Corticosteroid Treatment and Methicillin, Quinolone or Multidrug Resistance in Dogs

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ABSTRACT

Antimicrobial resistance is of great concern in human and veterinary medicine. Corticosteroid treatment is often administered together with antibiotics or prior to antibiotic treatment which might influence antibacterial resistance to *Staphylococcus pseudintermedius*. The objective of this study was to examine whether there is an association between corticosteroid treatment and either methicillin, quinolone or multidrug resistance to *Staphylococcus pseudintermedius* in dogs presented to the Dermatology Department of the veterinary teaching hospital. Medical records were collected from 138 dogs with pyoderma and otitis externa from which *Staphylococcus pseudintermedius* was isolated. The bacteria was isolated by bacterial culture and sensitivity tests were performed. Information regarding prior systemic or topical antimicrobial exposure and prior systemic or topical corticosteroid exposure was collected. An association between antibiotic treatment (AB) and multidrug resistance (MDR) was found. There was a significant association between Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and prior antibiotic treatment and similarly between quinolone resistance and prior antibiotic treatment ($p<0.05$). More MDR cases received antibiotics within one month prior to culture and sensitivity (75.4%) in comparison to all other time frames ($p<0.05$). An association between intact female dogs and MRSP was found ($p<0.01$). Of the intact female dogs, 92.3% were MRSP, 76.9% of intact females were MDR ($p<0.01$). No association was found between prior corticosteroid treatment and MRSP, or quinolone resistance or MDR. An effort was made in order to expose potential confounding variables but none were found. Since a clear association was found between prior antibiotic treatment and resistant *S. pseudintermedius*, while no association was found between any prior treatment with corticosteroids and MRSP, quinolone resistance or MDR, we concluded that either there is no association between corticosteroids and bacterial resistance, or, at the very least, there is a weaker association than the association between antibiotic treatment and bacterial resistance.

Keywords: Antimicrobial Resistance; Multidrug Resistance; Corticosteroid Therapy; *Staphylococcus pseudintermedius*; Canine.

INTRODUCTION

Antibacterial resistance is of global concern in human and veterinary medicine (1, 2, 3, 4, 5). In veterinary medicine, methicillin resistance has been recognized as a serious and widespread problem. The primary cutaneous pathogen of dogs, and probably cats, has been recognized as the coagulase positive species *Staphylococcus pseudintermedius* (1, 6, 7, 8). Systemic antibiotics are commonly prescribed for superficial and deep pyoderma cases, while otitis externa cases are mostly treated with topical antibiotics (1, 9). Most methicillin resistant *Staphylococcus pseudintermedius* (MRSP) isolates co-express resistance to several other classes of antimicrobials,

such as the fluoroquinolones, macrolides, tetracyclines and aminoglycosides (2), commonly known as multi-drug resistance (MDR). This situation presents a serious therapeutic challenge.

Published studies have reported an association between antibacterial treatment and bacterial resistance (8, 10, 11). One study claims that the concurrent use of an immunomodulatory drug may be associated with an increased likelihood of MRSP isolation (8) and 3 other studies found no association (10, 12, 13). Since skin infections are common in allergic dogs, treatment for allergy often involves the use of immunomodulatory drugs such as corticosteroids along with the antibiotic treatment for secondary skin or ear infection. In this retrospective study, we investigated whether the use of corticosteroids concurrently or prior to antibacterial treatments was associated with antibacterial resistance found in dogs presented to the Dermatology Department at the veterinary teaching hospital (KSVM-VTH).

MATERIALS AND METHODS

Cases Included in the Study

Dogs presented to the Dermatology Department between the years 2007-2013 that were initially suspected to be infected with *Staphylococcus pseudintermedius* on cytology, and later confirmed by positive microbial isolations, were included. Availability of complete anti-bacterial susceptibility results and history of treatments were required for inclusion in the study. Cases from which mixed bacterial cultures were reported were excluded, as were cases that lacked detailed information concerning prior therapeutic regimens. All cases fulfilling these criteria were included.

