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RESEARCH ARTICLE

Isolation and Antimicrobial Susceptibility of Selected Bacterial Pathogens from Pneumonic Lung Samples of Sheep and Goats

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ABSTRACT

Pneumonia in sheep and goats causes significant economic losses worldwide. This study aimed to isolate and identify *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*) and *Trueperella pyogenes* (*T. pyogenes*), as well as to determine their antimicrobial susceptibilities in lung samples from sheep and goats that were slaughtered at the Siirt Municipal Slaughterhouse, where pneumonic lesions were detected post-mortem. In the study, 429 lung samples were collected from 270 goats and 159 sheep slaughtered in the Siirt Municipal Slaughterhouse. As a result of the PCR analysis, *P. multocida* was identified in 8 (1.86%) out of the lung samples, *M. haemolytica* in 8 (1.86%), *E. coli* in 10 (2.33%), *T. pyogenes* in 2 (0.46%), and *Klebsiella* spp. in 1 (0.23%), while *H. somni* was not identified. The antimicrobial susceptibilities of the obtained isolates were determined by the Kirby-Bauer disc diffusion method. According to antimicrobial susceptibility test results, it was determined that all *P. multocida* and *M. haemolytica* isolates were sensitive to cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin; all *T. pyogenes* isolates were determined to be susceptible to danofloxacin, amoxicillin, clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, sulfamethoxazole/trimethoprim; and all *E. coli* isolates were susceptible to ertapenem, piperacillin/tazobactam, and cefoxitin. Consequently, it was determined that especially *P. multocida, M. haemolytica* and *E. coli* strains may cause pneumonia cases in sheep and goats raised in the Siirt region. Furthermore, the isolated strains were generally susceptible to antibiotics as a result of antimicrobial susceptibility tests.

Keywords: Antimicrobial susceptibility, Goat, Pneumonia, Sheep.

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Koyun ve Keçilerin Pnömonik Akciğer Örneklerinden Bazı Bakteriyel Patojenlerin İzolasyonu ve Antimikrobiyal Duyarlılığı

ÖZ

Koyun ve keçilerde meydana gelen pnömoni dünya çapında önemli ekonomik kayıplara neden olur. Bu çalışmada, Siirt Belediye Mezbahasında kesilen ve post-mortem pnömonik lezyonlar tespit edilen koyun ve keçilerin akciğer örneklerinde *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*) ve *Trueperella pyogenes*'in (*T. pyogenes*) izolasyon ve identifikasyonunun yapılması ve antimikrobiyal duyarlılıklarının belirlenmesi amaçlanmıştır. Çalışmada, Siirt Belediye Mezbahasında kesilen 270 keçi ve 159 koyuna ait 429 akciğer örneği toplandı. Yapılan PCR analizi sonucunda, alınan akciğer örneklerinin 8 (%1.86)'inden *P. multocida*, 8 (%1.86)'inden *M. haemolytica*, 10 (%2.33)'undan *E. coli*, 2 (%0.46)'sinden *T. pyogenes*, 1 (% 0.23)'inde de *Klebsiella* spp. izole edilirken, örneklerde *H. somni* belirlenmedi. Elde dilen izolatların antimikrobiyal duyarlılıkları Kirby-Bauer disk difüzyon yöntemi ile belirlendi. Antimikrobiyal duyarlılık test sonuçlarına göre; *P. multocida* ve *M. haemolytica* izolatlarının tamamının sefpodoksim, seftiofur, enrofloksasin, florfenicol ve spektinomisine, *T. pyogenes* izolatlarının tamamının danofloksasin, amoksisilin klavulanik asit, oksitetrasiklin, eritromisin, seftiofur, florfenikol, sulfametoksazol/trimetoprime; *E. coli* izolatlarının tamamının ise; ertapenem, piperasilin/tazobaktam ve sefoksitine duyarlı olduğu belirlendi. Sonuç olarak bu çalışmada Siirt bölgesinde yetiştiriciliği yapılan koyun ve keçilerde meydana gelen pnömoni olgularına özellikle *P. multocida*, *M. haemolytica* ve *E. coli* suşlarının neden olabileceği belirlendi. Ayrıca izole edilen suşların yapılan antimikrobiyal duyarlılık testleri sonucunda antibiyotiklere genel olarak duyarlı olduğu sonucuna varıldı.

Anahtar Kelimeler: Antimikrobiyal duyarlılık, Keçi, Koyun, Pnömoni.

