mucosal and skin commensals affecting people and other animals.1 The main coagulase positive *Staphylococcus* (CoPS) species in humans is *S. aureus2* and in dogs is *S. pseudintermedius,3* previously *S. intermedius.4* *S. pseudintermedius* is the most frequent cause of canine pyoderma.5,6 The population prevalence of mucosal *S. pseudintermedius* carriage in healthy dogs is between 11% and 87.4%7-12 with increased carriage with in dogs with pyoderma.13,14 Fewer healthy dogs carry *S. aureus* (6.5% to 14%)7,8,11,12,15 and such isolates are likely to originate from in-contact humans.16 In addition, coagulase negative staphylococci (CoNS) are common mucosal commensals in humans and other animals, with a population prevalence in dogs between 38% (isolated from nasal swabs)12 and 95% (from nasal and perineal swabs).11

Meticillin resistant (MR) and multi-drug resistant (MDR) staphylococci are increasingly isolated from dogs,17 rendering many antimicrobials ineffective. Meticillin resistant staphylococci (MRS) carry the *mecA* gene, which encodes an altered penicillin binding protein (PBP2a) and confers resistance to all beta-lactam antimicrobials.18 The *mecA* gene is carried on a large mobile genetic element (MGE), the staphylococcal cassette chromosome *mec* (SCC*mec*), which can be transferred horizontally between staphylococci.19 Tsubakishita *et al*.,20 suggested that CoNS (commonly MR and/or MDR21,22), were the original source of the *mecA* gene and may act as reservoirs of AMR genes for CoPS.22-25 SCC*mec* may also carry resistance genes for other antimicrobials26 and MRS can harbour other MGEs and chromosomal mutations giving rise to MDR. Hence MRS are frequently MDR, and commonly fluoroquinolone resistant (FQR).24

The population prevalence found in most studies of meticillin resistant *S. pseudintermedius* (MRSP) carriage in healthy dogs is between 0 and 4.5%,8,11,12,27-30 but prevalence can be higher, between 8 and 34%8,14,31,32 and up to 66% in one Japanese study33 in dogs with skin disease and/or exposed to other potential risk factors. Reported risk factors for mucosal carriage of, or infection with MRS in dogs include antimicrobial therapy, contact with veterinary premises, and hospital admissions.31,34-40 Similar risk factors have been reported or proposed for MR *S. aureus* (MRSA) and MR coagulase negative staphylococci (MR-CoNS) carriage in humans;23,38 the MR-CoNS population prevelance in healthy dogs is reported to be up to 42%.11,12,30 Carriage may lead to transfer of staphylococci between individuals or to the environment, with long-term persistence being reported in both circumstances.41-45

Most previous studies that have examined MRS risk factors for dogs have found either no association with antimicrobial therapy or reported a general association, and none have examined longitudinal carriage. This study aimed to: 1) examine AMR mucosal staphylococcal longitudinal carriage prevalence after antimicrobial administration; 2) determine the risk factors for detection of such bacteria; and 3) compare the impact of commonly used antimicrobials on the selection and persistence of AMR.

**Materials and Methods**

**Study Population**

Dogs attending veterinary consultations at three centres (a first opinion clinic, a referral practice and a centre with both first opinion and referral patients), in the North West of England between June 2011 and September 2012 were recruited for the study. The inclusion criteria were, diagnosis of a bacterial infection (skin, soft tissue, urinary tract, dental, respiratory tract, orthopaedic, gastrointestinal or ocular) and a requirement for systemic antimicrobial therapy with one of six antimicrobials authorised for use in dogs in the UK (cefalexin, clavulanate-amoxicillin, cefovecin, clindamycin, or a fluoroquinolone [enrofloxacin or marbofloxacin]). Treatment groups were based on the antimicrobial prescribed. Exclusion criteria were: antimicrobial therapy or admission within three-months prior to consultation, and dogs aged less than 12 months (fluoroquinolones are contraindicated in this group). Dogs were also excluded if they were administered additional systemic antimicrobials during the course of the study. Samples were collected pre-treatment, immediately post-treatment and at one- and three-months post-treatment. The University Veterinary Ethics Committee approved the study protocol.

**Staphylococcal isolation**

One nasal swab and one perineal swab was collected from each dog (Copan Eswab LQ Amies Minitip Nylon Flocked Applicator, Appleton Woods, Birmingham, UK) at each time point. A sterile swab was inserted 5mm into one nostril and a second swab rubbed on the skin of the perineum for 5 seconds before being placed in Amies transport media, stored at 4˚C, and processed within 36 hours of collection. Each swab was separately incubated aerobically overnight at 37˚C in nutrient broth with 6.5% sodium chloride. The overnight broth for each swab (one nasal and one perineal sample per dog) was cultured on mannitol salt agar (MSA), oxacillin resistance screening agar (ORSA) supplemented with 2μg/ml of oxacillin, and Columbia 5% horse blood agar (CAB), and incubated aerobically overnight at 37˚C. Several isolates of different morphologies, but resembling a staphylococcal phenotype, were selected from each agar plate (six agar plates per sample), and sub-cultured onto CAB for aerobic overnight incubation at 37˚C. Fresh staphylococcal cultures on CAB were subjected to Gram stain (Sigma-Aldrich Company Ltd., Gillingham, UK), tested for catalase (Sigma-Aldrich Company Ltd., Gillingham, UK) and free coagulase production (Rabbit plasma, Pro-Lab, Bromborough, UK), according to the manufacturer’s instructions. Isolates were stored at -80˚C in Microbank vials (Pro-Lab, Bromborough, UK) for further processing. All culture media were obtained from LabM Ltd., Bury, UK.

**Antimicrobial susceptibility testing**

Prior to cryopreservation, disc diffusion testing was performed on all confirmed *Staphylococcus* species isolates in accordance with accepted standards,46 as described previously11 with the following discs: 1μg oxacillin, 1μg ciprofloxacin (screening for fluoroquinolone resistance), 10μg gentamicin, 10μg fusidic acid, 30μg cefalexin, 30μg cefovecin, 25μg trimethoprim-sulfamethoxazole, 10μg tetracycline, 2μg clindamycin and 5μg vancomycin. All discs were purchased from MAST Group Ltd., Liverpool, UK, except for cefovecin, which were obtained from Oxoid, Basingstoke, UK. The reference strain *S. aureus* ATCC®25923 (LGC Standards, Teddington, UK) was used for quality control of zone diameter determinations.

**Polymerase Chain Reaction (PCR) for *nuc* and *mecA* genes**

Prior to cryopreservation, DNA extraction was performed as previously described.11 PCR assays to detect the presence of the *nuc* genes of *S. pseudintermedius, S. aureus* and *S. schleiferi* were performed on all CoPS47 using Qiagen® Multiplex PCR Mix (Qiagen, Crawley, UK). PCR assays were also used to detect the presence of the *mecA* gene in staphylococcal isolates that were phenotypically resistant to oxacillin48 using Solibiodyne® FiREPol PCR Mix RTL 12.5 mM MgCL2, according to the manufacturer’s instructions with minor modifications.11 PCR products were analysed by agarose gel (1.5%) electrophoresis and the DNA fragments were visualised under UV light after peqGREEN (Peqlab Ltd., Fareham, UK) staining.

