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RESEARCH ARTICLE

Capsular Typing and Antimicrobial Susceptibility of *Pasteurella multocida* Isolated from Different Hosts

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Summary

In this study, 75 *Pasteurella multocida* isolates from different animal species of cattle, sheep, goat and rabbit isolated during 2001-2012 were identified and characterized by multiplex PCR for 5 capsular types of A, B, D, E, and F. Thirty six of isolates were isolated from cattle (19 lungs, 12 nasal swabs and 5 milk samples), 33 from sheep lungs, 3 from goat lungs and 3 from rabbit lungs. Each isolates was obtained from different herds/flocks except 9 bovine nasal swab isolates (6 and 3), which were from two herds. All *P. multocida* isolates identified by conventional methods were confirmed by multiplex PCR. Thirty three (91.6%) of 36 bovine isolates were serogroup A; 11 (33.3%) of 33 sheep isolates were serogroup D, 2 (6.0%) were serogroup A, 1 (3.0%) was serogroup F and 19 (57.5%) were untypable; 2 of 3 goat isolates were serogroup D. All of 3 rabbit isolates were untypable. Overall 26 (34.6%) isolates were untypable. No strain was detected from serogroup B and E. Antimicrobial susceptibility test results of 50 *P. multocida* strains using disk diffusion test showed that all strains were susceptible to ceftiofur, enrofloxacin, florfenicol and trimethoprim-sulfamethoxazole. Six (12.0%) strains were resistant to spectinomycin, 6 (12.0%) to tetracycline, 3 (6.0%) to tilmicosin and 3 (6.0%) to erythromycin. Multiple resistance was detected in 7 (4 tetracycline+spectinomycin, 2 tilmicosin+erythromycin, 1 tetracycline + tilmicosin + erythromycin) of 11 resistant strains isolated from 10 cattle and 1 sheep.

Keywords: *Pasteurella multocida, Capsular typing, PCR, Antimicrobial susceptibility, Cattle, Sheep*

Farklı Hayvan Türlerinden İzole Edilen *Pasteurella multocida* Suşlarının Kapsüler Tiplendirilmesi ve Antimikrobiyal Duyarlılıkları

Özet

Bu çalışmada 2001-2012 yılları arasında sığır, koyun, keçi ve tavşan gibi değişik hayvan türlerinden izole edilen *Pasteurella multocida* suşlarının multipleks PCR ile identifikasyonu ve kapsüler serogrupları (A, B, D, E ve F) araştırıldı. Toplam 75 suşun 36'sı (19 akciğer, 12 burun svabı, 5 süt) sığır, 33'ü koyun, 3'ü keçi ve 3'ü de tavşan akciğerlerinden izole edildi. İki işletmeden alınan 9 (6 ve 3'er adet) sığır burun svabı dışındaki örneklerin her biri farklı işletmelerden gelen hayvanlara aitti. Konvansiyonel yöntemlerle *P. multocida* olarak identifiye edilen suşların tümü multipleks PCR ile doğrulandı. Kapsüler tiplendirme sonucu 36 sığır suşunun 33 (%91.6)'ü serogroup A; 33 koyun suşunun 11 (%33.3)'i serogrup D, 2 (%6.0)'si A, 1(%3.0)'i F olarak belirlendi ve 19 (%57.5)'u tiplendirilemedi; 3 keçi suşunun 2'si serogrup D olarak tiplendirildi; 3 tavşan suşunun hiçbiri tiplendirilemedi. Toplam 26 (%34.6) suş tiplendirilemedi. Serogrup B ve E'ye ait suş tespit edilmedi. Disk difüzyon yöntemiyle 50 suşa yapılan antimikrobiyal duyarlılık test sonucunda suşların tümü ceftiofur, enrofloxacin, florfenicol ve trimethoprim-sulfamethoxazole'e duyarlı bulundu. Spectinomycin ve tetracycline'e 6 (%12.0)'şar suş, tilmicosin ve erythromycin'e 3 (%6.0)'er, suş dirençli bulundu. Dirençli 11 (10 sığır, 1 koyun) suşun 7'sinde çoklu direnç (4 suşta tetracycline + spectinomycin, 2 suşta tilmicosin + erythromycin, 1 suşta tetracycline + tilmicosin + erythromycin) tespit edildi.