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INTRODUCTION

Small ruminant breeding, which is adapted to the geographical conditions of Türkiye and can make use of pastures throughout the year and allows the utilisation of inarable lands, takes place among important economic activities (Bakır and Mikail 2019). However, small ruminant breeding has been negatively affected and suffers major economic losses due to respiratory diseases that are effective on a herd basis (Abera and Mossie 2023). Although the prevalence of respiratory diseases in small ruminants is around 5.6%, 50% of the herd mortality is caused by respiratory tract infections (Chakraborty et al. 2014; Rahman et al. 2022).

Respiratory diseases in sheep and goats are caused by immunosuppression, bacterial and viral infections, parasitic infestations, environmental factors, and stress (Mahmoud et al. 2005; Chakraborty et al. 2014; Valadan et al. 2014; Nejiban and Al-Amery 2018; Abera and Mossie 2023). *Pasteurella multocida* (*P. multocida*), *Mannheimia haemolytica* (*M. haemolytica*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Histophilus somni* (*H. somni*), *Bibersteinia trehalosi* (*B. trehalosi*), *Staphylococcus aureus* (*S. aureus*), *Proteus vulgaris* (*P. vulgaris*) and *Trueperella pyogenes* (*T. pyogenes*) are frequently isolated and identified in pneumonia cases among small ruminants (Wang et al. 2012; Marru et al. 2013; Hanthorn et al. 2014; Headley et al. 2018; Babu et al. 2019; Mahrous et al. 2023).

M. haemolytica, which causes significant acute pneumonia outbreaks in newborn and weaned lambs, has become one of the important pathogens of the respiratory system, causing serious respiratory diseases in ruminants (Fernandez et al. 2016). In addition, *M. haemolytica* causes *P. multocida* to better colonise in the lungs and cause serious disease (Dabo et al. 2007). *E. coli* and *K. pneumoniae* are among the pathogens isolated from pneumonia cases with high mortality and morbidity rates (Wang et al. 2012). Particularly, *E. coli* and *K. pneumoniae* isolates producing extended-spectrum β-lactamase (ESBL) are of great importance because they cause the development of multiple resistance (Krishnamurthy et al. 2013). The global spread of ESBLs creates public health concerns in both developed and developing countries in terms of the development of multiple drug resistance (Chong et al. 2011; Alali et al. 2021).

National and international studies have reported that *P. multocida*, *M. haemolytica*, *E. coli, K. pneumoniae, H. somni* and *T. pyogenes* strains, which play an important role in respiratory diseases, have been isolated from lung samples of sheep and goats, and their antimicrobial susceptibilities have been determined by the disc diffusion method. Related studies have reported that the isolation rate of *P. multocida* from sheep and goats varies between 0.7% and 20% (Özbey and Muz 2004; Tel and Keskin 2010; Jarikre et al. 2018; Singh et al. 2019). Similarly, the isolation rate of *M. haemolytica* ranges between 2% and 40%, *E. coli* between 2% and 15.38%, *K. pneumoniae* between 0.7 and 3%, *T. pyogenes* between 0 and 20%, and *H. somni* between 2% and 8.7% (Özbey and Muz 2004; Tel and Keskin 2010; Tesfaye et al. 2013; Gulaydın and Gurtürk 2018; El-Mashad et al. 2019; Thakur et al. 2019).

The number of ovine animals in Türkiye has been reported to be 52.363.410, while the number of ovine animals in the Siirt region is 1.197.307 (TÜİK 2023). Siirt has great importance for livestock activities due to its topographical structure and geography. Small ruminant breeding in the region provides an important source of livelihood for the people of the Siirt region, especially due to the production of meat, milk, leather, and fleece (Semerci and Çelik 2016; Bakır and Mikail 2019). Finding solutions to the problems in small ruminant breeding, an important source of livelihood for the people of the region would make significant contributions to the economy of both the region and the country. From this perspective, it is important to identify the aetiology of respiratory diseases prevalent among sheep and goats that are raised in the region and to evaluate effective treatment options. It was found that there had been no previous study on the subject in the region. Therefore, this study aimed to isolate and identify *P. multocida*, *M. haemolytica*, *K. pneumoniae*, *E. coli*, *H. somni* and *T. pyogenes* the causative bacteria of pneumonia in sheep and goats from lung samples and to determine their susceptibility to various antimicrobial agents.