**Prevalence of staphylococci and resistance**

Staphylococci were classed as resistant or not resistant based on the Clinical Laboratory Standards Institute (CLSI) guidelines 2013.46 As CLSI standards were not available for interpretation of ciprofloxacin and fusidic acid, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) zone diameter interpretive standards were used.49 The breakpoints for interpretation of oxacillin resistance were a zone of inhibition of ≤17 mm for *S. pseudintermedius,50* and CoNS, and ≤10 mm for *S. aureus*. A sample was classified as AMR if it had at least one AMR staphylococcal isolate.

The percentage (with 95% confidence intervals) of samples with CoPS, CoNS, *S. pseudintermedius*, *S. aureus*, *S. schleiferi* subspecies *coagulans* or with ‘no growth’ and the percentage of samples with staphylococci resistant to each tested antimicrobial, MRS (phenotypic oxacillin resistance and carriage of the *mecA* gene) and MDR (resistance to three or more antimicrobial classes)51 were calculated at each sample time point for each treatment group and treatment overall using Clopper-Pearson exact interval.52

**Statistical analysis**

A questionnaire, examining potential risk factors for the carriage of AMR bacteria was administered at the start of the study and for each follow-up sample. Data were collected describing patient signalment, diet, previous veterinary history (including previous antimicrobial therapy and veterinary admission), the presence, number and type of in-contact pets, previous medical history of the household (including antimicrobial therapy or hospitalisation of humans or other pets) and whether any household member worked with farm animals or in healthcare. The attending veterinary surgeon also completed a one-page questionnaire confirming the diagnosis, the prescribed antimicrobial and therapeutic regime and any previous antimicrobial therapy within the last 12 months. Independent risk factor variables were created from information obtained from the owner and veterinary surgeon questionnaires.

Except for the age of the dog, all variables were dichotomous or categorical in nature. The variables ‘treatment duration’ and ‘body weight’ were divided into three categories: ≤1 week, >1 week but ≤3 weeks, and >3 weeks, and small (<11 kg), medium (11-20 kg) and large (>20kg), respectively. Samples were recruited from three veterinary practices (Sites 1, 2 and 3). The type of veterinary consultation was divided into two categories: first opinion and referral. Initial statistical analyses included Spearman’s rho to examine risk factor variable correlation. Differences between treatment groups at pre-treatment for each risk factor were investigated using Pearson’s chi-square or Fisher’s exact tests (if n<5) for categorical variables or one-way between-groups analysis of variance (ANOVA) for continuous age data (Table 1). Multivariable logistic regression was then used to investigate any differences in antimicrobial resistance outcomes between treatment groups at pre-treatment, after accounting for other independent variables. Statistical tests were performed using the SPSS software package (SPSS 20.0 for Mac, SPSS Inc, Chicago, Illinois).

To examine the effect of treatment-group/time-point, and other independent variables on the presence of resistance outcomes (presence or absence of CoPS, MRS, MDR or FQR in mucosal staphylococci), data were analysed using multilevel logistic regression models with a binomial distribution and logit link function. Due to repeated samples over time, data were clustered within dogs (level two units) and this clustering was accounted for by inclusion of dog as a random intercept in all models. Swab samples were considered the level one unit of interest. All dogs at pre-treatment were classed as untreated (n=127 dogs). In total, in addition to pre-treatment (the reference time-point), there were 15 categories created to account for the different combinations of time (n=3) and treatment group (n=5). Initially all variables were analysed using unadjusted multilevel models. Those that showed some association with each outcome on unadjusted analysis (*P*-value <0.25)53 were considered for incorporation into a final multilevel, multivariable model. Treatment-group/time-point were always retained in final models. Final models were constructed using a manual backwards stepwise procedure where variables with a Wald *P*-value <0.05 were retained. Once a final multivariable model was produced, all variables that were significantly (*P*<0.05) different between treatment groups at pre-treatment were forced back into the model to assess their effect on remaining variables, and in particular, treatment group. Multilevel models were analysed using the MLwiN statistical software package (MLwiN Version 2.28 Centre for Multilevel Modelling, University of Bristol). Univariable and multivariable calculations used penalised quasi-likelihood estimates (2nd order PQL for all outcomes). First order interaction terms were tested for all variables remaining in the final models. The residuals (+/- 1.96 standard deviation) were calculated and presented in ranked order in caterpillar plots for each dog, to check for outliers. If present, outliers were temporarily removed and the models were rerun to assess the impact upon results.

**Results**

**Study population**

One hundred and twenty-seven dogs were enrolled in this study from three centres: 1 (n=43), 2 (n=52) and 3 (n=32), which represented 72 first opinion and 55 referral cases. The dogs were treated with one of the following antimicrobials: cefalexin (n=31), clavulanate-amoxicillin (n=29), cefovecin (n=26), clindamycin (n=28), or a fluoroquinolone (n=13). Treatment was prescribed for ≤1 week in 33 dogs, >1 week but ≤3 weeks in 48 dogs, and >3 weeks in 46 dogs. Indication based prescription consisted of pyoderma (n=81 dogs), bite wound (n=4), post-surgical infection/ prophylaxis (n=8), infected skin tumour/cyst (n=2), gingivitis (n=9), anal sac abscess (n=4), otitis externa (n=2), pedal abscess/nail bed injury (n=4), respiratory tract infection (n=2), corneal ulcer (n=1), gastritis (n=1), or urinary tract infection (n=9). The dogs were aged from 12 to 204 months (mean=62 months) with 75 males and 52 females, 21 small, 16 medium and 90 large dogs.

One hundred and twenty-seven dogs provided samples at pre-treatment and immediately post-treatment, 106 dogs provided samples at one-month, and 103 dogs at three-months post-treatment respectively, resulting in a total of 463 samples (Table 2).

Owners of 24 dogs worked in health care environments; four owners in veterinary healthcare; 18 owners in human healthcare (hospital, GP surgery, community nursing, paramedical, pharmaceutical, research or nursing home staff); and two owners in undefined healthcare. Missing samples from one-month post-treatment (n=21) were due to prescription of further antimicrobials (n=6), euthanasia or death due to unrelated reasons (n=2), or owner non-compliance (n = 13). Missing samples from three-months post-treatment (n=24) were due to prescription of further antimicrobial courses (n=10), euthanasia or death due to unrelated reasons (n=3), re-homing (n=3) or owner non-compliance (n=8).

**Prevalence of staphylococci**

Overall, staphylococci were isolated in 88% samples from 99% dogs during the full study period. These included CoNS, isolated in 59% samples from 93% dogs, and CoPS, isolated in 62% samples and from 87% dogs. CoPS isolates were *S. aureus,* isolatedin 9% of samples from 21% of dogs, *S. schleiferi* subspecies *coagulans*,isolated in 3% samples from 7% of dogs and *S. pseudintermedius*, isolated in 56% samples from 83% dogs (Supplementary Information Table S1); 67% of samples positive for *S. pseudintermedius* were isolated from the nares and 79% were isolated from the perineum (data not shown). Changes in the percentage of samples with CoPS, *S. pseudintermedius* or CoNS during the full study are shown in Figure 1 and Supplementary Information Table S1 (Table S1 also shows samples with ‘no staphylococcal growth’).

