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Identification and Antimicrobial Susceptibility of Microorganisms Isolated from the Preputium of Healthy Dogs

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Abstract: This study was designed to study the microbiological status of the foreskin mucosa in 100 healthy dogs and determine their antibiotic sensitivity. The most frequently identified organism in the dog foreskin was *Staphylococcus* sp. (36%) followed in order of frequency by *E. coli* (30%), *Proteus* sp. (16%), *Pseudomonas* sp. (6%), *Streptococcus* sp. (6%), *Bacillus* sp. (4%) and *Corynebacterium* sp. (2%). The isolated strains were subjected to antibiotic susceptibility testing. *Staphylococcus* sp., *E. coli*, *Streptococcus* sp., *Bacillus* sp. and *Corynebacterium* sp. were very frequently susceptible to danofloxacin, amoxicillin/clavulanic acid, cloxacillin, amoxicilin and ceftiofur. It is concluded that the use of danofloxacin, amoxicillin/clavulanic acid, cloxacillin, amoxicilin and ceftiofur can be useful in the treatment of infections of the male genital organ.

Key words: Infection, antibiotic sensitivity, amoxicillin, treatment, male genital organ, Turkey

INTRODUCTION

Infections of the penis and foreskin are relatively frequent in the dog; they represent 20% of canine penile and preputial lesions. The slightly purulent preputial oozing observed in the normal adult dog is not a result of penile or preputial inflammation (Boothe, 2003).

Doig *et al*. (1981) investigated the mycoplasma and ureaplasma flora in healthy and sick dogs. They identified mycoplasma in 85% of 136 preputial swabs. It is reported that the presence of this organism is significantly more frequent in infertile than in healthy animals. It is also observed that mycoplasma are often found as saprophytes in the lower genital organs of both male and female animals (Doig *et al*., 1981).

Ling and Ruby (1978) studied aerobic bacterial flora in samples collected from the preputium and penis in a study performed in 20 healthy dogs. The researchers identified 14 different species of microorganisms in this study. They report a frequency of 60% for *Staphylococcus aureus* and 35% for *Mycoplasma* sp. Another report indicates that *Mycoplasma* sp. were isolated in six of twelve healthy dogs (Rosendal, 1973). In yet, another study performed in 15 stud dogs, samples were collected at various times over a period of a year and a half. No bacteria were isolated in 33 out of 232 (14%) preputial swab samples. *Pasteurella multocida* was identified in all subjects. Beta-hemolytic streptococci were isolated from 32.8% of 76 preputium samples. *Pseudomonas* sp. were isolated in one subject and *Streptococcus* sp. in four. The bacteria isolated from the dogs' preputium and urethra were reported to be opportunistic pathogens (Bjurstrom and Linde-Forsberg, 1992).

Bibliography searches showed that reports describing the preputial flora of healthy dogs was relatively limited. The purpose of this study was to determine the bacterial flora of the preputial mucosa of healthy dogs brought to the hospital and the antibiotics to which isolated microorganisms are susceptible thus, identifying the antibiotic groups which can be used in the male genital organ infections.

MATERIALS AND METHODS

Preputial swab sampling of the dogs: The material for this study consisted of 100 swab samples taken from the foreskin of healthy dogs that were brought to the Surgery Department of the Afyon Kocatepe University Veterinary Faculty. Preputium swab samples were collected as follows: The dogs were held in supine position, their penis freed from the preputium and swabbed in a circular motion from the penile basis to the preputium.

The preputial swabs were placed in transport medium and brought under refrigeration to the Microbiology Department Laboratory of Afyon Kocatepe University Veterinary Faculty.

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Table 1: Bacteria isolated from preputial swab samples of dogs

Bacteria	n	Percentage
Staphylococcus sp.	18	36.0
E. coli	15	30.0
Proteus sp.	8	16.0
Pseudomonas sp.	3	6.0
Streptococcus sp.	3	6.0
Corynebacterium sp.	1	2.0
Bacillus sp.	2	4.0
Total	50	100.0

Isolation and identification of aerobic bacteria from preputial swabs: The samples collected from dog foreskins on sterile swabs were inoculated on blood agar (Oxoid CM55), MacConkey agar (Oxoid CM0109) and Eosin Methylene Blue (EMB) sgar (Oxoid CM0069) and incubated for 24-48 h under aerobic conditions at 37°C. The colony morphology and hemolysis characteristics of growth on blood agar were examined. Pure cultures were obtained by blood agar passage after gram staining of the first colonies. The identification of isolated bacteria followed classical methods as shown in Table 1 (Quinn *et al*., 2002; Holt *et al*., 1994).

Antimicrobial susceptibility testing: *In vitro* antimicrobial susceptibility testing was conducted using the Disk Diffusion Method on Mueller Hinton Agar, according to the National Committee for Clinical Laboratory Standards (Thomas and NCCLS, 2002). The antimicrobial susceptibility of isolated bacteria was tested for danofloxacin, rifaximin, amoxicillin/clavulanic acid, cloxacillin, amoxicillin, penicillin, erythromycin, streptomycin, ceftiofur (Oxoid Ltd., Basingstoke, UK).

RESULTS AND DISCUSSION

The most frequently identified organism in the dog foreskin was *Staphylococcus* sp. (36%) followed in order of frequency by *E. coli* (30%), *Proteus* sp. (16%), *Pseudomonas* sp. (6%), *Streptococcus* sp. (6%), *Bacillus* sp. (4%) and *Corynebacterium* sp. (2%). The prevalence of microorganisms isolated from the healthy dog foreskin was found to be statistically significant ($p<0.05$). The isolated strains were subjected to antibiotic susceptibility testing. *Staphylococcus* sp., *E. coli*, *Streptococcus* sp. *Bacillus* sp. and *Corynebacterium* sp. were very frequently susceptible to danofloxacin, amoxicillin/ clavulanic acid, cloxacillin, amoxicilin and ceftiofur.

