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Original article

Bacterial flora and antibiogram sensitivity in the preputium samples of healthy rams

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Abstract

This study was conducted to determine the presence of bacterial flora in the preputium samples of healthy rams in the province of Afyonkarahisar and identify the antibiotic sensitivity of the isolates. Preputial swab samples were collected from a total of 50 healthy 2-3-year-old rams in the center, districts, and villages of Afyonkarahisar, including 20 Merino, 10 Hampshire, and 20 Pirlak breed rams. Seventy-eight isolates obtained from the 50 clinically healthy rams were identified using standard microbiological and biochemical methods, as well as the VITEK-2 automated system device. Forty-four isolates were Gram-positive bacteria (56.4%), 29 isolates were Gram-negative bacteria (37.2%), and 5 isolates were yeasts (6.4%). Seventeen genera consisting of *Acinetobacter lwoffii*, *Aerococcus viridans*, *Aeromonas hydrophila*, *Bacillus* spp., *Candida* spp., *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Kocuria kristinae*, *Kocuria rosea*, *Kytococcus sedentarius*, *Lactococcus lactis*, *Mannheimia haemolytica*, *Neisseria animaloris*, *Salmonella enterica* ssp. *diarizonae*, *Sphingobacterium thalpophilum*, *Sphingomonas paucimobilis*, *Staphylococcus capitis*, *Staphylococcus cohnii* spp. *urealyticus*, *Staphylococcus epidermidis*, *Staphylococcus lentus*, *Staphylococcus xylosus*, *Streptococcus ovis*, and *Streptococcus thoraltensis* were isolated. The most frequently isolated species in the tested animals were *Staphylococcus* spp. (25.6%), *E. coli* (21.8%), *Streptococcus* spp. (7.7%), *A. viridans* (6.4%), *Lactococcus lactis* ssp. (6.4%), and *Candida* spp. (6.4%).

The susceptibility of the isolates to ampicillin, enrofloxacin, erythromycin, florfenicol, gentamicin, tetracycline, ceftiofur, and trimethoprim-sulfamethoxazole was tested using the Kirby-Bauer disk diffusion method (Bauer et al. 1966). Most isolates were susceptible to ceftiofur, enrofloxacin, gentamicin, and florfenicol, while most were resistant to erythromycin, tetracycline, and ampicillin.

Keywords: antibiotic sensitivity, identification of microorganisms, vitek-2, preputium, ram

Introduction

The preputium is formed by the coverage of the glans penis by the skin of the abdominal wall which folds and surrounds the corpus penis. It completely surrounds the head of the penis like a sheath. The inner surface of the preputium is covered in glands that secrete a sebaceous substance. This secretion forms a structure with a strong and specific odor known as 'smegma' by mixing with shedding epithelial tissue and the present bacterial flora (Rickwood 1999, Boukhliq et al. 2017, Freitas et al. 2022).

Posthitis is the inflammation of the preputium, balanitis is the inflammation of the head of the penis, and balanoposthitis is the inflammation of both. In balanoposthitis cases, warmth, painful, and diffuse swollen areas are seen in the anterior of the preputium (Abdullah et al. 2014, Stewart and Shipley 2021). The frequently encountered causes of posthitis, balanoposthitis, orchitis, and epididymitis in small ruminants include infections caused by *Brucella ovis, B. melitensis, Corynebacterium pseudotuberculosis*, *C. renale*, *Histophilus somni*, *Pasteurella* spp., *Actinobacillus* spp., *Mycoplasma* spp., *Trueperella pyogenes*, and *Herpesvirus* (Bath and De Wet 2000, Al-Katib and Dennis 2009, McEntee 1990, Foster 2016, Susan 2016).

Reproductive disorders in rams and male goats (bucks) caused by microorganisms most of which are contagious reduce animal welfare and affect small ruminant farms negatively in the financial sense (Scott et al. 2007). Aydin et al. (2020), collected 191 preputial swab samples from healthy bucks to determine the presence of *Campylobacter* spp. in these samples and reported that 27 of the samples (14.13%) were positive. In another study, *C. pseudotuberculosis* was identified in sperm samples collected from orchitis and epididymitis cases consisting of 2 bucks and a ram (Stewart et al. 2018).