**Prevalence of antimicrobial resistance**

During the full study period, MRS were isolated in 26% samples from 63% dogs, however MR-CoPS were only isolated in 10% dogs. Of the MR-CoPS, 11 dogs carried MRSP and two dogs carried MRSA; all *S. schleiferi* subspecies *coagulans* were meticillin susceptible. Of the 11 dogs with MRSP, the first isolation was immediately post-treatment in two dogs, and at one-month post-treatment in four dogs. After initial isolation, MRSP was also isolated in subsequent samples in four dogs (Supplementary Table S2). Changes in the percentage of samples with MRS, MDR and FQR at different time points are shown in Figure 2 and for each tested antimicrobial in Supplementary Figure S3).

**Multilevel logistic regression**

There were significant differences at pre-treatment, between treatment groups (cefalexin, clavulanate-amoxicillin, cefovecin, clindamycin or fluoroquinolones) in: age, weight, treatment duration, recruitment site, type of veterinary consultation, whether a ‘diagnosis of pyoderma’ was made, previous systemic antimicrobial treatment, in-contacts admitted to hospital/veterinary premises, and dogs regularly eating animal stools (Table 1). There were however, no significant differences among pre-treatment variables for each treatment group for each resistance outcome (MRS, MDR or FQR) after accounting for all variables in multivariable models.

Multilevel multivariable model results suggested that individual treatment groups, cefalexin, CVN and CD, were significantly associated with decreased CoPS isolation immediately post-treatment. Furthermore, fluoroquinolone therapy was a significant risk for the detection of MRS, MDR or FQR staphylococci, and cefalexin was a risk for the detection MDR staphylococci immediately post-treatment (Tables 3 and 4).

At one-month post-treatment, there were no significant differences compared to all pre-treatment samples, for any treatment group or AMR outcome measure other than an increased risk for the detection of MDR staphylococci after fluoroquinolone treatment, and decreased CoPS after clindamycin. At three-months post-treatment, the only significant difference, compared to all pretreatment samples, was increased risk of MDR staphylococci following cefalexin treatment.

Other results from the multivariable multilevel models: dogs living in a multi-dog household had significantly decreased MRS compared to single-dog households; small and medium dogs compared to large dogs had significantly increased FQR staphylococci; female dogs, dogs that regularly ate animal stools or dogs that lived with in-contacts admitted to hospital/veterinary premises had significantly increased carriage of MDR staphylococci; recruitment site significantly impacted CoPS detection with reduced isolation in dogs from site 3 (referral only) than site 1 (mixed referral and first opinion); and a ‘diagnosis of pyoderma’ significantly increased the risk of identifying CoPS (Tables 3 and 4).

**Discussion**

This study is the first prospective, longitudinal survey to examine the effects of different routine antibiotic therapies on canine mucosal staphylococci and the development of AMR. The results suggest that levels of resistance increased in staphylococci immediately post-treatment for most of the antibiotics investigated, and at the same time, the level of CoPS decreased. In addition, levels of AMR returned to pre-treatment prevalence by one to three-months post-treatment, for most antibiotics and types of resistance.

**Prevalence of staphylococci**

Mucosal CoNS and CoPS, including *S. pseudintermedius* (93%) and *S. aureus* (21%), were isolated from a high proportion of dogs during the study period. *S. pseudintermedius* has been isolated from up to 87.4% and *S. aureus* from up to 14% of healthy dogs.7,10 Repeated sampling is likely to have yielded a high prevalence of staphylococci in this study; in particular *S. pseudintermedius* carriage in dogs has been reported to be diverse and changing over time9,41 and could be missed with cross-sectional sampling. Moreover, the majority of enrolled dogs were suffering from pyoderma, which may have increased detection of mucosal staphylococci,36 and in particular, *S. pseudintermedius.*13,14 The overall study prevalence of CoNS which we have identified (93%), matches previous results (95%, from n=73 individuals), using the same methodology in healthy dogs;11 this is much higher (38%) than the prevalence in a larger cross-sectional study (n=724) in which only the nares were sampled using different methodology.12 A previous study also reported improved sensitivity to detect canine staphylococcal carriage by sampling two anatomical sites (in particular the mouth along with the perineum or nares).54 The high prevalence detected in this study is not unexpected for commensal bacteria.

The decrease in CoPS and increase in CoNS noted immediately post-treatment could be due to inhibition of potentially more AMR susceptible CoPS and selection of more AMR resistant CoNS. CoNS are more commonly MR and MDR,21,22 so one concern is that they may act as reservoirs of AMR genes for CoPS22,24 during co-colonisation. Although CoNS are often thought to be non-pathogenic compared to CoPS, CoNS can possess virulence factors and have been reported to cause serious disease in both human and animal hosts.21,22 Furthermore CoN-*S*. *schleiferi* subspecies *schleiferi* has been increasingly isolated from infections in dogs,55 particularly following antimicrobial therapy. However, in this study we did not examine the species of CoNS detected in samples; this is a study limitation and an area of research that merits further investigation.

**Prevalence of AMR staphylococci**

The prevalence of MR (62%), MDR (65%) and FQR (48%) amongst staphylococci was higher in the overall study period compared to previous work in healthy dogs,11,12 possibly due to longitudinal sampling of individual dogs. These data however, were in line with reports in dogs with recurrent pyoderma exposed to antimicrobial therapy.36 MRSP was isolated in 9% of dogs overall, which is double that previously reported in healthy dogs;7,8,11,12,27-30 likely due to recent antimicrobial and/or veterinary premises exposure and longitudinal sampling. A similar prevalence of MRSP (7.4%) was reported in a study of dogs admitted to a small animal hospital in which antimicrobial therapy and hospitalisation were both identified as risk factors.31 The initial detection of MRSP occurred immediately post-treatment in two dogs, and at one-month post-treatment in four of 11 dogs. This may be because antimicrobial therapy will inhibit susceptible mucosal staphylococci, creating vacant ecological niches that can be filled by resistant variants transmitted from other hosts or the environment. MRSP was isolated in subsequent samples in the same four dogs, despite no further antimicrobials being prescribed, supporting the potential for extended carriage periods following treatment of clinical infections.40

**Impact of fluoroquinolone therapy**

Fluoroquinolone therapy was a significant risk factor for the detection of MRS, MDR staphylococci, and not surprisingly, FQR staphylococci. Selection of MRS and MDR staphylococci is likely to have been due to co-selection and other studies have reported this for MRSA or MR-*S. epidermidis* in humans, and MRSA in dogs prescribed fluoroquinolones.35,56-58 Furthermore, fluoroquinolones may select for meticillin resistant isolates by up-regulating adhesion factors,57 increasing mutational rates or stimulating bacterial ‘SOS’ response,59 or by repressing *mecA* regulator genes.60