Limited information is available on the preputial flora of male dogs. Thats why this study was performed to identify the preputial aerobic flora of healthy dogs brought to the hospital.

Bjurstrom and Linde-Forsberg (1992) have reported that bacteria isolated from the foreskin of male dogs are similar to those found in the genital organs of female dogs inhabiting the same shelter (*P. multocida*, β-hemolytic streptococci and *E. coli*) (Table 1).

Elad *et al.* (1992) reported the presence of Corynebacterium group D2, *Pseudomonas aeruginosa* and *Proteus* sp. in one of two dogs with urinary tract infection while *P. aeruginosa* and Corynebacterium group D2 were isolated in the other animal. In this study, *Proteus* sp. were isolated in eight, *Pseudomonas* sp. in three and *Corynebacterium* sp. in one of 100 dogs. The study results are similar in this respect to those of Elad *et al.* (1992) even though these authors had isolated the microorganisms from the urine samples of two dogs.

The study by Gouletsou *et al.* (2006) of samples from the scrotal; skin of rams reported the presence of *Staphylococcus* sp. in 48, *Bacillus* sp. in 32 (33%) and *A. pyogenes* in 16. The same researchers report that the organisms frequently isolated from preputial cavity samples are *Staphylococcus* sp. in 16, *E. coli* in 15 and *A. pyogenes* in four rams. They also report that *A. pyogenes* caused orchitis in the rams (Gouletsou *et al*., 2006). It should be underlined that the dogs in the experiment reported here were clinically healthy with no scrotal disease.

Approximately 10-15% of dogs are reported to be experiencing urinary tract infection during their entire lifetime. Also, it is stressed that the female animals are more frequently affected (Bartges, 2004; Lulich and Osborne, 1995). Published reports indicate that urinary tract infections may be concomitant with genital tract infections. In this study, microorganisms were identified in 50 the of 100 dogs. These dogs however, were not submitted to urine examination because they were considered healthy. The clinical studies show that penile and preputial infection is not frequent in the dog, a statement supported by the available published reports.

The flora of the lower genitourinary system exerts a protective effect by preventing the cellular adhesion and inhibiting the growth of pathogenic microorganisms. Microorganisms present in healthy dogs are only rarely reported to cause urinary tract infection (Lulich and Osborne, 2004; Osborne and Lees, 1995). In this study, no complaints suggesting urinary tract infection was elicited from the owners of dogs brought to the clinic. This finding supports the published data (Table 2).

Data on the antimicrobial susceptibility of microorganisms isolated from the healthy dog foreskin was not encountered in the literature search that the researchers performed. The present study reports these tests.

The *Staphylococcus* sp. isolated from 18 of the dogs were sensitive to rifaximin in 95% and ceftiofur in 93.3%. *E. coli*, isolated in 15 animals was sensitive to

Table 2: Antimicrobial susceptibility of isolated microorganisms

Microorganism	Strain no.	Danofloxacin	Rifaximin	Amoxicillin clavulanic acid	Cloxacillin	Amoxicillin	Tetracycline	Penicillin G	Erythromycin	Streptomycin	Ceftiofur
		(%)									
Staphylococcus sp.	18	91.6	95.0	90.0	83.3	85.0	63.3	58.3	50.0	46.6	93.3
E. coli	15	86.6	73.3	80.0	73.3	66.6	46.6	33.3	53.3	33.3	80.0
Proteus sp.	8	25.0	0.0	0.0	0.0	0.0	75.0.	0.0	0.0	0.0	0.0
Pseudomonas sp.	3	33.3	33.3	33.3	33.3	0.0	0.0	0.0	0.0	0.0	33.3
Streptococcus sp.	3	100.0	100.0	100.0	100.0	100.0	33.3	33.3	33.3	33.3	100.0
Corynebacterium sp.	1	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0
Bacillus sp.	2	100.0	100.0	100.0	100.0	100.0	50.0	50.0	50.0	50.0	100.0

danofloxacin in a proportion of 86.6% and to ceftiofur in 80%. *Proteus* sp. isolated from eight dogs were sensitive to tetracycline in 75% while the *Pseudomonas* sp. found in 3 subjects were sensitive in 33.3% to danofloxacin, rifaximin, amoxicillin/clavulanic acid, cloxacillin and ceftiofur.

Streptococcus sp. were also found in 3 subjects were 100% sensitive to danofloxacin, rifaximin, amoxicillin/clavulanic acid, cloxacillin amoxicillin and ceftiofur. Isolated in one dog included in the study, *Corynebacterium* sp. were sensitive (100%) to danofloxacin, rifaximin, amoxicillin/clavulanic acid, cloxacillin amoxicillin and ceftiofur. Finally, *Bacillus* sp. which were grown from 2 cases were found sensitive to danofloxacin, rifaximin, amoxicillin/clavulanic acid, cloxacillin amoxicillin and ceftiofur in both i.e., 100%.

CONCLUSION

The study shows that *Staphylococcus* sp. were the most frequently isolated microorganisms from the foreskin of healthy dogs in this study followed by *E. coli* (30%). The antimicrobial susceptibility tests determined that ceftiofur followed by rifaximin and amoxicillin/clavulanic acid could be used in cases of infection.

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