MATERIALS and METHODS

Materials

The study material consisted of 429 lung samples collected from 270 goats and 159 sheep that were slaughtered in Siirt municipal slaughterhouse and whose lungs showed pneumonic lesions on macroscopic examination. Palm-sized lung samples were taken and were placed in phosphate buffer saline (PBS) containing 20% glycerine and stored at -20 °C until they were used.

Methods

Collection of lung samples

Lung samples were collected from sheep and goats slaughtered at the Siirt municipal slaughterhouse in Siirt province. As a result of the examinations, palm-sized sections were taken from the lungs where pneumonic lesions were observed macroscopically and samples were collected. The samples were transferred to sample collection containers with PBS containing 20% glycerine and delivered under cold chain conditions to the Department of Microbiology Laboratory of the Faculty of Veterinary Medicine at Siirt University.

Isolation and Identification of Bacterial Agents

The surface of the lung samples was cauterised and cut with a sterile scalpel, and a swab sample was taken from the inner part of the organ and transferred to the

Brain Heart Infusion Broth (Himedia, M210, Mumbai, India). Their medium was incubated at 37 °C for 24 hours (Besser et al. 2012; El-Mashad et al. 2019). At the end of the incubation, samples were inoculated onto MacConkey agar (Himedia, MH081, Mumbai, India), Eosin Methylene Blue (EMB) agar (Oxoid, CM0069, Hampshire, England) and the blood agar (Oxoid, CM0271, Hampshire, England), containing 5% sheep blood and incubated at 37 °C for 24-48 hours in an aerobic environment (Quinn et al. 2011). Also, they were inoculated on Columbia agar (Oxoid, CM0331, Hampshire, England) containing 5% sheep blood for isolation of *H. somni* and *T. pyogenes* and incubated at 37 ℃ in an atmosphere of 5-10% CO_2 for 24-48 hours (Humphrey and Stephens 1983; Ward et al. 2006). The colonies were Gram-stained. Catalase, oxidase, and various biochemical properties of the isolates were determined (Quinn et al. 2011).

Identification by Polymerase Chain Reaction

Suspected isolates were confirmed by PCR using species-specific primers. Table 1 shows the information about the specific primers to be used for this purpose.

DNA Extraction:

Genomic DNA was extracted using a commercial DNA isolation kit (GeneAll, ExgeneTM Clinic SV Mini, 108.101, Seoul, Korea).

PCR Amplification:

Commercial mastermix (2X PCR Mastermix, BioLabs, 1333-HY-100, Van, Turkiye,) was used to prepare the PCR mixture. For optimisation of the mixture, 5 µl of genomic DNA and 1.5 µl from each of the primers 10 (µM) were added to 12.5 µl of mastermix, and the total volume was completed up to 25 µl with PCR water. During the amplification process, the binding temperature was optimised according to the recommendations of the company where the primers were synthesised (Table 1). The optimization process included an, initial denaturation step at 94 °C for 10 min and a final extension step at 72 °C for 10 min. Amplification procedures are given in Table 1. Amplicons generated PCR were electrophoresed in agarose gel and analysed in a gel imaging system (Gen-Box ImagER, Ankara, Türkiye). *P. multocida* subsp. *multocida* ATCC® 43137, *M. haemolytica* ATCC®33396, *T. pyogenes* ATCC® 19411, *E. coli* ATCC® 25922, and *K. pneumoniae* strain identified by MALDI-TOF found in the culture collection in the microbiology laboratory were used as positive controls in the PCR analysis, and DNA-free PCR water was used as a negative control

Table 1 Primers and sequences used in the identification of isolates for PCR.

Species	Target Gene	Oligonukleotid (5'-3')	Amplification Protocol	Amplicon size (bp)	References
P. multocida	KMT1	F: ATCCGCTATTTACCCAGTGG R: GCTGTAAACGAACTCGCCAC	94 ℃ 60 s 58 ℃ 60 s 72 ℃ 60 s (35 cycles)	460	Townsend et al. 1998
M. haemolytica	PHSSA	F: TTCACATCTTCATCCTC R: TTTTCATCCTCTTCGTC	94 ℃ 60 s 48 ℃ 60 s 72 ℃ 60 s (35 cycles)	325	Hawari et al. 2008
E. coli	16S rRNA	F: CCCCCTGGACGAAGACTGAC R: ACCGCTGGCAACAAAGGATA	94 ℃ 60 s 55 ℃ 60 s 72 ℃ 60 s (35 cycles)	401	Wang XiaoRong et al. 2012