Gangwar et al. (2020), screened preputial swab samples collected from 32 healthy breeding stock bucks for zoonotic and abortus-associated factors. They found the preputial swab samples to be negative for *Brucella* spp. and *Coxiella* spp., while 17 (53.13%) samples were positive for *Chlamydia* spp., and two (6.25%) were positive for *Campylobacter* spp. It was reported that *Mycoplasma* and *Ureaplasma* are frequently isolated from preputial flora and cause nonspecific balanoposthitis (Doig et al. 1981). Gocmen et al. (2020), obtained samples from clinically healthy animals consisting of 17 Saanen breed bucks and 10 Kivircik breed rams and reported the presence of *Mycoplasma* spp. in three of the 27 preputial swab samples (11.1%), excluding those with orchitis symptoms. In a study conducted to identify the yeast species prevalent in healthy dogs, in 93 preputial samples, 21 samples showed *Malassezia pachydermatis*, and one showed *Candida parapsilosis* and *C. tropicalis* (Brito et al. 2009).

This study aimed to identify species isolated from preputial swab samples collected from clinically healthy rams in Afyonkarahisar to determine the presence of aerobic bacterial flora constituting the preputial microflora of rams and test the antibiotic sensitivity of the identified isolates.

Materials and Methods

For this study, permission was obtained from Afyon Kocatepe University Animal Experiments Local Ethics Committee with the number 10 (AKUHADYEK 25.02.2016-49533702-10).

Preputial swab samples

Preputial swab samples were collected from a total of 50 healthy 2-3-year-old rams in the center, districts, and villages of Afyonkarahisar, including 20 Merino, 10 Hampshire, and 20 Pirlak breed rams (Table 1). The swab samples were brought to the laboratory in Stuart's transport medium (Oxoid, CM0111) in the cold chain and prepared for isolation tests.

Isolation and identification

The samples that were collected using sterile swabs were inoculated into 5% Columbia Blood agar (Oxoid, CM0331), McConkey agar (Oxoid, CM0115), and Eosin Methylene Blue (EMB CM0069) agar media for aerobic bacteria isolation. The inoculated samples were incubated under aerobic conditions at 37°C for 24-48 hours. The colony morphologies and hemolytic characteristics of the bacteria that reproduced in the Columbia Blood agar were examined. Gram staining was performed on the obtained cultures. Cultures from the colonies were streaked into 5% sheep blood agar (Oxoid, CM0055), and pure cultures were kept at -20°C. To isolate yeast species, chloramphenicol-added (0.05 mg/mL) Sabouraud dextrose agar (SDA, Merck 105438) was used. The swab samples were inoculated into SDA and left to incubate at 28°C for 7-10 days. After incubation, the reproduced colonies were identified by examining their macroscopic and microscopic morphologies and subjecting them to classical methods and the VITEK-2 (bioMérieux, France) automated system (Holt et al. 2000, Larone 2002, Quinn et al. 2011).

Table 1. Distribution of samples collected from rams according to age.

Breed of Rams	2 years (n)	3 years (n)	Total (n)
Merino	14	6	20
Hampshire	8	2	10
Pirlak	16	4	20
Total	38	12	50

Antibiotic sensitivity test

The antibiotic sensitivity tests of the isolated strains were determined according to the Kirby-Bauer disk diffusion method (Bauer et al. 1966). For this purpose, the 18-hour fresh culture of the microorganism to be tested in the 7% sheep blood agar was suspended inside 2 ml sterile normal saline at a ratio corresponding to the McFarland Standard No. 5 and inoculated onto the Muller-Hinton agar by spreading. The agar surface was dried at room temperature and incubated at 37°C for 24 hours after the placement of different antibiotic disks. At the end of the incubation period, the inhibition zones around the disks were measured, and the results were evaluated based on the Clinical and Laboratory Standards Institute (CLSI 2015) standards.