Anahtar sözcükler: *Pasteurella multocida, Kapsüler tiplendirme, PCR, Antimikrobiyal duyarlılık, Sığır, Koyun*

INTRODUCTION

Pasteurella multocida causes important diseases as primary or secondary pathogen in many domestic or wild mammalian and avian hosts. Major diseases include haemorrhagic septicaemia in cattle and buffaloes, atrophic

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rhinitis in swine, snuffles in rabbit and pneumonias in cattle and sheep and, fowl cholera in poultry [1,2]. Occasionally, it can cause mastitis, localized infections and abortion in cattle [3]. *P. multocida* is also commonly found in the upper respiratory tract flora of many animal species [4-6]. Capsule and lipopolysaccaride (LPS) are the major components of *P. multocida* cell surface [7]. *P. multocida* are classified into 5 serogroups as A, B, D, E, and F based on capsule structures and further 16 serotypes based on LPS composition [1]. Capsule is considered as one of important virulence determinants that allow *P. multocida* to avoid innate host defense systems [4,8,9]. All *P. multocida* capsules are carbohydrate polymers, but composition of capsule is different in each serogroup. It was shown that, capsular material of serogroup A strains contains hyaluronic acid, serogroup D heparine, serogroup F chondroitin sulfate, serogroup B was composed of arabinose, mannose (or N-acetyl mannosaminuronic acid) and galactose [1,7,8,10]. Conflicting reports exist on capsular polysaccharides of *P. multocida* as protective antigen. Although pure polysaccharides are nonimmunogenic, antigens responsible for the capsule specific reactions are not completely understood [1,10]. It was suggested that immunogenicity of capsule is associated with LPS and purified capsule antigens behave as haptens in certain animals [1].

Capsular serogrouping is frequently used for typing of *P. multocida,* and capsular types generally correlate with particular disease and host [4,8]. The original and most common method for capsular serogrouping is indirect haemagglutination test. One of the limitations of the test is difficulties in preparation of high titer specific capsule antisera and their commercially unavailability [1]. In addition, inagglutinability of some capsulated fresh cultures with homolog capsular antiserum or reduced capsular material by serial subculture often renders such strains untypable in this method [1,6]. These problems of indirect haemagglutination test caused to attempts to devise non-serological tests. Non-serological tests such as hyaluronidase for serogroup A and acriflavin flocculation test for serogroup D were shown to be unreliable in a comparative study with PCR [11]. Multiplex PCR facilitates capsular serogrouping and provides fast, reliable and inexpensive typing [10].

Respiratory diseases including *P. multocida* infections in livestock are one of the most common causes of antimicrobial use for prophylaxis, metaphylaxis and treatment. However, imprudent use of antimicrobials creates a high risk of selecting resistance [12,13]. Continuous monitoring of antimicrobial susceptibilities of important animal pathogens with standard methods provides useful information on trends in resistance development [14]. In practice, in the case of respiratory problems veterinarians prefer to start antimicrobial treatment immediately, before disease does not progress. Therefore national or regional antimicrobial susceptibility data can help practitioners in their initial antibiotic choice.

Little is known about the serotype and antimicrobial susceptibility profiles of *P. multocida* isolates in Turkey. Except very few studies in cattle performed by conventional serological methods [15,16], no study is present about capsular types of *P. multocida* from animal species using molecular methods. In other countries most of studies were carried out with bovine isolates and little is known about serogroups and antimicrobial susceptibilities of *P. multocida* isolated from small ruminants. The aim of this study was to investigate capsular serogroups of *P. multocida* isolated from different animal species during 2001-2012, by multiplex PCR and to determine their antimicrobial susceptibility profiles.