Klebsiella spp.	*glyA*	F: CGCGTACTATACGCCATGAACGTA R: ACCGTTGATCACTTCGGTCAGG	94 ℃ 60 s 62 ℃ 60 s 72 ℃ 60 s (35 cycles)	441	Sikrodia et al. 2022
K. pneumoniae	16S rRNA	F: ATTTGAAGAGGTTGCAAACGAT R: TTCACTCTGAAGTTTTCTTGTGTTC	94 ℃ 60 s 56 ℃ 60 s 72 ℃ 60 s (35 cycles)	130	Turton et al. 2010
H. somni	16S rDNA	F: GAAGGCGATTAGTTTAAGAG R: TTCGGGCACCAAGTATTCA	94 ℃ 60 s 55 ℃ 60 s 72 ℃ 60 s (35 cycles)	407	Angen et al. 2003
T. pyogenes	16S-23S rDNA	F:GTTTTGCTTGTGATCGTGGTGGTT ATGA R: AAGCAGGCCCACGCGCAGG	94℃ 60 s 63 ℃ 60 s 72 ℃ 60 s (35 cycles)	122	Ülbegi-Mohyla et al. 2010

Note: *P. multocida*: *Pasteurella multocida*; *M. haemolytica*: *Mannheimia haemolytica*; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; *H. somni*: *Histophilus somni*; *Trueperella pyogenes*: *T. pyogenes*.

Determination of Antimicrobial Susceptibility

The susceptibility of the isolates to various antimicrobial agents was determined by the disc diffusion method.

Accordingly, ceftiofur (30 μg, Bioanalyse, Ankara, Türkiye), enrofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), spectinomycin (100 μg, Liofilmchem, Roseto degli Abruzzi, Italy), cefpodoxime (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), tulathromycin (30 μg, Bioanalyse, Ankara, Türkiye), and florfenicol (30 μg, Bioanalyse, Ankara, Türkiye) were used for *P. multocida* and *M. haemolytica*.

Cefpodoxime (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), ceftazidime (30 μg, Bioanalyse, Ankara, Türkiye), aztreonam (30 μg, Bioanalyse, Ankara, Türkiye), cefotaxime (30 μg, Hımedia, Mumbai, India), ceftriaxone (30 μg, Oxoid, Hampshire, UK), cefoxitin (30 μg, Bioanalyse, Ankara, Türkiye), enrofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), gentamicin (10 μg, Bioanalyse, Ankara, Türkiye), piperacillin-tazobactam (100/10 μg, Bioanalyse, Ankara, Türkiye), chloramphenicol (30 μg, Bioanalyse, Ankara, Türkiye), doxycycline (30 μg, Liofilmchem, Roseto degli Abruzzi, Italy), imipenem (10 μg, Liofilmchem, Roseto degli Abruzzi, Italy), streptomycin (10 μg, Bioanalyse, Ankara, Türkiye), trimethoprim-sulfamethoxazole (1.25/23.7 μg, Bioanalyse, Ankara, Türkiye) antibiotic discs were used for *E. coli* and *K. pneumoniae.*

Danofloxacin (5 μg, Bioanalyse, Ankara, Türkiye), amoxicillin-clavulanic acid (20/10 μg, Bioanalyse, Ankara, Türkiye), oxytetracycline (30 μg, Oxoid, Hampshire, UK), erythromycin (15 μg, Liofilmchem, Roseto degli Abruzzi, Italy), Ceftiofur (30 μg, Bioanalyse, Ankara, Türkiye), florfenicol (30 μg, Bioanalyse, Ankara, Türkiye), and trimethoprim-sulfamethoxazole (1.25/23.7 μg, Bioanalyse, Ankara, Türkiye) antibiotic discs were used for *T. pyogenes*.

At the end of the incubation period, the zone diameters that were formed around the antibiotic discs were measured. The measured values were compared with the criteria established by CLSI (2002; 2003; 2018; 2023) and EUCAST (2023), and the susceptibility, intermediate susceptibility, or resistance of the pathogens to antibiotics was determined.