Statistical analysis

Differences in the positivity rates of the samples and microbiological analysis results were analyzed using chi-squared tests (SPSS 13.0 for Windows/SPSS Inc, USA). The level of statistical significance was accepted as $p<0.05$. The statistical analysis of antibiotic susceptibility data was initially conducted by testing the assumption of normality. All data demonstrating a normal distribution were subjected to ANOVA. Since the assumption of equal variances was not met, post hoc comparisons between groups were performed using Tamhane's T2 test.

Results

Isolation and identification

Seventy-eight isolates obtained from the 50 clinically healthy rams were identified using standard microbiological and biochemical methods, as well as the VITEK-2 (bioMérieux, France) automated system device. Forty-four isolates were Gram-positive bacteria (56.4%), 29 isolates were Gram-negative bacteria (37.2%), and 5 isolates were yeasts (6.4%). According to the identifications of the isolated microorganisms, 17 (21.8%) were *Escherichia coli,* 8 (10.3%) were *Staphylococcus epidermidis,* 5 (6.4%) were *Aerococcus viridans*, 5 (6.4%) were *Candida* spp., 5 (6.4%) were *Lactococcus lactis* ssp. *lactis*, 4 (5.1%) were *Staphylococcus capitis*, 4 (5.1%) were *Staphylococcus cohnii* ssp. *urealyticus*, 4 (5.1%) were *Streptococcus ovis*, 3 (3.8%) were *Bacillus* spp., 3 (3.8%) were *Staphylococcus xylosus*, 3 (3.8%) were *Sphingomonas paucimobilis*, 2 (2.6%) were *Streptococcus thoraltensis*, 2 (2.6%) were *Kocuria kristinae*, 2 (2.6%) were *Acinetobacter lwoffii*, 2 (2.6%) were *Aeromonas hydrophila*, 2 (2.6%) were *Neisseria animaloris*, 1 (1.3%) was *Kocuria rosea*, 1 (1.3%) was *Kytococcus sedentarius*, 1 (1.3%) was *Erysipelothrix rhusiopathiae*, 1 (1.3%) was *Staphylococcus lentus*, 1 (1.3%) was *Sphingobacterium thalpophilum*, 1 (1.3%) was *Salmonella enterica* ssp. *Diarizonae*, and 1 (1.3%) was *Mannheimia haemolytica* (Table 2). No statistical significant difference ($p>0.05$) was found between the mean number of bacterial isolated in samples from the rams, age and breeds.

Antibiotic sensitivity levels of isolated strains

The 73 aerobic bacteria isolates obtained from the preputial swab samples were susceptible to ceftiofur by 95.9%, enrofloxacin by 94.5%, florfenicol by 89%, gentamicin by 89%, ampicillin by 78.1%, trimethoprim-sulfamethoxazole by 74%, tetracycline by 60.3%, and erythromycin by 49.3% (Table 3).

Upon examination of the results, it was observed that antibiotics such as Enrofloxacin, Gentamicin, Ceftiofur, and Trimethoprim-Sulfamethoxazole exhibited very low p-values ($p<0.0001$), indicating a strong statistical difference between the groups tested for these antibiotics. Additionally, Ampicillin and Tetracycline also showed significant differences ($p=0.03$ and $p=0.02$, respectively). However, for Erythromycin, the p-value was 0.127, suggesting that there was no significant difference between the groups, with the data appearing more homogenous. In conclusion, while significant differences in resistance profiles were observed for certain antibiotics, such as Enrofloxacin and Gentamicin, the data for Erythromycin and some other antibiotics showed a more uniform distribution (Table 4).

Table 2. Distribution of aerobic bacteria and yeasts isolated from the prepuce of healthy rams.