**Impact of beta-lactams**

Only cefalexin therapy was statistically significant for the detection of MDR staphylococci immediately post-treatment. It was surprising that beta-lactam therapy was not associated with MRS detection in multivariable models, however results of previous risk factor analyses have also shown inconsistent results. A few studies have identified previous antimicrobial administration as a risk for MRS/MRSP carriage/infection,31,34,38,39,61 but not individual drugs and/or classes, while other studies have not found a significant association at all.37,62,63 Other studies however, have reported beta-lactam therapy as a risk factor for MRSA infection in humans and dogs.35,64 Another study reported beta-lactams were a risk for MRSP infections in dogs that were mainly receiving cephalosporins (less so clavulanate-amoxicillin)65 and Fungwithaya *et al*.66 reported the detection of nasal MRSP in dogs within one week of cefalexin treatment in 10 dogs. Penicillin-based or cephalosporin treatment was also found to be a risk for MR-*S. schleiferi* infection in dogs.54

In our study, clavulanate-amoxicillin appeared to have less effect on MRS detection than cephalosporins. The reasoning for this is likely to be multifactorial, including the level of antimicrobial resistance present before therapy, and the spectrum of activity, pharmacokinetics and pharmacodynamics of the different antimicrobials, and warrants further investigation. Another consideration could be treatment length, as the majority of dogs in the clavulanate-amoxicillin group only received up to one-week of treatment, while those prescribed cephalosporins received two or more weeks. Although extended courses of antimicrobials would be expected to prolong selection pressure,67 we found that duration of therapy was not significantly associated with the detection of resistance, however this may be a result of small sample sizes.

**Impact of clindamycin**

Clindamycin therapy was not significantly associated with selection of AMR. Other authors found that people with skin and soft tissue infections, treated with clindamycin, had earlier clearance of MRSA mucosal/skin carriage68 and were less likely to have persistent MRSA colonisation;69 treatment was also found to be protective against MDR MRSA environmental contamination.70 Clindamycin was however a significant risk factor for reduced CoPS immediately after and at one-month post-treatment in this study. These findings suggest an extended effect compared to other tested antimicrobials. To the author’s knowledge, the impact of antimicrobials on canine mucosal microbiota has not been reported previously, but a recent human study described increased proportions of AMR nasal anaerobic bacteria for at least 12 months, following a ten-day course of clindamycin.71

**Antimicrobial exposure recovery period**

By one-month post-treatment, for most treatment groups, the percentage of samples with the AMR outcomes had started to decline, and had typically recovered to pre-treatment levels by three-months post-treatment for AMR outcomes MRS and FQR; at this stage, there was no significant difference compared to all pre-treatment samples for all treatment groups. The decline in the percentage of samples with MDR staphylococci however, was not as consistent. This finding was reflected in the multilevel modelling results, where fluoroquinolone therapy was significant for MDR detection at one-month and cefalexin therapy was significant for MDR detection at three-months post-treatment.

The mechanism of increased AMR following therapy and subsequent recovery was not investigated in this study, but is likely to be multi-factorial. Staphylococci contain a number of MGEs that may be involved in horizontal transfer of AMR determinants between isolates.72 SCC*mec* cassettes carry the *mecA* gene, and may carry other AMR determinants,26,73 but are generally less mobile than other MGEs.74 Furthermore, chromosomal mutations, giving rise to clinically relevant FQR, may take time to accumulate.75 Therefore selection and co-selection of pre-existing, possibly unisolated, MDR strains may have occurred. Additionally treatment with broad-spectrum antimicrobials may result in vacant mucosal niches that could be filled by exogenous strains acquired from the environment or in-contact hosts.

**Other findings**

In addition to antimicrobial therapy, a number of other factors were found to increase the risk of antimicrobial resistance. Dogs from multi-dog households were less likely to be positive for mucosal MRS in this study. This finding was unexpected, as sharing of staphylococci, including MRSP, has been reported between in-contact individuals and pets,41,42,76 particularly in multi-dog households.77 Further, females were more likely to carry MDR isolates than males, which disagree with previous findings for MRS.36,39 We found that dogs reported as eating animal stools or living with hosts with hospital contact had increased risk of detecting MDR staphylococci. These findings are line with other studies describing animal stools as a source of AMR Gram-positive bacteria,78 and veterinary premises or hospital contact as a risk factor for MRS, often MDR staphylococci,21,79,80 in dogs31,34,81 or humans.76,82,83

**Limitations**

Whilst our work is extensive, study power may have been limited due to small sample-sizes in treatment groups, however we identified a number of significant risk factors. The P-value was not corrected based on the number of statistical tests (four) as this can increase Type II error.84 Although our ‘clinically-led’ recruitment process was potentially biased, we accounted for any differences in pre-treatment variables within models. This study did not speciate the numerous CoNS detected from samples due to time and economic constraints. Unfortunately, we therefore cannot report on the longitudinal prevalence of potential CoNS pathogens, for example, *S*. *schleiferi* subspecies *schleiferi* following antimicrobial therapy. Longitudinal AMR prevalence however was analysed at the genus level and was therefore not affected.

**Conclusions**

This study prospectively examined the relationship between the persistence of staphylococcal AMR and antimicrobial therapy in 127 dogs (463 samples) over the full time-course and post-treatment exposure period. These results concur with previous reports that antimicrobial therapy increases the risk of AMR staphylococcal carriage, including MRS and MDR in dogs.36 As the infecting isolates often originate from an individual’s own mucosal flora, carriage of resistant staphylococci is a risk for resistant infections and therapeutic failure. This is of particular concern in dogs that may be immune suppressed or predisposed to recurrent infections such as pyoderma. AMR mucosal staphylococci may be shared between individuals and environments including hospitals, home or the wider community,42 meaning that adherence to strict hand hygiene and barrier nursing protocols could prevent dissemination, and have impact upon veterinary and public health. Finally, our results have implications for antimicrobial prescribing guidelines and stewardship programs for appropriate use of antibiotics for the veterinary community at large.

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**Tables and Figures**

**Table 1.** The pre-treatment variables considered for inclusion in the final multivariable model, with the number and percentage (%) of samples in each treatment group and variable category.

**Table 2.** The number of samples provided at each time point in each treatment group.

**Table 3.** Multilevel multivariable results for outcomes meticillin resistant staphylococci (MRS) and fluoroquinolone resistant staphylococci (FQR) in samples from 127 dogs.

**Table 4.** Multilevel multivariable results for outcomes multidrug resistant (MDR) staphylococci or the presence of coagulase positive staphylococci (CoPS) in samples from 127 dogs.

**Figure 1.** The percentage of samples with a) coagulase positive staphylococci (CoPS) or b) *S. pseudintermedius* or c) coagulase negative staphylococci (CoNS) at each time point in each treatment group (error bars=95% confidence intervals (CI)).

**Figure 2.** The percentage of samples with a) meticillin resistant staphylococci (MRS) or b) multidrug resistant (MDR) or c) fluoroquinolone resistant staphylococci at each time point for each treatment group and treatment overall (error bars = 95% confidence interval (CI)).

**Supplementary Information including:**

**Supplementary Table S1.** The percentage of samples with CoPS, *S. pseudintermedius*, *S. aureus*, *S. schleiferi coagulans*, CoNS or where ‘no staphylococci’ were isolated , at each time point for each treatment group and treatment overall (error bars = 95% confidence interval).

**Supplementary Table S2.** Percentage of samples with MRS, MDR, fluoroquinolone resistant staphylococci and CoPS-MRS, MRSP, CoPS-MDR or CoPS-fluoroquinolone resistant staphylococci in each treatment group and for treatment overall at each time point (95% confidence interval).