Details regarding prior use of antimicrobial products were obtained from the history form in the case records and/or referral letter in cases where treatment had not been prescribed at the KSVM-VTH. Data collected included the drug(s) prescribed, the total number of exposures to each drug, duration of each therapeutic regimen and the time elapsed since last exposure.

Bacteriological Methods

Specimens from the cohort of cases were submitted for culture and susceptibility tests in accordance with published guidelines (14). Pyoderma cases were sampled from pustules and/or epidermal collarettes, as described (15, 16), whereas otitis externa cases were sampled from the distal horizontal canal using sterile cotton swabs. Samples were inoculated into Amies Transport Medium without charcoal (Copan, Brescia, Italy) and were submitted within 24 hours to the diagnostic laboratory at the Department of Clinical Bacteriology and Mycology, The Kimron Veterinary Institute, Veterinary Services, Ministry of Agriculture, The State of Israel. Isolation and identification of bacteria was completed in accordance with standard methods (17) and the disk diffusion test was used for antimicrobial susceptibility testing in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines.

Data Classification and Statistical Analysis

Details regarding prior antimicrobial treatment and/or prior corticosteroid treatment were obtained from the medical records at the KSVM-VTH. Data collected included the following: signalment, site of samples (skin or ear canal or both), antibacterial and corticosteroid treatment methods (topical, systemic or both), year of *Staphylococcus pseudintermedius* isolation, resistance to methicillin and/or resistance to quinolone and/or multidrug resistance to 3 or more drugs (MDR). Antibacterial treatment prior to bacterial and sensitivity culture were classified as follows; >1 month, 1-3 months, 3-6 months, >6 months or never received antibiotics. Corticosteroid treatment prior to bacterial and sensitivity culture were classified as follows; >1 month, 1-3 months, 3-6 months, >6 months or never received corticosteroids. Data on other immunosuppressive drugs, topical or systemic (if any) were also collected.

Statistical Methods

The data was evaluated for normality of continuous parameters (age) by comparing the mean to median and visually by inspection of the histogram. In order to compare continuous variables (e.g. age) between two groups (e.g. MDR positive or not), the two sample Student t-test or Mann-Whitney U test was used, as appropriate for the distribution. In order to test the association between categorical variables Chi square or the Fisher's exact test was used, as appropriate. Trends were evaluated by the Mantel-Haenszel Chi squared test for trend. To adjust for confounding variables, a logistic regression model employing the enter method was used. Statistical significance was set at $p<0.05$. Calculations were made using IBM SPSS version 21 statistical software.

Table 1: Sex of the study population

Sex	Percentage
Intact male	33.3%
Neutered females	31.9%
Neutered males	25.4%
Intact females	9.4%

Table 2: Effect size based on logistic regression model

Odds ratios and 95% confidence intervals for the significant associations with antibiotic resistance (MRSP, MDR and Quinolone Resistance) are listed in the following table:

Logistic regression models for bacterial resistance	Odds ratio for resistance	95% confidence interval of Odds ratio
MRSP: Intact female	14.5	1.6-127.3
MDR: Intact female	4.7	1.1 – 21.1
Antibiotic treatment	3.7	1.1-12.5
Quinolone resistance: Antibiotic treatment	3.1	1.1-9.0

Models included time from antibiotic treatment to culture and sensitivity and sex. MRSP- Methicillin-resistant *Staphylococcus pseudintermedius*, MDR- Multidrug-resistant.

RESULTS

Population Characteristics

The most common breed in the group was German Shepherds (13.8%). The mean age was 6.3 years (95% confidence interval (95%CI) 5.7-6.9). The sex distribution of the study population is listed in Table 1. The sample locations were from skin, ears or both 46.4%, 9.6%, 44.2%, respectively.