Isolate	Hampshire (n=10)	Merino (n=20)	Pirlak (n=20)	Total (n=50)	
	n	n	n	n	(%)
Staphylococcus epidermidis	-	5	3	8	10,3
Aerococcus viridans	1	2	2	5	6,4
Lactococcus lactis ssp. *lactis*	1	2	2	5	6,4
Staphylococcus capitis	-	2	2	4	5,1
Staphylococcus cohnii ssp. *urealyticus*	1	3	-	4	5,1
Streptococcus ovis	1	3	-	4	5,1
Bacillus spp.	1	-	2	3	3,8
Staphylococcus xylosus	-	-	3	3	3,8
Streptococcus thoraltensis	-	2	-	2	2,6
Kocuria kristinae	1	-	1	2	2,6
Kocuria rosea	-	1	-	1	1,3
Kytococcus sedentarius	-	1	-	1	1,3
Erysipelothrix rhusiopathiae	-	1	-	1	1,3
Staphylococcus lentus	1	-	-	1	1,3
Gram positive bacteria				**44**	**56,4**
Escherichia coli	4	6	7	17	21,8
Sphingomonas paucimobilis	1	1	1	3	3,8
Acinetobacter lwoffii	-	2	-	2	2,6
Aeromonas hydrophila	-	-	2	2	2,6
Neisseria animaloris	1	1	-	2	2,6
Sphingobacterium thalpophilum	-	1	-	1	1,3
Salmonella enterica ssp. *diarizonae*	1	-	-	1	1,3
Mannheimia haemolytica	-	1	-	1	1,3
Gram negative bacteria				**29**	**37,2**
***Candida* spp.**	1	2	2	**5**	**6,4**
Total	**15**	**36**	**27**	**78**	100

Discussion

Pathogenic strains found in the reproductive system of male animals are among the main causes of testis-, penis-, and preputium-related fertility problems. Diseases of these organs have serious and permanent effects on fertility and lead to significant economic losses (Koc and Alkan 2001, Gouletsou and Fthenakis 2015, Stewart and Shipley 2021).

In this study, the most frequently isolated species in the tested animals were *Staphylococcus* spp. (25.6%), *E. coli* (21.8%), *Streptococcus* spp. (7.7%), *A. viridans* (6.4%), *Lactococcus lactis* ssp. (6.4%), and *Candida* spp. (6.4%). In their study on scrotum and preputium samples obtained from 48 rams, Gouletsou et al. (2006), reported the most frequently isolated microorganisms as *Staphylococcus* spp. (27.1%), *E. coli* (25.4%), *Bacillus* spp. (11.9%), and *Streptococcus* spp. (5.1%) in the preputium and *K. rosea* (2.3%), *Acinetobacter* spp. (6.5%), and *M. haemolytica* (3%) in the posterior scrotum. Daher et al. (2018), divided preputial swab samples that they collected from 56 healthy rams into two groups; the first group consisted of the samples of 28 young rams (8-12 months old), and the second group consisted of the samples of 28 adult rams (3-5 years old). They reported the microorganisms isolated from the young rams as *Staphylococcus* spp. (85.7%), *E. coli* (57.1%), *S. pyogenes* (50%), *Proteus mirabilis* (32.1%), and *Pseudomonas auruginosa* (14.2%), whereas those isolated from the adult rams were *S. aureus* (100%), *E. coli* (92.8%), *S. pyogenes* (71.4%), *P. mirabilis* (57.1%), *P. auruginosa* (28.5%), *Klebsiella pneumoniae* (21.4%), and *B. melitensis* (3.5%) (Daher et al. 2018). Jarvinen and Kinyon (2010), studied the preputial microflora of 17 llamas and 13 alpacas aged 1 day to 16 years, seven of which were castrated. The microorganisms isolated from the preputial swab samples of the llamas were identified as *Streptococcus* spp. (17.6%),

Table 3. Results of antibiotic susceptibility profiles of microorganisms isolated from the prepuce of healthy rams.