**Supplementary Figure S3.** The percentage of samples with a) clindamycin or b) trimethoprim sulfamethoxazole resistant staphylococci at each time point for each treatment group and treatment overall (error bars=95% confidence interval).

**Table 1.** The pre-treatment variables considered for inclusion in the final multivariable model, with the number and percentage (%) of samples in each treatment group and variable category.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **CFX** | **AC** | **CVN** | **CD** | **FQ** | **All Dogs** | ***P*-value** |
| Mean agea (months) | 44 | 50 | 68 | 79 | 83 | 62 | **0.002** |
| Weight: |  | | | | | | **0.003** |
| Small (< 11kg) | 1 (5) | 3 (14) | 4 (19) | 10 (48) | 3 (14) | 21 (17) |  |
| Medium (11-20kg) | 4 (25) | 3 (19) | 0 | 7 (44) | 2 (13) | 16 (13) |  |
| Large (> 20kg) REF | 26 (29) | 23 (26) | 22 (24) | 11 (12) | 8 (9) | 90 (71) |  |
| Gender: |  | | | | | | 0.925 |
| Male REF | 18 (24) | 17 (23) | 14 (19) | 17 (23) | 9 (12) | 75 (59) |  |
| Female | 13 (25) | 12 (23) | 12 (23) | 11 (21) | 4 (8) | 52 (41) |  |
| Treatment duration: |  | | | | | | **0.001** |
| 1 week REF | 5 (15) | 17 (52) | 0 | 10 (30) | 1 (3) | 33 (26) |  |
| >1 or < 3 weeks | 12 (25) | 9 (19) | 10 (21) | 11 (23) | 6 (13) | 48 (38) |  |
| ≥ 3 weeks | 14 (30) | 3 (7) | 16 (35) | 7 (15) | 6 (13) | 46 (173) |  |
| Recruitment site: |  |  |  |  |  |  | **0.001** |
| Site 1 REF | 8 (18) | 1 (2) | 15 (34) | 8 (18) | 12 (27) | 44 (35) |  |
| Site 2 | 10 (20) | 19 (37) | 6 (12) | 16 (31) | 0 | 51 (40) |  |
| Site 3 | 13 (41) | 9 (28) | 5 (16) | 4 (13) | 1 (3) | 32 (25) |  |
| First opinion/ referral: |  | | | | | | **0.001** |
| First opinion practice REF | 23 (32) | 28 (39) | 4 (6) | 17 (24) | 0 | 72 (57) |  |
| Referral consultation | 8 (15) | 1 (2) | 22 (40) | 11 (20) | 13 (24) | 55 (43) |  |
| A diagnosis of pyoderma was made at enrolment | 27 (33) | 3 (4) | 25 (31) | 15 (18) | 12 (15) | 82 (65) | **0.001** |
| Previous systemic antimicrobial treatment1 | 15 (26) | 10 (17) | 15 (26) | 9 (16) | 9 (16) | 58 (46) | **0.031** |
| Previous beta-lactam antimicrobial treatment1 | 11 (29) | 7 (18) | 7 (18) | 6 (16) | 7 (18) | 38 (30) | 0.111 |
| Previous hospital admission1 | 4 (16) | 3 (12) | 6 (24) | 8 (32) | 4 (16) | 25 (20) | 0.287 |
| In-contacts received antimicrobials2 | 7 (25) | 6 (22) | 4 (14) | 6 (21) | 5 (18) | 28 (22) | 0.720 |
| In-contacts admitted to hospital or veterinary premises1 | 16 (40) | 12 (30) | 5 (13) | 6 (15) | 1 (2.5) | 40 (31) | **0.015** |
| Owner works in healthcare | 5 (21) | 2 (8) | 5 (21) | 9 (38) | 3 (13) | 24 (19) | 0.133 |
| Multi-dog household | 17 (30) | 15 (27) | 12 (21) | 8 (14) | 4 (7) | 56 (44) | 0.241 |
| Dog regularly eats animal stools | 7 (18) | 5 (13) | 8 (21) | 10 (26) | 8 (21) | 38 (30) | **0.025** |

CFX=cefalexin; AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; aAge was the only continuous variable and is represented by the mean age of dogs in each treatment group (ANOVA); REF=the reference category for non-dichotomous variables; 1Within 12-months but more than three months as per enrolment criteria; 2Within 12-months of enrolment; significant at *P*<0.05 (Pearson’s chi-square or Fisher’s Exact Test).

Table 2. The number of samples provided at each time point in each treatment group.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antimicrobial treatment group** | | | | | | |
| **Time point** | **CFX** | **AC** | **CVN** | **CD** | **FQ** | **Total** |
| D0 | 31 | 29 | 26 | 28 | 13 | 127 |
| End | 31 | 29 | 26 | 28 | 13 | 127 |
| M1 | 26 | 27 | 20 | 24 | 9 | 106 |
| M3 | 24 | 25 | 21 | 24 | 9 | 103 |
| Total | 112 | 110 | 93 | 104 | 44 | 463 |

CFX=cefalexin; AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; D0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; two swabs per sample.

**Table 3.** Multilevel multivariable results for outcomes meticillin resistant staphylococci (MRS) and fluoroquinolone resistant staphylococci (FQR) in samples from 127 dogs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **MRS** | | | **FQR** | | |
|  | **OR** | **95% CI** | ***P*-value** | **OR** | **95% CI** | ***P*-value** |
| Time D0 (REF) | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ |
| Time End and CFX | 2.23 | 0.86-5.77 | 0.100 | 1.56 | 0.53-4.63 | 0.421 |
| Time End and AC | 0.98 | 0.31-3.12 | 0.977 | 0.68 | 0.17-2.70 | 0.584 |
| Time End and CVN | 2.42 | 0.84-6.95 | 0.100 | 2.36 | 0.80-6.92 | 0.118 |
| Time End and CD | 0.63 | 0.18-2.21 | 0.466 | 0.29 | 0.06-1.46 | 0.133 |
| Time End and FQ | **9.09** | 2.22-37.23 | \*0.002 | **4.98** | 1.39-17.80 | \*0.013 |
| Time M1 and CFX | 1.70 | 0.59-4.93 | 0.328 | 2.43 | 0.84-7.02 | 0.102 |
| Time M1 and AC | 0.55 | 0.14-2.22 | 0.399 | 0.23 | 0.03-2.07 | 0.190 |
| Time M1 and CVN | 1.27 | 0.34-4.72 | 0.717 | 1.03 | 0.24-4.34 | 0.968 |
| Time M1 and CD | 1.39 | 0.45-4.30 | 0.571 | 1.00 | 0.31-3.27 | 0.994 |
| Time M1 and FQ | 3.22 | 0.69-15.01 | 0.137 | 4.27 | 0.95-19.23 | 0.059 |
| Time M3 and CFX | 2.09 | 0.74-5.91 | 0.163 | 1.24 | 0.35-4.38 | 0.740 |
| Time M3 and AC | 1.22 | 0.38-3.94 | 0.744 | 0.83 | 0.21-3.33 | 0.792 |
| Time M3 and CVN | 1.06 | 0.29-3.89 | 0.931 | 0.58 | 0.11-3.06 | 0.519 |
| Time M3 and CD | 1.06 | 0.32-3.47 | 0.928 | 1.30 | 0.42-4.00 | 0.644 |
| Time M3 and FQ | 0.47 | 0.04-4.99 | 0.530 | 1.60 | 0.28-9.36 | 0.599 |
| Time treatment overall | ⎯ | ⎯ | 0.207 | ⎯ | ⎯ | 0.082 |
| Weight (large) (REF) | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ |
| Weight (small) | ⎯ | ⎯ | ⎯ | **3.04** | 1.45-6.36 | \*0.003 |
| Weight (medium) | ⎯ | ⎯ | ⎯ | **2.48** | 1.11-5.52 | 0.026 |
| Weight overall | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | 0.005 |
| Multi-dog household | **0.56** | 0.31-0.99 | \*0.045 | ⎯ | ⎯ | ⎯ |