RESULTS

Isolation and Identification

In the study, a total of 429 lung samples (159 sheep and 270 goats) with macroscopic findings of pneumonia were collected. As a result of bacteriological examination of the lung samples collected from sheep using conventional methods, 13 (8.17%) isolates were suspected of *P. multocida*, 10 (5.03%) isolates suspected of *M. haemolytica*, 10 (6.28%) isolates suspected of *E. coli*, 2 (1.25%) isolates

suspected of *Klebsiella* spp., 2 (1.25%) isolates were suspected of *H. somni*, and 5 (3.14%) isolates were suspected of *T. pyogenes* (Table 2). As a result of the PCR analysis, it was found that among 159 sheep lung samples, *P. multocida* was identified in 7 (4.40%) isolates, *M. haemolytica* in 7 (4.40%) isolates, *E. coli* in 6 (3.77%) isolates, *Klebsiella* spp. in 1 (0.62%) isolate, and *T. pyogenes* in 1 (0.62%) isolate. Bacteriological examination of 270 lung samples collected from goats by conventional methods indicated 5 (1.85%) isolates suspected of *P. multocida*, 3 (1.11%) isolates suspected of *M. haemolytica*, 4 (1.48%) isolates suspected of *E. coli*, 3 (1.11%) isolates suspected of *H. somni*, and 14 (5.18%) isolates suspected of *T. pyogenes* (Table 2).

Table 2: Distribution of isolates obtained from sheep and goat lung samples (n=429)

Agent	Sheep (%)	Goat (%)	Total (%)
P. multocida	4.40	0.37	1.86
M. haemolytica	4.40	0.37	1.86
E. coli	3.77	1.5	2.33
Klebsiella spp.	0.62	0	0.23
T. pyogenes	0.62	0.37	0.46
H. somni	0	0	0

Note: *P. multocida: Pasteurella multocida; M. haemolytica: Mannheimia haemolytica; E. coli: Escherichia coli; H. somni: Histophilus somni; Trueperella pyogenes: T. pyogenes.*

PCR analysis showed that 1 (0.37%) isolate tested positive for *P. multocida*, 1 (0.37%) isolate tested positive for *M. haemolytica*, 4 (1.5%) isolates tested positive for *E. coli*, and 1 (0.37%) isolate tested positive for *T. pyogenes* (Fig. 1). However, none of the isolates from both sheep and goat lung samples were not tested positive for *H. somni*. *K. pneumonia* was not isolated in goats by conventional and molecular methods.

Antimicrobial Susceptibility

All *P. multocida* and *M. haemolytica* isolates (100%) were susceptible to cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin, while 25% of *P. multocida* isolates were resistant to tulathromycin and 25% of *M. haemolytica* isolates were moderately susceptible to tulathromycin.

All *T. pyogenes* isolates that were identified in the study were susceptible to danofloxacin, amoxicillin/clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, and sulfamethoxazole+trimethoprim.

All *E. coli* isolates (100%) were susceptible to cefoxitin, piperacillin/tazobactam, and ertapenem, while 10% of the isolates were resistant to cefpodoxime, aztreonam, gentamicin, enrofloxacin, sulfamethoxazole+trimethoprim, chloramphenicol, and ciprofloxacin.

On the other hand, *Klebsiella* spp. strain isolated from the lung samples were susceptible to cefoxitin, piperacillin/tazobactam, and ertapenem, and resistant to chloramphenicol and sulfamethoxazole+trimethoprim.