Microorganisms	No. of isolates (n)	Antimicrobial Agents*																							
		Ampicillin 25 µg (n)			Enrofloxacin 5 µg (n)			Erythromycin 15 µg (n)			Florfenicol 30 µg (n)			Gentamicin 10 µg (n)			Ceftiofur 30 µg (n)			Trimethoprim--Sulfamethox-azole 25 µg (n)			Tetracycline 30 µg (n)		
		R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
A. lwoffii	2	1	-	1	-	-	2	1	1	-	-	-	2	-	-	2	-	-	2	1	1	-	2	-	-
A. viridans	5	1	-	4	-	-	5	1	1	3	-	1	4	-	-	5	-	-	5	1	-	4	1	-	4
A. hydrophila	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	2
Bacillus spp.	3	-	-	3	-	-	3	1	-	2	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3
E. coli	17	7	1	9	4	-	13	9	2	6	3	1	13	2	-	15	3	-	14	5	1	11	8	2	7
E. rhusiopathiae	1	-	-	1	-	-	1	1	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1
Kocuria spp.	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3
K. sedentarius	1	-	-	1	-	-	1	1	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1
L. lactis ssp. *lactis*	5	-	-	5	-	-	5	3	1	1	-	-	5	-	-	5	-	-	5	1	-	4	3	1	1
M. haemolytica	1	-	-	1	-	-	1	1	-	-	-	-	1	-	-	1	-	-	1	-	-	1	1	-	-
N.animaloris	2	-	1	1	-	-	2	-	1	1	1	1	-	-	-	2	-	-	2	1	1	-	2	-	-
S. enterica ssp. *diarizonae*	1	1	-	-	-	-	1	-	1	-	-	1	-	-	-	1	-	-	1	1	-	-	1	-	-
S. thalpophilum	1	-	-	1	-	-	1	1	-	-	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1
S. paucimobilis	3	-	-	3	-	-	3	2	-	1	-	-	3	-	-	3	-	-	3	-	-	3	-	-	3
Staphylococcus spp.	20	2	-	18	-	-	20	5	-	15	-	-	20	3	-	17	-	-	20	3	-	17	6	-	14
Streptococcus spp.	6	2	-	4	-	-	6	4	-	2	-	-	6	3	-	3	-	-	6	3	-	3	2	-	4
Total (n)	**73**	**14**	**2**	**57**	**4**	**0**	**69**	**30**	**7**	**36**	**4**	**4**	**65**	**8**	**0**	**65**	**3**	**0**	**70**	**16**	**3**	**54**	**26**	**3**	**44**
Total Ratio (%)	**100**	**19,2**	**2,7**	**78,1**	**5,5**	**-**	**94,5**	**41,1**	**9,6**	**49,3**	**5,5**	**5,5**	**89,0**	**11,0**	**-**	**89,0**	**4,1**	**-**	**95,9**	**21,9**	**4,1**	**74,0**	**35,6**	**4,1**	**60,3**

* R = Resistant, I = Intermediate, S = Susceptible strains, µg = Microgram

Table 4. Statistics of antibiotic susceptibility profiles of microorganisms isolated from the prepuce of healthy rams.

Groups	Ampicillin 25 µg (n)	Enrofloxacin 5 µg (n)	Erythromycin 15 µg (n)	Florfenicol 30 µg (n)	Gentamicin 10 µg (n)	Ceftiofur 30 µg (n)	Trimethoprim--Sulfametho-xazole 25 µg (n)	Tetracycline 30 µg (n)
Resistant	0,88±1,78^{ab}	0,25±1,00^{a}	1,88±2,39	0,25±0,77^{a}	0,50±1,09^{a}	0,19±0,75^{a}	1,00±1,46^{a}	1,63±2,33^{a}
Intermediate	0,13±0,34^{a}	0,00±0,00^{a}	0,44±0,62	0,25±0,44^{a}	0,00±0,00^{a}	0,00±0,00^{a}	0,19±0,40^{a}	0,19±0,54^{a}
Susceptible strains	3,56±4,44^{b}	4,31±5,17^{b}	2,25±3,76	4,06±5,29^{b}	4,06±4,85^{b}	4,38±5,29^{b}	3,38±4,50^{b}	2,75±3,56^{b}
p	0,03	<0,0001	0,127	0,001	<0,0001	<0,0001	0,006	0,02

Different letters between the columns indicate statistical significance ($p<0.05$).