MATERIAL and METHODS

Samples

A total of 75 *P. multocida* isolated from cattle, sheep, goat and rabbit submitted to diagnostic laboratory of Konya Veterinary Control Institute during 2001-2012 were tested *(Table 1)*. Among 75 isolates, 36 were from cattle (19 lung, 12 nasal swab, and 5 milk samples), 33 from sheep lungs, 3 from goat lungs and 3 from rabbit lungs. Except 6 and 3 nasal swab samples of calves taken from per herd, each isolate was belonging to animals from different herds. All lungs and nasal swab samples were from animals (aged 1-7 months) with respiratory problems as evidenced by clinical, macroscopic and microscopic findings after necropsy and, milk samples from animals with mastitis. Samples submitted mainly from Konya province in Central

***Table 1**. Distribution of P. multocida isolates according to hosts species and years*
***Tablo 1.** P. multocida suşlarının konakçı türleri ve yıllara göre dağılımı*

Years	Animal Species				Total Number of Isolates
	Cattle	Sheep	Goat	Rabbit	
2001	1	2			3
2002	2	1			3
2003	2	1		3	6
2004	3	6	1		10
2005	3		2		5
2006	5	2			7
2007	2	1			3
2008	1	4			5
2009	10	14			24
2010	2				2
2011	3	1			4
2012	2	1			3
Total	36	33	3	3	75

Anatolia, however, in recent years bovine herds are enlarging and animals from other provinces are gathered into herds, because of the widespread animal movements, origin of some animals may belong to other provinces. No reliable information was available for previous antibiotic use in animals.

Isolation and Identification

Swab and organ specimens were plated onto blood agar base No.2 (Oxoid) with 5% sheep blood. Agar plates were incubated at 37°C overnight. Presumptive identification of isolates as *P. multocida* was made on the basis of colony characteristics, no haemolysis on blood agar, no growth on MacConkey agar, Gram staining and microscopic morphology, positive catalase and oxidase tests [1,17]. Following presumptive identification, isolates were stored at -85°C in Microbank (Pro-Lab Diagnostics) tubes.

PCR for Identification and Capsular Typing

For the confirmation of isolates and determination of their capsular types, a multiplex PCR using primers specific for *P. multocida* [18] and for 5 capsular serogroups [10] based on capsule biosynthesis genes were used. To perform PCR, isolates were recovered from stocked cultures by streaking on blood agar, after overnight incubation at 37°C several colonies were suspended in PCR grade water and used as template following incubation at 100°C for 10 min.

PCR mixture contained 2 mM $MgCl_2$, 200 µM dNTPs, 3.2 pmol of each primers (*Cap*A, B, D, E and F; KMT1T7, KMT1SP6), 1.25 U Taq DNA polymerase (Bioron), 5 µl template DNA in total volume of 50 µl. Cycling parameters were as follows: initial denaturation at 95°C for 5 min; 30 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 90 sec. Products were electrophoresed on 1.5% agarose gel in TBE containing 0.5 µg/ml ethidium bromide for 1 h at 90 V, with 100 bp DNA ladder. All isolates were also tested second time without *P. multocida*-specific primers (KMT1T7, KMT1SP6) using only capsular primers to get clear amplicon bands for capsular serogroups. The vaccine strain of serogroup B provided from Pendik Veterinary Control Institute, İstanbul was used as control strain.

Antimicrobial Susceptibility Testing

A total of 50 *P. multocida* strains from 24 cattle, 20 sheep, 3 goats and 3 rabbits were tested by disc diffusion test in Mueller-Hinton agar (Oxoid) supplemented with 5% defibrinated sheep blood according to Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, CLSI: M31-A3 [19]. *S. aureus* ATCC 25923 was used as quality control strain. Following antimicrobial discs (Oxoid) were used: spectinomycin, ceftiofur, enrofloxacin, trimethoprim-sulfamethoxazole, tilmicosin, florfenicol, tetracycline, erythromycin and penicillin. To interpret results zone diameters described in CLSI: M31-A3 were used *(Table 2)*.