Between the years 2007-2013, *Staphylococcus pseudintermedius* was isolated in 138 dogs out of approximately 1,700 cases in which bacteria were identified by cytology from the skin or ear canals. Sixty percent of the dogs received systemic corticosteroids and 2.2% topical corticosteroids prior to culture and sensitivity. Seventy one percent of the dogs received systemic antibiotics and 0.7% received topical antibiotics prior to culture and sensitivity. Sixty one (60.9%) percent of the dogs received antibiotics within one month prior to culture and sensitivity, 13% between 1-3 months, and 12.3% between 3-6 months. Fourteen percent (13.8%) did not receive any antibiotics or received antibiotics more than 6 months prior to culture and sensitivity.

Almost forty nine percent (48.6%) of the dogs had quinolone resistant *S. pseudintermedius,* 50% were positive for MRSP and 41.3% were MDR positive.

Associations Between Antibiotic Treatment and MDR

An association was found between antibiotic treatment and multidrug resistance (MDR). Of cases in which MDR was diagnosed 52.4% received antibiotics within one month prior to culture and sensitivity which was significantly greater than in any other time interval ($p<0.05$). Of those dogs that received no antibiotics at all or received antibiotics greater than 6 months prior to culture and sensitivity, 20% were found to have MDR to *S. pseudintermedius*. Of the cases which received antibiotics, both 1-3 months or 3-6 months prior to culture and sensitivity were found to have MDR staphylococ-

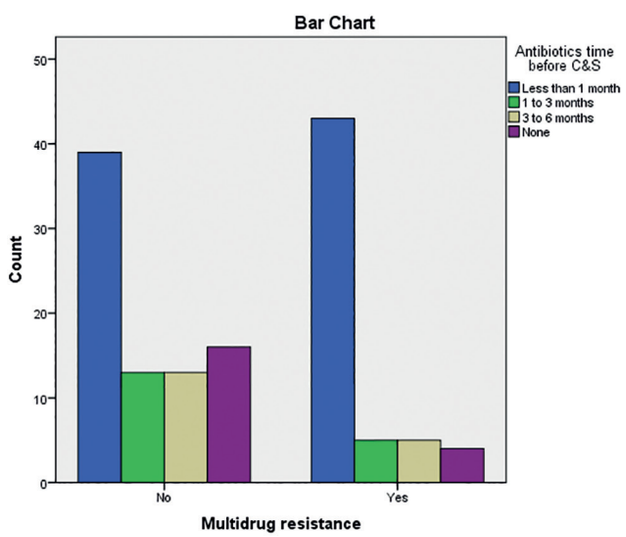

Figure 1: The number of dogs with or without multi drug resistance (MDR) according to antibiotics time before culture and sensitivity.

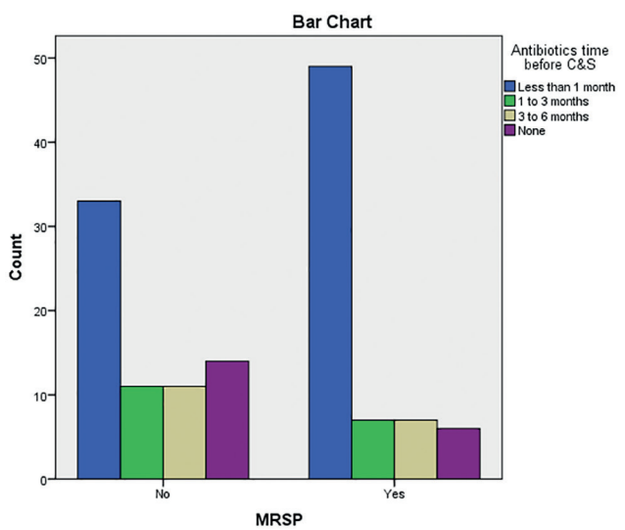

Figure 2: The number of dogs with or without MRSP positive isolations according to antibiotics time before culture and sensitivity.

cus (27.8%), showing a significant trend so that the longer the time between AB treatment and culture and sensitivity, the lower the rate of MDR ($p<0.01$) (Figure 1).