Bacillus spp. (14.7%), *Staphylococcus* epidermidis (13.2%), *Arcanobacterium pyogenes* (7.4%), *Bacteroides* spp. (5.9%), *Actinomyces* spp. (2.9%), and *E. coli* (2.9%). Those isolated from the preputial swab samples of the alpacas were identified as *Staphylococcus* spp. (22.7%), *Bacteroides* spp. (15.9%), *Bacillus* spp. (13.6%), *Streptococcus* spp. (6.8%), and *Actinomyces* spp. (6.8%) (Jarvinen and Kinyon 2010). The rates reported by Gouletsou et al. (2006) for *Staphylococcus* spp. (27.1%), *E. coli* (25.4%), and *Streptococcus* spp. (5.1%) were compatible with our results, the rates they reported for *Bacillus* spp. (11.9%), *Acinetobacter* spp. (6.5%), *K. rosea* (2.3%), and *M. haemolytica* (3%) were lower, and the rates they reported for *S. xylosus* (2.6%) and *S. ovis* (0.4%) were close. The rates reported by Daher et al. (2018), in their study on young rams for *Staphylococcus* spp. (85.7%), *E. coli* (57.1%), and *Streptococcus* spp. (50%) were higher than those in our study. Jarvinen and Kinyon (2010), found a smaller rate of *E. coli* (2.9%) than the one in our study, while they noted a *Streptococcus* spp. rate (17.6%) higher than the one in our study in their llama samples and similar rates of *Streptococcus* spp. (6.8%) and *Staphylococcus* spp. (22.7%) in their alpaca samples.

Some frequently isolated bacteria from the preputial samples of dogs were stated as *Staphylococcus* spp. (36%), *E. coli* (30%), *Proteus* spp. (16%), *Pseudomonas* spp. (6%), and *Corynebacterium* spp. (2%) (Sarıtas et al. 2012). In another study, in preputial samples of 51 dogs, *Staphylococcus* spp., **β-**hemolytic *Streptococcus* spp., and *E. coli* were isolated (Allen and Dagnall 1982). Ling and Ruby (1978), listed *S. aureus* and *Mycoplasma* spp. among the frequently isolated species in the penis and preputium cultures of 20 adult dogs. In another study, in breeding stock dogs, *Pasteurella multocida*, β-hemolytic *Streptococcus* spp., and *E. coli* were identified as very frequently isolated species (Bjurstrom and Linde-Forsberg 1992). In their study on the preputial samples of 93 healthy dogs, Brito et al. (2009), isolated 21 *M. pachydermatis*, one *C. parapsilosis*, and one *C. tropicalis* strains. In the aforementioned studies, *Staphylococcus* spp., *Streptococcus* spp., and *E. coli* isolates have been the mainly isolated strains. The strains isolated in our study, including *Staphylococcus* spp., *E. coli*, *Streptococcus* spp., and *Candida* spp., were similar to those reported in other preputial flora studies (Ling and Ruby 1978, Allen and Dagnall 1982, Bjurstrom and Linde-Forsberg 1992, Gouletsou et al. 2006, Jarvinen and Kinyon 2010, Sarıtaş et al. 2012, Daher et al. 2018). The varying rates of other microorganism strains in different studies may have been caused by geographical differences, different species, and different breeds (Brito et al. 2009, Gouletsou and Fthenakis 2015, Foster 2016).