OR=odds ratio; 95% CI=95% confidence interval; *P* values are from the Wald chi-squared test; CFX=cefalexin, AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; Day 0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; MRS=meticillin resistant staphylococci; FQR=fluoroquinolone resistance; \*significant at *P* <0.05

**Table 4.** Multilevel multivariable results for outcomes multidrug resistant (MDR) staphylococci or the presence of coagulase positive staphylococci (CoPS) in samples from 127 dogs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **MDR** | | | **CoPS** | | |
|  | **OR** | **95% CI** | ***P*-value** | **OR** | **95% CI** | ***P*-value** |
| Time D0 (REF) | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ |
| Time End and CFX | **2.35** | 1.21-8.52 | 0.019 | **0.15** | 0.05 - 0.45 | \*0.001 |
| Time End and AC | 0.95 | 0.09-2.26 | 0.341 | 1.20 | 0.35 - 4.03 | 0.776 |
| Time End and CVN | 1.86 | 0.95-7.81 | 0.063 | **0.12** | 0.04 - 0.42 | \*0.001 |
| Time End and CD | 0.39 | 0.23-2.70 | 0.698 | **0.03** | 0.01 - 0.10 | \*0.000 |
| Time End and FQ | **3.04** | 2.11-32.2 | \*0.002 | 0.23 | 0.05 - 1.14 | 0.072 |
| Time M1 and CFX | 0.92 | 0.56-5.06 | 0.358 | 0.53 | 0.15 - 1.88 | 0.329 |
| Time M1 and AC | 0.92 | 0.09-2.34 | 0.361 | 0.65 | 0.19 - 2.19 | 0.487 |
| Time M1 and CVN | 1.08 | 0.58-6.56 | 0.281 | 0.51 | 0.11 - 2.31 | 0.384 |
| Time M1 and CD | 1.18 | 0.65-5.65 | 0.239 | **0.16** | 0.05 - 0.53 | \*0.003 |
| Time M1 and FQ | **1.98** | 1.02-19.97 | \*0.048 | 1.84 | 0.12 - 28.22 | 0.661 |
| Time M3 and CFX | **2.31** | 1.19-9.25 | \*0.021 | 1.14 | 0.28 - 4.59 | 0.857 |
| Time M3 and AC | 0.29 | 0.35-4.22 | 0.768 | 1.72 | 0.44 - 6.78 | 0.439 |
| Time M3 and CVN | 1.78 | 0.90-8.70 | 0.074 | 0.45 | 0.10 - 2.02 | 0.301 |
| Time M3 and CD | 0.18 | 0.26-3.13 | 0.860 | 0.40 | 0.12 - 1.40 | 0.153 |
| Time M3 and FQ | 0.94 | 0.43-10.94 | 0.349 | 0.26 | 0.04- 1.91 | 0.186 |
| Time treatment overall | ⎯ | ⎯ | \*0.03 | ⎯ |  | \*0.001 |
| Pyoderma diagnosis | ⎯ | ⎯ | ⎯ | **6.16** | 2.26 - 16.80 | \*0.000 |
| Recruitment site 1 (REF) | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ |
| Recruitment site 2 | ⎯ | ⎯ | ⎯ | 1.93 | 0.68 - 5.51 | |  | | --- | | 0.22 | |
| Recruitment site 3 | ⎯ | ⎯ | ⎯ | **0.33** | 0.12 - 0.95 | \*0.039 |
| Recruitment site overall | ⎯ | ⎯ | ⎯ | ⎯ | ⎯ | \*0.006 |
| Gender (male REF) | **2.07** | 1.23-3.50 | \*0.006 | ⎯ | ⎯ | ⎯ |
| Dog eats animal faeces | **1.93** | 1.11-3.35 | \*0.02 | ⎯ | ⎯ | ⎯ |
| In-contact been hospitalised2 | **2.01** | 1.12-3.69 | \*0.019 | ⎯ | ⎯ | ⎯ |

1 Within 12 months but more than three months as per enrolment criteria; 2Within 12 months of enrolment; OR=odds ratio; 95% CI=95% confidence interval; *P* values are from the Wald chi-squared test; CFX=cefalexin, AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; Day 0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; MDR=multidrug resistance (resistance to three or more antimicrobial classes); CoPS=coagulase positive staphylococci; \*significant at *P*<0.05

**Figure 1.** The percentage of samples with a) coagulase positive staphylococci (CoPS) or b) *S. pseudintermedius* or c) coagulase negative staphylococci (CoNS) at each time point in each treatment group (error bars=95% confidence intervals (CI)).

a)

b)

c)

CFX=cefalexin, AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; Day 0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; CoPS=coagulase positive staphylococci; CoNS=coagulase negative staphylococci; \*non-overlapping CI (Day 0 compared to End for each antibiotic treatment group).

**Figure 2.** The percentage of samples with a) meticillin resistant staphylococci (MRS) or b) multidrug resistant (MDR) or c) fluoroquinolone resistant staphylococci at each time point for each treatment group and treatment overall (error bars = 95% confidence interval (CI)).

a)

b)

c)

CFX=cefalexin; AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; Day 0=pre-treatment; End=immediately-post-treatment; M1=one-month post treatment; M3=three-months post-treatment; MRS=meticillin resistant staphylococci; MDR=multidrug resistant staphylococci (resistance to three or more antimicrobials); \*non-overlapping CI (Day 0 compared to End for each antibiotic treatment group).

# Supplementary Information

**Further material and methods**

***Multilevel modeling: questionnaire***

About your dog.

1. Age Years Months Weeks  Don’t Know 
   1. Is this age Exact  Estimate
2. Breed Pedigree *(please specify)*

Cross *(please specify)*

1. Sex Male  Female  Neutered
2. How long have you owned him/her?

About your dog’s diet.

1. What is s/he fed? Tinned meat  Dry mixer

(*Tick all that apply)* Dry Complete  Raw chicken  Cooked chicken  Raw red meat

Cook red meat  Don’t Know

Other

1. Is s/he fed commercial dog treats? Never  Rarely  Sometimes  Often  Don’t know
2. Is s/he fed human titbits/ scraps? Never  Rarely  Sometimes  Often  Don’t know
3. Does your dog ever eat stools (faeces)? Never  Rarely  Sometimes  Often  Don’t know  If so what types of stools? *(Please tick all that apply)* Rabbit  Cat  Dog  Horse  Cow  Sheep  Badger  Other

About your household.