Association Between MRSP and Prior Antibiotic Treatment

An association between MRSP and prior antibiotic treatment was found ($p<0.05$). Of cases in which MRSP was cultured, 71% had received antibiotics within one month of culture and sensitivity, 10.1% between 1-3 months and 10.1% between 3-6 months. Of those that received antibiotics within 1 month of culture and sensitivity, 59.8% were MRSP positive. A significant trend was found, in that the longer the time between antibiotic treatment and culture and sensitivity, the lower the rate of MRSP ($p<0.01$) (Figure 2).

Association Between Quinolone Resistance and Prior Antibiotic Treatment

An association between quinolone resistance and prior antibiotic treatment was found ($p<0.05$). Of the quinolone resistant cases, 71% received antibiotics within one month prior to culture and sensitivity. Of those that received antibiotics within 1 month of culture and sensitivity, 59.8% were positive for quinolone resistance, 44.4% between 1-3 months and 33.3% between 3-6 months. A trend was found indicating that the longer the time lapse between culture and antibiotic treatment, the lower the rate of quinolone resistance ($p<0.01$). (Figure 3).

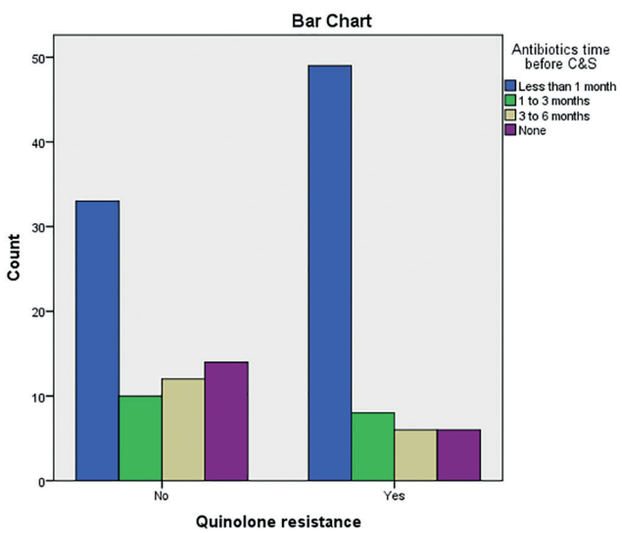

Figure 3: The number of fluoroquinolone resistance according to antibiotics time before culture and sensitivity.

Confounding Variables

No association was found between age or year of culture and sensitivity on drug resistance, however, there was a significant association between sex and drug resistance. (Figure 4).

Association Between Intact Female Dogs and Bacterial Resistance

A significantly larger proportion of resistant bacteria (MRSP) was found in intact females than for any other sex group (Figure 4). Similar results were found for MDR, however, this did not hold true for quinolone resistant bacteria (Table 2). When logistic regression models were constructed that included both time of administration of antibiotic and sex, in the case of MRSP isolation, only variable for sex remained in the model. In the case of quinolone resistance, only antibiotic time remained in the model, and in the case of MDR, both antibiotic time of administration and sex remained in the model. The effect sizes (odds ratios) for the significant associations are listed in table 2.

Prior Corticosteroid Treatment and Drug Resistance

No association was found between corticosteroid treatment and drug resistance. No confounding variables were found, except that statistically significantly more cases that received corticosteroids, had also received systemic antibiotics prior to culture and sensitivity ($p<0.01$) but still no association was found between corticosteroid treatment and drug resistance. When both variables were included in a logistic regression

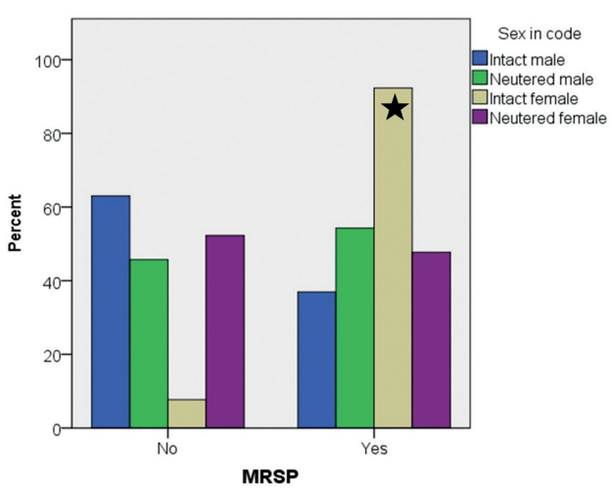

Figure 4: MRSP Positive and Negative According to Dog Sex. ★ = significant p-value <0.01

model, however, only systemic antibiotics prior to culture and sensitivity remained significant. Also, more intact females received systemic corticosteroid treatment (84.6% compared to 60% in all other sex categories) but this was without statistical significance ($p>0.05$).