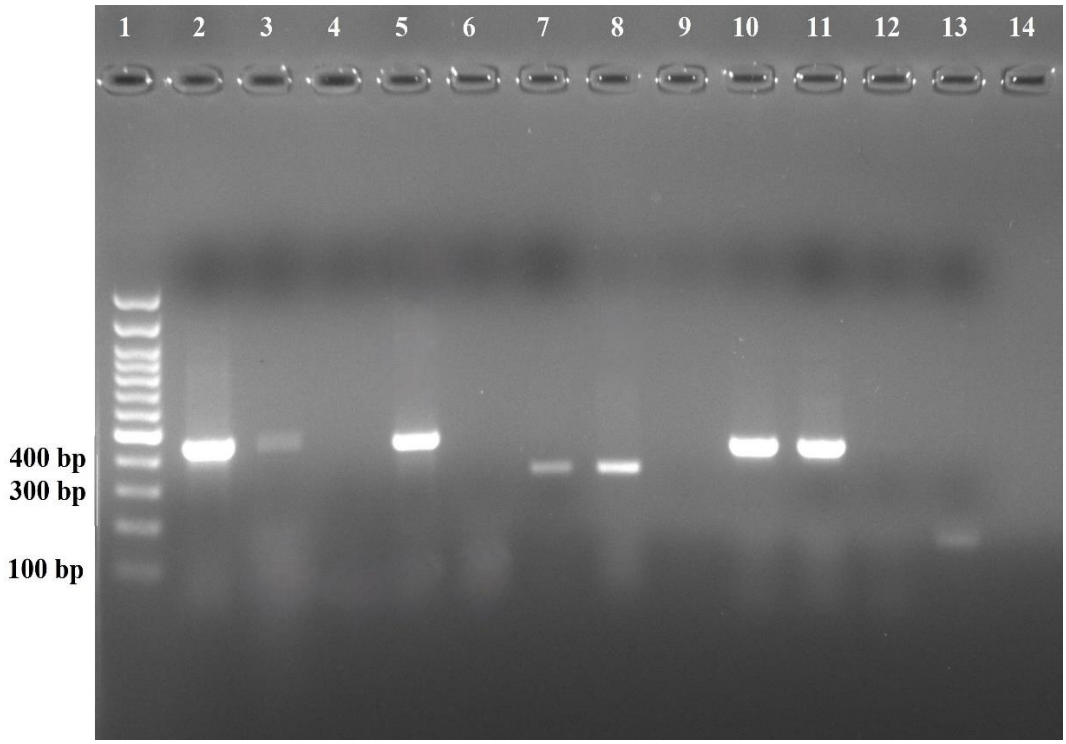

Figure 1: Agarose gel image of amplicons obtained as a result of PCR [1: 100 bp DNA marker; 2-3: *P. multocida* positive isolate (460 bp); 4: *P. multocida* negative control; 5: *Klebsiella* spp. positive isolate (441 bp); 6: *Klebsiella* spp. negative control; 7-8: *M. haemolytica* positive isolate (325 bp); 9: *M. haemolytica* negative control; 10-11: *E. coli* positive isolate (401 bp); 12: *E. coli* negative control; 13: *T. pyogenes* positive isolate (122 bp), 14: *T. pyogenes* negative control].

DISCUSSION

Respiratory tract infections in ovines have a multifactorial aetiology. To establish effective prevention-control strategies and treatment options against the disease, it is important to understand the aetiology (Asaye et al. 2015; Baykan et al. 2023). Antimicrobial treatment is inevitably essential for the effective treatment of bacterial pneumonia cases. However, the resistance in bacteria constrains the treatment options. It is necessary to monitor the resistance rates that develop in bacteria in order to achieve effective treatment of infectious diseases (Kılıç 2004; Gulaydin et al. 2021). This study aimed to identify *P. multocida*, *M. haemolytica*, *E. coli*, *K. pneumoniae*, *T. pyogenes* and *H. somni* isolates that cause respiratory tract infections in sheep and goats by PCR method as well as bacteriological methods and to determine the antimicrobial susceptibility results of the isolates.

A number of national studies have been conducted on the subject. In a prevalence study conducted by Ülgen et al. (1997) in Bursa, *K. pneumoniae* was isolated in 12.85% of 71 lamb lung samples, *E. coli* in 11.43%, *M. haemolytica* in 10% and *Histophilus* spp. in 5.71%. In a study conducted in Elazığ, *P. multocida* was detected in 15 (4.28%) of 350 sheep lung samples, *M. haemolytica* in 8 (2.3%) and *E. coli* in 10 (2.8%) (Özbey and Muz, 2004). A study conducted by Oruc (2006) in Konya reported that *M. haemolytica* was identified in 56.14% of 262 lung samples with pneumonia, *E. coli* was identified in 24.56%, and *P. multocida* was identified in 10.52%. A study conducted by Tel and Keskin (2010) in Şanlıurfa reported that they isolated and identified *M. haemolytica* at 12.5% and *P. multocida* at 31.6% from lung samples of sheep, and another study conducted by Özavcı et al. (2022) in the Aegean region reported that they isolated and identified *M. haemolytica* at 10% and *P. multocida* at 11% from lung samples of sheep and lamb with pneumoniae. Another study conducted in the Marmara region indicated that they identified *M. haemolytica* in 35.37%, *Klebsiella* spp. in 6.10%, and *P. multocida* in 8.54% of lung samples from sheep with pneumonia (Baykan et al. 2023). A study conducted by Tesfaye et al. (2013) in Ethiopia reported that they isolated and identified *P. multocida* in 2.4%, *M. haemolytica* in 25.4%, *H. somni* in 8.7%, and *Klebsiella* spp. in 1.6% of 960 nasal swab samples collected from sheep. In their study conducted in Egypt, El-Mashad et al. (2019) found *E. coli* in 15.38% and *K. pneumoniae* in 3.84% of 134 sheep lung samples. Likewise, a study conducted in India reported that they isolated and identified *M. haemolytica* in 18.75% and *P. multocida* in 14.58% of 96 sheep lung samples using bacteriological and molecular methods (Singh et al. 2019). Akane et al. (2022) reported that they identified *M. haemolytica* in 32.62% of 141 nasal swab samples.