RESULTS

All isolates were confirmed as *P. multocida* which produced about 460 bp *P. multocida*-specific fragment in multiplex PCR *(Fig. 1)*. In capsular typing, 33 of 36 bovine isolates were determined as serogroup A. Among bovine isolates, only 2 milk and 1 nasal swab samples were untypable. Of 33 sheep isolates 11 (33.3%) were serogroup D, 2 (6.0%) serogroup A, 1 (3.0%) serogroup F and 19 (57.5%) was untypable *(Table 3)*. Two of 3 goat isolates were serogroup D. All of 3 rabbit isolates were untypable. Serogroup B and E were not determined. An amplified product of about 1044 bp, 657 bp, 851 bp and 760 bp for serogroup A, D and F strains, and for serogroup B of the control strain were obtained respectively (*Fig. 1, 2* and *3*). When samples were tested without *P. multocida* specific

Table 2. *Zone diameters and interpretation criteria of tested antimicrobial agents according to CLSI Standard M31-A3 (2008) used in the study*
Tablo 2. *Test edilen antimikrobiyal etkenlerin CLSI Standardı M31-A3 (2008)'de belirtilen zon çapları ve değerlendirme kriterleri*

Antimicrobial Agent	Disk Content	CLSI Zone Diameter (mm)		
		Susceptible	Intermediate	Resistant
Spectinomycin	100 µg	≥ 14	11-13	≤ 10
Ceftiofur	30 µg	≥ 21	18-20	≤ 17
Enrofloxacin	5 µg	≥ 21	17-20	≤ 16
Trimethoprim-sulfamethoxazole*	1.25/23.75 µg	≥ 16	11-15	≤ 10
Tilmicosin	15 µg	≥ 11	-	≤ 10
Florfenicol	30 µg	≥ 19	15-18	≤ 14
Tetracycline*	30 µg	≥ 19	15-18	≤ 14
Erythromycin*	15 µg	≥ 23	14-22	≤ 13
Penicillin*	10 units	≥ 29 ≥ 15	- -	≤28 (for Staphylococci) ≤ 14 (for Enterococci)

** Because no zone diameter was determined specifically for P. multocida in CLSI Standard, zone diameters defined for other bacteria were used*

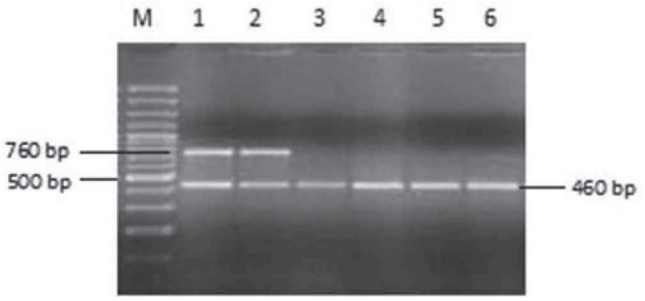

Fig 1. Multiplex PCR for *P. multocida*. M: 100 bp marker; Lane 1-2: Positive control (capsular type B), *P. multocida* specific 460 bp+760 bp bands; Lane 3-6: Untypable isolates, only *P. multocida* specific 460 bp bands

Şekil 1. *P. multocida* multipleks PCR. M: 100 bp marker; 1-2: Pozitif kontrol (kapsüler tip B), *P. multocida* spesifik 460 bp+760 bp bandlar; 3-6: Tiplendirilemeyen *P. multocida* suşları, sadece *P. multocida* spesifik 460 bp bandlar

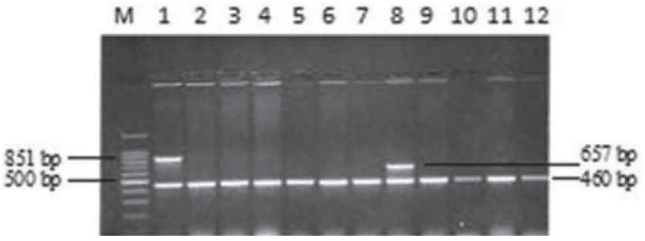

Fig 2. Multiplex PCR with *P. multocida* isolated from sheep. M: 100 bp marker; Lane 1-12: *P. multocida* specific 460 bp band; Lane 1: 460 bp+851 bp bands (capsular type F); Lane 8: 460 bp+657 bp bands (capsular type D)