1. Are there any other dogs in the household?

Yes  No  Don’t Know  If yes, how many? 1  2  3 or more

1. Do you own any other animals (other than dogs)?

Yes  No  Don’t Know  If yes, what animals? *(Please tick all that apply)*

Cat  Bird  Rabbit

Rodent *(e.g. hamster)*  Reptile *(e.g. snake)*

Don’t know  Other

1. Does anyone in your household work with farm animals?

Yes  No  Don’t know  If yes, please state which species are worked with

1. Has anyone in your family (including other pets) to your knowledge in the last month taken antibiotics?

Yes  No  Don’t know

* 1. If yes, was this a Family Member  Pet
  2. Which antibiotic was prescribed *(if known)*

1. Does anyone in your household work in medical or veterinary healthcare?

Yes No  Don’t Know

* 1. If yes, in what setting? Hospital  Community Nursing

GP surgery  Nursing Home

Dentist  Veterinary practice

Don’t Know  Other

1. Has anyone in your household attended hospital in the last month?

Yes  No  Don’t Know

* 1. If yes, why? Admission to hospital  Visit

Outpatient appointment  Don’t Know

Other

**Further results**

***Prevalence of staphylococci***

Supplementary Table S1: The percentage of samples with CoPS, *S. pseudintermedius*, *S. aureus*, *S. schleiferi coagulans*, CoNS or where ‘no staphylococci’ were detected, at each time point for each treatment group and treatment overall (error bars=95% confidence interval)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Time and total number of samples** | **Percentage of positive samples (95% CI)** | | | | | |
|  |  | CoPS | SP | SA | SSC | CoNS | NG |
| **CFX** | **Day 0**  **(n=31)** | 67.7  (51.3-84.2) | 67.7  (51.3-84.2) | 6.45  (0-15) | 3  (0.6-16) | 61.3  (44-78.4) | 13  (1-24.7) |
|  | **End**  **(n=31)** | 45.2  (27.6-62.7) | 41.9  (24.6-59.3) | 3.2  (0-9.5) | 3  (0.6-16) | 54.8  (37.3-72.4) | 29  (13-45) |
|  | **M1**  **(n= 26)** | 69.2  (51.4-87) | 65.4  (47-83.7) | 7.7  (0-17.9) | 4  (0.7-19) | 69.2  (51.5-87) | 12  (0-23.8) |
|  | **M3**  **(n=24)** | 79.2  (62.9-95.4) | 75  (57.7-92.3) | 16.7  (0-31.6) | 0 | 58  (38.6-78) | 4.2  (0-12.2) |
| **AC** | **D0**  **(n=29)** | 58.6  (40.7-76.6) | 58.6  (40.7-76.6) | 10.3  (0-21.4) | 0 | 48.3  (30-66.5) | 20.7  (6-35.4) |
|  | **End**  **(n=29)** | 58.6  (40.7-76.6) | 48.3  (30-66.5) | 6.9  (0-16) | 0 | 72.4  (56.2-88.7) | 3.5  (0-10) |
|  | **M1**  **(n=27)** | 55.6  (36.8-74.3) | 52  (33-70.7) | 7.4  (0-17.3) | 0 | 51.9  (33-70.7) | 11  (0-23) |
|  | **M3**  **(n=25)** | 64  (45.2-82.8) | 56  (36.5-75.5) | 8  (0-18.6) | 4  (0.7-19) | 56  (36.5-75.5) | 16  (1.6-30.4) |
| **CVN** | **D0**  **(n=26)** | 88.5  (76.2-100) | 84.6  (70.8-98.5) | 7.7  (0-17.9) | 4  (0.7-19) | 50  (30.8-69.2) | 3.9  (0-11.2) |
|  | **End**  **(n=26)** | 57.7  (38.7-76.7) | 38.5  (19.8-57.2) | 7.7  (0-17.9) | 4  (0.7-19) | 61.5  (42.8-80.2) | 11.5  (0-23.8) |
|  | **M1**  **(n=20)** | 75  (56-94) | 60  (38.5-81.5) | 10  (0-23) | 10  (3-30) | 60  (38.5-81.5) | 5  (0-14.6) |
|  | **M3**  **(n=21)** | 76.2  (58-94.4) | 47.6  (26.3-69) | 14.3  (0-29.3) | 5  (0.9-23) | 47.6  (26.3-69) | 0 |
| **CD** | **D0**  **(n=28)** | 78.6  (63.4-93.8) | 71.4  (54.7-88.2) | 7  (0-16.7) | 4  0.6-18 | 60.7  (42.6-78.8) | 0 |
|  | **End**  **(n=28)** | 21.4  (6.2-36.6) | 17.9  (3.7-32) | 3.6  (0-10.5) | 0 | 60.7  (42.6-78.8) | 32  (14.8-49.4) |
|  | **M1**  **(n=24)** | 45.8  (25.9-65.8) | 45.8  (25.9-65.8) | 12.5  (0-25.7) | 0 | 50  (30-70) | 16.7  (1.8-31.6) |
|  | **M3**  **(n=24)** | 62.5  (43-81.9) | 62.5  (43-81.9) | 8.3  (0-19.4) | 0 | 58  (38.6-78) | 12.5  (0-25.7) |
| **FQ** | **D0**  **(n=13)** | 69.2  (44-94.3) | 61.5  (35-88) | 23  (0-46) | 8  (1.4-33) | 53.9  (26.8-81) | 15.4  (0-35) |
|  | **End**  **(n=13)** | 53.9  (26.8-81) | 53.85  (26.8-81) | 7.7  (0-22) | 8  (1.4-33) | 76.9  (54-99.8) | 15.4  (0-35) |
|  | **M1**  **(n=9)** | 88.9  (68.4-100) | 77.8  (50.6-100) | 22.2  (0-49.4) | 11  (2-44) | 66.7  (35.9-97.5) | 11  (0-31.6) |
|  | **M3**  **(n=9)** | 55.6  (23-55.6) | 55.6  (23-88) | 22  (0-49.4) | 11  (2-44) | 77.8  (50.6-100) | 0 |
| **All** | **D0**  **(n=127)** | 72.4  (64.7-80.2) | 70  (62-78) | 9.5  (4.4-14.5) | 4  (1.2-7.8) | 55  (46.5-63.8) | 10.2  (5-15.5) |
|  | **End**  **(n=127)** | 46.5  (37.7-55) | 38.6  (30-47) | 5.5  (1.5-9.5) | 2  (0.8-7) | 63.8  (55.4-72) | 18.9  (12-25.7) |
|  | **M1**  **(n=106)** | 63.2  (54-72.4) | 57.6  (48-67) | 10.4  (4.6) | 4  (1.5-9) | 58.5  (49-67.9) | 12.3  (6-18.5) |
|  | **M3**  **(n=103)** | 68.9  (60-77.9) | 60.2  (50.7-69.7) | 12.6  (6.2-19) | 3  (1-8) | 57.3  (47.7-66.8) | 7.8  (2.6-13) |
| **Total % of samples** | **(n=463)** | 62  (57.9-66.7) | 56  (51.8-60.8) | 9  (7.0 – 12.3) | 3  (1.8-5) | 59  (54-63) | 12  (9.5-15.7) |
| **Total % of dogs** | **(n=127)** | 87  (80.5-92.2) | 83  (75.2-88.3) | 21  (14.5-29.4) | 7  (4-13) | 93  (87-96.2) | 35  (26.4-43.6) |