When the results obtained from sheep lung samples were compared with the results of other studies, the rate of obtaining *E. coli* was found to be low compared to the results of the studies of Ülgen et al. (1997), Oruc et al. (2006) and El-Mashad et al. (2019). It was found to be higher according to the results of Özbey and Muz (2004). The rate of *M. haemolytica* presence was evaluated as low according to the results of Ülgen et al. (1997), Oruc (2006), Tel and Keskin (2010), Tesfaye et al. (2013), Singh et al. (2019), Akane et al. (2022) and Baykan et al. (2023), and higher according to the study results of Özbey and Muz (2004). In this study, the acquisition rate of *P. multocida* was evaluated as low compared to the results of Oruc (2006), Tel and Keskin (2010), Singh et al. (2019) and Baykan et al. (2019). Similarly *Klebsiella* spp. detection rate was found to be low compared to the study results of El-Mashad et al. (2019), Baykan et al. (2023) to the results of Tesfaye et al. (2013).

Studies have also been carried out to determine the aetiology of respiratory diseases in goats. In their study conducted in Elazığ, Özbey and Muz (2004) reported that they isolated and identified *P. multocida* in 0.7%, *M. haemolytica* in 4%, *E. coli* in 2.7%, and *Klebsiella* spp. in 0.7% of 150 goat lung samples. A study conducted by Baykan et al. (2023) in the Marmara region in 2023 reported that they examined a total of 25 goat lung samples and identified *P. multocida* in 8%, *M. haemolytica* in 20%, and *Klebsiella* spp. in 8% of the samples. Similarly, a study conducted by Ferdausi et al. (2008) in Bangladesh reported that they isolated *Pasteurella* spp. and *E. coli* in 11.7% and 6.7% of goat lung samples, respectively. Another study conducted in Mexico reported that *H. somni* was identified in 2.38% of 42 goat nasal swab samples (Pérez-Romero et al. 2011). Rashid et al. (2013) reported that *E. coli* was detected in 25% and *Pasteurella* spp. in 15% of goat lung samples in which gross abnormalities were detected, while Jarikre et al. (2018) detected *E. coli* in 13%, *P. multocida* in 20% and *M. haemolytica* in 40% of 150 goat lung samples. A study conducted by El-Mashad et al. (2019) in Egypt stated that they isolated *E. coli* in 7.69% of lung samples. Another study conducted by Adam et al. (2023) in Nigeria reported that they isolated and identified *Klebsiella* spp. in 51.72% of 58 goat lung samples and *E. coli* in 52.94% of 17 liver samples.

Within the scope of the study, the rate of obtaining *P. multocida* from goat lung samples (1.85%) was determined to be lower than the data obtained by Özbey and Muz (2004), Ferdausi et al. (2008), Rashid et al. (2013), Jarikre et a.l (2018) and Baykan et al. (2023). The *M. haemolytica* isolation rate obtained in the study was found to be lower compared to the results of the studies conducted by Özbey and Muz (2004), Jarikre et al. (2018) and Baykan et al. (2023). Similarly, the *E. coli* isolation rate in this study was found to be lower compared to other studies [Özbey and Muz (2004), Ferdausi et al. (2008), Rashid et al. (2013), Jarikre et al. (2018), El-Mashad et al. (2019), Adam et al. (2023)].

On the other hand, a study conducted by Thakur et al. (2019) in India reported that they collected 50 samples (44 nasal swabs and 6 lung samples) from sheep and

goats and found *H. somni* in 1 (2%) goat nasal swab sample, *T. pyogenes* in 10 (20%), and *Mycoplasma* spp. in 11 (22%), but they could not identify *H. somni, T. pyogenes,* and *Mycoplasma* spp. from any of the samples collected from sheep. Similarly, a study conducted by Babu et al. (2019) in India reported the identification of *P. multocida* in 9.30%, *M. haemolytica* in 11.62%, and *E. coli* in 46.5% of 43 lung swab samples collected from sheep and goats.