Şekil 2. Koyunlardan izole edilen *P. multocida* suşları ile multipleks PCR. M: 100 bp marker; 1-12: *P. multocida* spesifik 460 bp band; 1: 460 bp+851 bp bandlar (kapsüler tip F); 8: 460 bp+657 bp bandlar (kapsüler tip D)

primers (only by capsular primers) in multiplex PCR, number of serogroup A positive samples increased from 1 to 33 *(Fig. 3)* and number of serogroup D and F positive samples did not changed.

In 9 (9/75) cases (in 5 sheep, 3 calf and 1 kid lung samples) *P. multocida* was isolated together with *Mannheimia haemolytica* and in 4 cases (in 2 sheep, 1 calf and 1 kid lung samples) it was isolated with *Arcanobacterium (Trueperella) pyogenes*.

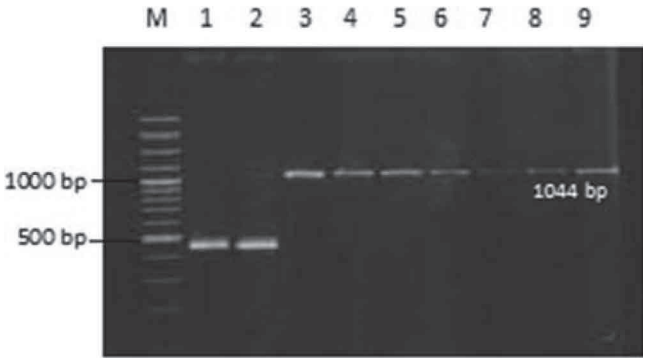

Fig 3. Multiplex and capsular PCR for *P. multocida*. M: 100 bp marker; Lane 1-2: Products of multiplex PCR including primers specific for *P. multocida* and for 5 capsular types (only 460 bp bands); Lane 3-9: Products of PCR with primers for capsular serogroups without *P. multocida* specific primers (capsular type A specific 1044 bp bands)

Şekil 3. *P. multocida* multipleks ve kapsüler PCR. M: 100 bp marker; 1-2: *P. multocida* spesifik ve 5 kapsüler primeri içeren multipleks PCR ürünleri (sadece *P. multocida* spesifik 460 bp bandlar); 3-9: Sadece 5 kapsüler serogrup primerleri ile yapılan PCR ürünleri (kapsüler tip A spesifik 1044 bp bandlar)

Antimicrobial susceptibility test results of 50 *P. multocida* strains from 24 cattle, 20 sheep, 3 goats and 3 rabbits are shown in *Table 4*. All isolates were susceptible to ceftiofur, enrofloxacin, florfenicol and trimethoprim-sulfamethoxazole. All isolates had ≥ 20 mm zone diameter for penicillin. In total, 11 strains were resistant to antimicrobial agents and 7 of them were multiresistant *(Table 5)*.

DISCUSSION

Studies indicate that *P. multocida* is one of the most common bacterial pathogens of bovine respiratory disease complex in different countries [20-23] and even in some studies its isolation from calf pneumonia appear to be increasing [24]. In a previous study carried out in Konya province [25], and in other studies from Turkey [26,27], isolation rate of *P. multocida* from pneumonic cattle and sheep lungs was found higher than that of *M. haemolytica*. In the present study, in 12% (9/75) of cases *P. multocida* was

Table 3. *Multiplex capsular PCR results of P. multocida isolates from different animal species*
Tablo 3. *Farklı hayvan türlerinden izole edilen P. multocida suşlarının multipleks kapsüler PCR sonuçları*