Attempts to Find an Association Between Antibiotics and Corticosteroids

Since it is difficult to find statistical correlation with many groups, we reduced the number of categories to two, by combining them into corticosteroid administration <1 month prior to culture and sensitivity or >6 months prior to culture and sensitivity, however no associations were found to bacterial resistance.

After exclusion of topical corticosteroid treatment, no associations were found between systemic corticosteroids and quinolone or MRSP. Sixty percent of otitis externa cases with quinolone resistance received corticosteroid treatment less than one month prior to culture and sensitivity but no statistical association was found. All otitis externa cases with MDR received corticosteroids less than one month prior to culture and sensitivity, therefore no assessment of association could be carried out.

We excluded otitis externa cases and examined only pyoderma cases and no associations were found to administration of corticosteroids and bacterial resistance.

In order to evaluate whether the influence of corticosteroids was more significant when closer in time to the culture and sensitivity, we grouped the corticosteroid time prior to culture and sensitivity to <1 month and >1 month but no associations were found either with topical or with systemic corticosteroids.

DISCUSSION

The purpose of this study was to investigate whether there was an association between glucocorticoid treatment and antibiotic resistance in dogs. No association was found. During this investigation, we found significant associations regarding antibiotic treatment and resistance. These findings are consistent with previous studies about the association between prior antibiotic administration and resistance (8, 10, 11). We also found significant temporal trends in that the closer in time that the antibiotic treatment was administered in comparison to the sampling time (1 month, 1-3 months, 3-6 months, >6 months), the higher the rate of MRSP or fluoroquinolone resistance, as well as MDR isolation. Temporal trends support the hypothesis that prior antibiotic treatment actually causes resistance (18).

An unexpected finding in this study was an association between intact females and MDR isolation that was retained in a logistic regression model, along with antibiotic treatment, and between intact females and MRSP isolation that was retained in the model, while the antibiotic treatment was rejected. The odds ratio of 14.5, indicating that intact females have 14.5 times the odds of MRSP isolation, is a strong effect, however the 95% confidence interval is quite wide indicating a lack in precision of the results. Further studies are needed with a larger sample size in order to achieve more precise results. On the other hand, sex was not retained in the model of antibiotic treatment and quinolone resistance.

We did not find any reasonable explanation as to why intact female dogs were more prone to resistance. Weese *et al.,* 2012, found an association between castrated male dogs and MRSP infections but not with intact females and they also had no explanation for these findings (10).

No significant association was found between corticosteroid treatment and antibiotic resistance, despite the fact that more intact females were treated with corticosteroids, considering the association found between resistance to antibiotics and intact females, as pointed out in the previous paragraph. Four previous studies examined the effect of immunoregulatory drugs on MSRP. Three of them (Weese *et al.,* 2012 (10), Bryan *et al.,* 2012 (12), Beck *et al.,* 2012 (13)) also found no association between prior use of immunoregulatory drugs and bacterial resistance, however, the fourth study, Hensel *et al.,* 2016, found a significant association between prior use of immunoregulatory drugs and MRSP isolation (8). The authors propose that immunomodulatory drugs can mask clinical signs of pyoderma and mimic a resolution of clinical signs and that this could lead to a shorter duration of antibiotic therapy, thereby, promoting survival of more resistant strains. However, in their research the authors did not find an association between a shorter duration of antibiotic therapy and an increased likelihood of MRSP. The contradictory results of these studies may be explained by differences between the populations. As in the study reported here, the Weese *et al.* study included *S. pseudintermedius* infections from various body sites (10), while *Hensel et al.* sampled only pyoderma cases (8). In the Weese *et al.* study, most of the cases (69.6%) were from skin and ears