As a result of the studies, it was observed that the isolation rates of *P. multocida, M. haemolytica, E. coli, Klebsiella* spp., *Klebsiella* spp., *T. pyogenes,* and *H. somni* from sheep and goat lung samples varied between 0-31.6%, 0-56.14%, 2.8-52.94%, 0.7-51.72%, 0-20%, and 0-8.7%, respectively. The identification rates of bacterial agents obtained as a result of this study are shown in Table 2. In this study, sheep and goat lung samples were tested negative for *H. somni.* in this study, *K. pneumonia* was not isolated in goats conventional and molecular methods.

This may be attributed to geographical differences, different regional climatic characteristics, as well as different sample sizes and diagnostic methods used. Furthermore, it was also considered that the detection of more parasitic infestations in the lung samples with macroscopic lesions (unpublished data) may have caused this condition. Moreover, it was concluded that the maintenance of the nomadic breeding style in Siirt region for most of the year may lower the bacterial pneumonia rates by allowing the animals to stay less in the cramped barn environment.

Antimicrobial resistance may develop in bacteria due to unnecessary and incorrect use of antibiotics used for the treatment of bacterial diseases. In such cases, it is possible to develop resistance not only in pathogenic bacteria but also in normal flora bacteria. Developed antimicrobial resistance causes significant yield and economic losses due to the prolonged treatment period of animals and the increase in treatment costs (Gulaydin et al. 2021).

There are national and international studies on the subject. Accordingly, Rashid et al. (2013) reported that E. coli isolates obtained from lung tissue samples with pneumonic lesions were sensitive to streptomycin and ciprofloxacin. Also, in their study, Babu et al. (2019) reported that 100% of *P. multocida* and *M. haemolytica* strains and 70% of *E. coli* strains were susceptible to enrofloxacin. A study conducted by Özavcı et al. (2022) reported that all *M. haemolytica* isolates and 90% of *P. multocida* isolates were susceptible to tulathromycin, florfenicol, and amoxicillin-clavulanic acid. In their study, Rashid et al. (2013) showed that the susceptibility rate of *E. coli* isolates to streptomycin in was higher than the results of this study, while a similar rate of susceptibility was found against ciprofloxacin. While the results of the study by Babu et al. (2019) indicated that susceptibility rates of *P. multocida* and *M. haemolytica* isolates to enrofloxacin were lower than those in this study, the results of the study conducted by Özavcı et al. (2022) showed that susceptibility rates of *P. multocida* and *M. haemolytica* isolates to tulathromycin, florfenicol, and enrofloxacin were lower than those in this study. It was determined that antimicrobial resistance rates of bacterial agents isolated from different regions may differ. It was considered that this may be attributed to the habits of antibiotic use among veterinarians or livestock breeders in the region.

CONCLUSION

Consequently, this study revealed the presence of *P. multocida*, *M. haemolytica*, *E. coli* and *T. pyogenes* which were the causative agents of pneumonia in sheep and goats, leading to significant yield losses, by using phenotypic and genotypic methods. Furthermore, based on antimicrobial susceptibility testing, the following antibiotics were found to be effective: cefpodoxime, ceftiofur, enrofloxacin, florfenicol, and spectinomycin for *P. multocida* and *M. haemolytica*; piperacillin/tazobactam, ertapenem, and cefoxitin for E. coli; and danofloxacin, amoxicillin/clavulanic acid, oxytetracycline, erythromycin, ceftiofur, florfenicol, and sulfamethoxazole+trimethoprim for *T. pyogenes*. Due to the frequent and indiscriminate use of antibiotics in pneumonia cases, multi-resistant strains are often detected. Antibiotic susceptibility varies between farms and bacterial agents. For this reason, it can be suggested that antibiotic sensitivity testings should definitely be carried out and antibiotics should be used according to the results.

In addition, in order to obtain reliable data, it was concluded that conventional bacteriological methods should be supported by molecular methods.

Author's Contributions: MY and ÖG contributed to the project idea, design and execution of the study. MY contributed to the acquisition of data. MY analysed the data. MY drafted and wrote the manuscript. MY and ÖG reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical Approval: This study was carried out at Siirt University Veterinary Faculty Department of Microbiology. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Siirt University (SİÜ_HADYEK, Ref No: 2023/04/28, Tarih: 28/09/2023)

Conflict of Interest: The authors have no conflicts of interest to report.

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