Animal Species	No. of Isolates	Isolated Samples	Capsular PCR Typed Isolates		Untypable Isolates	
			No	%	No	%
Cattle	36	19 Lung	19 serogroup A	100	-	8.3
		12 Nasal swab	11 serogroup A	91.6	1	
		5 Milk	3 serogroup A	60.0	2	
Sheep	33	Lung	11 serogroup D	42.4	19	57.5
			2 serogroup A			
			1 serogroup F			
Goat	3	Lung	2 serogroup D	66.6	1	33.3
Rabbit	3	Lung	-	-	3	100
Total	75		49	65.3	26	34.6

Table 4. *Antimicrobial susceptibility test results of 50 P. multocida strains isolated from cattle (24), sheep (20), goat (3) and rabbit (3) during 2001-2012*

Tablo 4. *2001-2012 yıllarında sığır (24), koyun (20), keçi (3) ve tavşan (3)'lardan izole edilen 50 P. multocida suşunun antimikrobiyal duyarlılık test sonuçları*

Antimicrobial Agent	Number of Strains		
	Susceptible	Intermediate	Resistant
Spectinomycin	44 (88%)	-	6 (12%)
Ceftiofur	50 (100%)	-	-
Enrofloxacin	50 (100%)	-	-
Trimethoprim-sulfamethoxazole	50 (100%)	-	-
Tilmicosin	38 (76%)	9 (18%)	3 (6%)
Florfenicol	50 (100%)	-	-
Tetracycline	42 (84%)	2 (4%)	6 (12%)
Erythromycin	7 (14%)	40 (80%)	3 (6%)
Penicillin	50 strains: ≥ 20 mm (22: 20-24 mm, 28: ≥ 25 mm)		

however, no such an interference was occurred with the other serogroups.

In the study, 91.6% (100% of lung isolates) of bovine isolates were capsular type A and only 3 isolates from 2 milk and 1 nasal swab samples were untypable. Similarly, studies from different countries showed that most of *P. multocida* isolates from cattle with respiratory disease complex including neonatal calf pneumonia and shipping fever were capsular type A and occasionally other capsular types have been isolated [28-31].

Contrary to previous studies [15,16] in Turkey which were carried out by conventional methods, capsular type B strains were not detected in this study. Serogroup B and E strains reported to cause haemorrhagic septicaemia generally in Asian and African countries respectively. An inactivated vaccine with Robert type 1 strain (serogroup B) was produced in Pendik Veterinary Control Institute, İstanbul until recently. As far as we know, in cattle no case

Table 5. *Properties and resistance profiles of P. multocida strains found resistant in antimicrobial susceptibility testing*

Tablo 5. *Antimikrobiyal duyarlılık testinde dirençli bulunan P. multocida suşlarının özellikleri ve direnç profilleri*

Resistant Strains	Isolation Year	Isolated Animal Species	Samples	Capsular Serogroup	Resistance Profile
1	2002	Cattle	Nasal svab	A	Tetracycline
2	2003	Cattle	Milk	A	Tetracycline + Spectinomycin
3	2003	Cattle	Lung	A	Tetracycline + Spectinomycin
4	2004	Cattle	Lung	A	Spectinomycin
6	2006	Cattle	Lung	A	Tilmicosin + Erythromycin
7	2007	Cattle	Lung	A	Tetracycline + Spectinomycin
8	2007	Cattle	Lung	A	Tetracycline + Spectinomycin
9	2008	Cattle	Milk	A	Spectinomycin
10	2011	Cattle	Lung	A	Tetracycline + Tilmicosin + Erythromycin
11	2006	Sheep	Lung	Untypable	Tilmicosin + Erythromycin

isolated together with *M. haemolytica* and in 4 cases it was isolated with *A. (Trueperella) pyogenes*. Results indicated that in majority (62/75) of samples *P. multocida* was isolated alone.