Various antibacterial agents are used to prevent and treat infections that are seen in animals. However, the irresponsible use of antibiotics brings about the problem of antibiotic resistance. The resistance developed by bacterial strains isolated in clinical samples against antimicrobial agents not only jeopardizes the chemical treatment of infections but also threatens human health directly or indirectly (Walther et al. 2017, Hernando--Amado et al. 2019). It was determined that the *Staphylococcus* spp. isolates obtained in this study were susceptible to enrofloxacin (100%), florfenicol (100%), ceftiofur (100%), ampicillin (90%), gentamicin (85%), trimethoprim-sulfamethoxazole (85%), erythromycin (75%), and tetracycline (70%). The isolated *E. coli* spp. strains were susceptible to gentamicin (88.2%), ceftiofur (82.3%), enrofloxacin (76.4%), and florfenicol (76.4%) and resistant to erythromycin (52.9%), tetracycline (47%), and ampicillin (41.1%). The *Streptococcus* spp. isolates were observed to be susceptible to enrofloxacin, florfenicol, and ceftiofur (100%), ampicillin (66.6%), gentamicin (50%), and trimethoprim-sulfamethoxazole (50%) and resistant to erythromycin (66.6%) and tetracycline (33.3%). The isolated *Bacillus* spp. strains were resistant to tetracycline at a rate of 33.3%.

In their study on preputial samples collected from dogs, Sarıtas et al. (2012), stated that *Staphylococcus* spp. isolates were susceptible to danofloxacin (91.6%), amoxicillin (85.0%), tetracycline (63.3%), erythromycin (50%), and ceftiofur (93.3%), *E. coli* isolates were susceptible to danofloxacin (86.6%), amoxicillin

(66.6%), tetracycline (46.6%), erythromycin (53.3%), and ceftiofur (80%), *Streptococcus* spp. isolates were susceptible to tetracycline and erythromycin (33.3%), and *Bacillus* isolates were susceptible to tetracycline and erythromycin (50%). In comparison to the results obtained in our study, it is seen that they found similar antibiotic sensitivity rates in *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, and *Bacillus* spp. isolates. It was observed that data and sources on preputial microbiology in rams are highly limited in the literature. This led us to compare our results to those reported for other animal species and make limited interpretations.

The results of this study highlight significant variations in the resistance profiles of different antibiotics tested, as indicated by the statistical analyses. Enrofloxacin, Gentamicin, Ceftiofur, and Trimethoprim-Sulfamethoxazole displayed strong statistically significant differences between resistant, intermediate, and susceptible strains, with p-values less than 0.0001. These findings suggest that these antibiotics have clear efficacy against certain bacterial strains, with a distinct differentiation between resistant and susceptible populations. The high variability in the susceptibility to these antibiotics may indicate differences in bacterial resistance mechanisms, environmental factors, or prior exposure to these drugs.

In contrast, Ampicillin and Tetracycline also showed significant differences ($p=0.03$ and $p=0.02$, respectively), though the magnitude of the differences was smaller compared to the aforementioned antibiotics. The significant differences observed in these antibiotics may reflect regional or species-specific resistance patterns, as well as the varying levels of selective pressure these antibiotics exert on bacterial populations.

Interestingly, Erythromycin showed no significant differences between the groups ($p=0.127$), suggesting that the resistance patterns for this antibiotic may be more uniform across the tested strains. This could be attributed to factors such as widespread resistance mechanisms or limited effectiveness of erythromycin in the target population, which warrants further investigation to explore the underlying causes of such homogeneity.

The overall findings underscore the importance of regularly monitoring antibiotic resistance profiles to inform treatment strategies. The variability observed across different antibiotics highlights the need for tailored approaches in clinical settings, taking into account the resistance patterns of the local bacterial populations. Further studies should focus on the molecular mechanisms underlying the observed resistance and susceptibility to better understand the factors driving these patterns and to guide the rational use of antibiotics in both human and veterinary medicine.

Conclusion

Consequently, in this study, it was determined that the microorganisms isolated from the preputium of rams were highly diverse, and they were microorganisms that could lead to various infections via primary and secondary mechanisms. Again, in this study, the in vitro antibiotic sensitivity levels of the microorganisms isolated from healthy rams were revealed. These opportunistic pathogens that are resistant to antimicrobial agents pose significant health risks in the fields of human medicine and veterinary medicine worldwide. It is believed to be important in terms of both human and animal health for veterinary clinicians to provide treatment to animals by keeping this situation in mind and after conducting antibiogram sensitivity tests. We expect that the findings obtained in this study will contribute to other studies to be conducted on preputial microbiology.

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