(10), which is similar to the study reported here. Also, the sample size in this study is larger, with 138 dogs, as compared to 53 dogs in the Hensel study which favors the results of this study (19). Therefore, perhaps the association found between immunomodulatory drugs and antibacterial resistance in the Hensel *et al.* study was limited by size and would have lost its significance in a larger, more comprehensive study. Also, their explanation for their findings were recently put into question by an interesting article in human medicine that claimed that completing a prescribed course of antibiotics to prevent antibiotic-resistance is a myth and not based on evidence (20). They considered that actually the opposite may to be true, namely, that taking antibiotics for longer than necessary increases the risk of developing resistance, which could be the same for veterinary medicine. This goes counter to the claim by Hensel *et al.* that shortening the duration of antibiotic therapy promotes survival of more resistant strains and can therefore explain why corticosteroids, given concurrently, are associated with resistance.

Of the two additional studies, mentioned above, Bryan *et al.,* 2012, describe a retrospective study that examined the outcome of MRSP and methicillin-sensitive staphylococcal (MSSP) in dogs diagnosed with pyoderma (12). In their study, allergic dogs were the majority of the population but it also included dogs with hypothyroidism, hyperadrenocorticism, neoplasia, Pemphigus foliaceus and other diseases. The dogs were receiving corticosteroids, cyclosporine and chemotherapeutics drugs at the time of the bacterial isolation. The authors found no association between these medications and the diagnosis of MRSP or MSSP pyoderma infections. They also found that cases with MRSP pyoderma and concurrent corticosteroid use were significantly associated with lack of resolution at the first recheck examination. They explained that these patients were likely to have an altered immune systems and, therefore may not respond to antimicrobial therapy as effectively as patients with healthy immune systems.

Beck *et al.,* 2012, described a prospective study examining the prevalence of MRSP in dogs diagnosed with pyoderma. They found a trend towards a decreased likelihood of MRSP infection with dogs treated with glucocorticoids or cyclosporine (13). The authors explained that their results were not surprising since 63% of the dogs in their study had atopic dermatitis and by controlling the underlying inflammation and pruritus, secondary infections could be reduced, thereby requiring less antibacterial use. In addition, as a prospective study, the Beck *et al.* study can be considered stronger evidence since retrospective studies suffer from recall bias (19). In fact, in the present study, despite the association between females and bacterial resistance and the fact that there were more females in the corticosteroid treated group, this fact did not result in confounding, indicating that possibly, the association between corticosteroids and resistance was a negative association, that had been lost in the confounding due to the preponderance of females in the group.

In many dermatological cases, it is common to treat with antibiotics concurrently with corticosteroids and so it is not surprising to find an association with MSRP cases. Recently a guideline with recommendations for approaches to MRS infections in small animals was published (2). No recommendation has been made regarding the use of glucocorticoids in relation to bacterial resistance. We hypothesize that what influences bacterial resistance is the use of antibiotics alone and that glucocorticoids have no contribution to that resistance. Moreover, we hypothesize also that if an association were to be found between corticosteroids and bacterial resistance in the future, that association will be weaker than the association between antibiotic treatment and bacterial resistance.

As a retrospective study, this study has its limitations. Many dogs were excluded because they did not fulfill all of the inclusion criteria. We did not evaluate the doses of antibiotics and corticosteroids in the study. However, review of all the medical records in this study, confirmed that, on the whole, antibiotics were given at the recommended dosages and that corticosteroids were given at an anti-inflammatory dose and not an immunosuppressive dose. In addition, the underlying diseases were not recorded and therefore, not evaluated for their association with culture and susceptibility results.

In summary, we found an association between antibiotic treatment and bacterial resistance as in previous studies, however, we did not find any association between prior treatment with corticosteroids and MRSP, quinolone resistance or MDR in this retrospective study. We also found an association between intact females and an increased risk for antibiotic resistance. The significance of this finding is unclear at this time.

CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

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