D0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; CFX=cefalexin; AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone (marbofloxacin or enrofloxacin); All=all treatment groups; CoPS=coagulase positive staphylococci, SP=*S. pseudintermedius*, SA=*S. aureus*, SSC=*S. schleiferi coagulans*; CoNS=coagulase negative staphylococci, NG=no growth of staphylococci

***Prevalence of antimicrobial resistance***

Supplementary Table S2: Percentage of samples with MRS, MDR, fluoroquinolone resistant staphylococci and CoPS-MRS, MRSP, CoPS-MDR or CoPS-fluoroquinolone resistant staphylococci in each treatment group and for treatment overall at each time point (95% confidence interval)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Time and number of samples** | **Percentage of positive samples (95% CI)** | | | | |
|  |  | **MRS** | **MDR** | **FQR** | **MR-CoPS** | **MRSP** |
| **CFX** | **Day 0 (n=31)** | 19.4  (5.5-33.3) | 9.7  (0-20.1) | 12.9  (1.1-24.7) | 0 | 0 |
|  | **End**  **(n=31)** | 35.5  (18.6-52.3) | 32.3  (15.8-48.7) | 22.6  (7.9-37.3) | 0 | 0 |
|  | **M1**  **(n=26)** | 30.8  (13-48.5) | 26.9  (9.9-44) | 26.9  (9.9-44) | 8  (2-24) | 8  (2-24) |
|  | **M3**  **(n=24)** | 33.3  (14.5-52.2) | 37.5  (18-56.9) | 16.7  (1.8-31.6) | 8  (2-26) | 8  (2-26) |
| **AC** | **D0**  **(n=29)** | 10.3  (0-21.4) | 13.8  (1.2-26.3) | 13.8  (1.2-26.3) | 3  (0.6-17) | 3  (0.6-17) |
|  | **End**  **(n=29)** | 13.8  (1.2-26.3) | 13.8  (1.2-26.3) | 10.3  (0-21.4) | 3  (0.6-17) | 3  (0.6-17) |
|  | **M1**  **(n=27)** | 14.8  (1.4-28.2) | 22.2  (0-37.9) | 3.7  (0-10.8) | 0 | 0 |
|  | **M3**  **(n=25)** | 24  (7.3-40.7) | 20  (4.3-35.7) | 12  (0-24.7) | 0 | 0 |
| **CVN** | **D0**  **(n=26)** | 23.1  (6.9-39.3) | 30.8  (13-48.5) | 19.2  (4-34.4) | 4  (0.6-19) | 4  (0.6-19) |
|  | **End**  **(n=26)** | 38.5  (19.8-57.2) | 46.2  (27-65.3) | 26.9  (9.9-44) | 12  (4-29) | 8\*  (2-24) |
|  | **M1**  **(n=20)** | 15  (0-30.7) | 30  (9.9-50.1) | 15  (0-30.7) | 5  (0.8-24) | 5  (0.8-24) |
|  | **M3**  **(n=21)** | 23.8  (5.6-42) | 42.9  (21.7-64) | 9.5  (0-22) | 10  (2.7-29) | 10  (2.7-29) |
| **CD** | **D0**  **(n=28)** | 25  (9-41) | 25  (8-41) | 17.9  (3.7-32) | 0 | 0 |
|  | **End**  **(n=28)** | 17.9  (3.7-32) | 17.9  (3.7-32) | 7.14  (0-16.7) | 0 | 0 |
|  | **M1**  **(n=24)** | 29.2  (11-47.4) | 37.5  (18-56.9) | 20.8  (4.6-37) | 4  (0.7-20) | 4  (0.7-20) |
|  | **M3**  **(n=24)** | 25  (7.7-42.3) | 25  (7.7-42.3) | 25  (7.7-42) | 4  (0.7-20) | 0\* |
| **FQ** | **D0**  **(n=13)** | 30.8  (5.7-55.9) | 38.5  (12-64.9) | 15.4  (0-35) | 8  (1.4-33) | 8  (1.4-33) |
|  | **End**  **(n=13)** | 69.2  (44-94.3) | 69  (44-94.3) | 46.2  (19-73.3) | 8  (1.4-33) | 8  (1.4-33) |
|  | **M1**  **(n=9)** | 44.4  (12-77) | 55.6  (23.1-88) | 44.4  (12-76.9) | 11  (2-44) | 11  (2-44) |
|  | **M3**  **(n=9)** | 11  (0-31.6) | 44.4  (12-77) | 22.2  (0-49.4) | 0 | 0 |
| **All** | **D0**  **(n=127)** | 20.5  (13.5-27.5) | 21.3  (14-28.4) | 15.8  (9.4-22) | 2  (0.8-8) | 2  (0.8-8) |
|  | **End**  **(n=127)** | **30.7**  **(22.7-38.7)** | 31.5  (23.4-39.6) | 19.7  (12.8-26.6) | 3  (1.2-8) | 3  (1.2-8) |
|  | **M1**  **(n=106)** | 24.5  (16.3-32.7) | 31  (22.3-40) | 18.9  (11.4-26.3) | 5  (2-11) | 5  (2-11) |
|  | **M3**  **(n=103)** | 25.2  (16.9-33.6) | 32  (23-41) | 16.5  (9.3-23.7) | 4  (2-10) | 4  (2-10) |
| **Total % of samples** | **(n=463)** | 25  (21.5-29.4) | 29  (24.8-33) | 18  (14.5-21.4) | 4  (2-5.6) | 3  (2-5.5) |
| **Total % of dogs** | **(n=127)** | 62  (53.5-70.2) | 65  (56-72.3) | 48  (40-56.7) | 10  (6-17) | 9  (5-15) |

D0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three-months post-treatment; CFX=cefalexin; AC=clavulanate-amoxicillin; CVN= cefovecin; CD=clindamycin; FQ=fluoroquinolone (marbofloxacin or enrofloxacin); All=all treatment groups; MRS=*mecA* gene positive phenotypic oxacillin resistance; MDR=multidrug resistant (resistance to three or more antimicrobial classes); FQR=fluoroquinolone resistant; MR\_CoPS=meticillin resistant coagulase positive staphylococci, MRSP=meticillin resistant *S. pseudintermedius*; MDR\_CoPS=coagulase positive and multidrug resistant staphylococci; FQR\_CoPS=coagulase positive and fluoroquinolone resistant staphylococci; \**S. aureus* was detected from a single dog at these treatment group/time-points.

Supplementary Figure S3: The percentage of samples with a) clindamycin or b) trimethoprim sulfamethoxazole resistant staphylococci at each time point for each treatment group and treatment overall (error bars=95% confidence interval (CI)).

a)

b)

CFX=cefalexin; AC=clavulanate-amoxicillin; CVN=cefovecin; CD=clindamycin; FQ=fluoroquinolone; Day 0=pre-treatment; End=immediately-post-treatment; M1=one-month post-treatment; M3=three=months post-treatment; non-overlapping CI