In this study, *P. multocida* isolates previously identified by phenotypic properties were confirmed by multiplex PCR. Capsular PCR method reported to be used reliably determining capsular serogroups of bovine, ovine, porcine and avian *P. multocida* isolates [6,28]. Results showed that in the multiplex PCR, *P. multocida* specific primers can be used in combination with 5 set of capsular primers except with serogroup A strains. *P. multocida* specific primers interfered with serogroup A primers and resulted in no or weak band. When all isolates were tested one more time using only capsular set of primers, serogroup A specific bands appeared in previously negative bovine isolates, related with haemorrhagic septicemia symptoms was observed for a long time in Turkey and the vaccine is not produced anymore. Capsular typing provides helpful information about distribution of isolates in different host with different disease conditions. It has been observed that haemorrhagic septicemia and pneumonic pasteurellosis are frequently confused. Use of reliable methods for capsular typing is quite important to determine real disease conditions in animal species. In this respect PCR is quite helpful.

In the study, 11 (33.3%) of 33 sheep isolates were serogroup D, 2 (6%) serogroup A, 1 (3%) serogroup F and majority of them (57.5%) were untypable. Similar to sheep isolates, 2 of 3 goat isolates were serogroup D. Little information is present worldwide about capsular serogroups of *P. multocida* from sheep and goats. In several

studies in Turkey, *P. multocida* was the most commonly isolated bacterial agent from sheep pneumonias [27,32]. Nevertheless, no study is present about capsular types of *P. multocida* from small ruminants in Turkey. Few studies examining limited number of isolates showed that in small ruminants, serogroup D [11] and A [30,33] were commonly detected capsular types. Capsular type D so far most frequently isolated from porcine atrophic rhinitis. Sheep and goat isolates of capsular type D should be further characterized for differentiation from porcine isolates.

Antimicrobial susceptibility test with commonly used antimicrobial agents for the therapy of respiratory diseases of livestock animals, such as enrofloxacin, florfenicol, ceftiofur, oxytetracycline, trimethoprim-sulfamethoxazole, tilmicosin, spectinomycin and erythromycin showed that resistance development among *P. multocida* isolates was generally low. Resistance was determined against spectinomycin, tetracycline, tilmicosin and erythromycin. Other studies [24,34,35] also pointed out that, tetracycline, tilmicosin and spectinomycin were less susceptible antimicrobial agents for *P. multocida* strains. In the study, majority of isolates (76%) were intermediately susceptible to erythromycin according to taken criteria *(Table 2)*. Similarly, in other studies [33,36], high rate of intermediate strains was reported among *P. multocida* strains. In other studies in Turkey, in sheep [27] and in cattle [36] resistance development was not high and erythromycin was less susceptible antimicrobial agent. This is not surprising, because erythromycin is an antimicrobial agent susceptible mostly for Gram positive microorganisms.

It is known that occurrence of antimicrobial resistance varies between countries and regions. Antimicrobial susceptibility data of *P. multocida* isolated from cattle over a three year period (2002-2004) in different European countries showed that resistance against *P. multocida* isolates were generally low and most common resistance observed against tetracycline which was 24% in Italy, 20% in Holland, 12% in Denmark and France in some years [14]. In a monitoring study [37] in Germany in 2002-2003, only 1.5% of 132 bovine *P. multocida* isolates were found resistant against ceftiofur and enrofloxacin. In a 4 year survey [38] in the USA including 318 *P. multocida* from cattle, similar to our study, it was reported that all strains were susceptible ceftiofur, highest resistance were detected against erythromycin (84%) followed by tilmicosin (41.1%), tetracycline (28.9%) and spectinomycin (23.6%). In the Netherland study [39], in agreement with this study, no resistance to ceftiofur and florfenicol but higher resistance to tetracycline was reported. In this study, while no resistance was detected against enrofloxacin and ceftiofur, tetracycline resistance was not as high as in other countries. High resistance rates against some antimicrobial agents may be due to intensive production systems and common use of antimicrobial in these types of productions. There is limited study on small ruminants. In a study [40] all of 28 *P. multocida* isolated from sheep and goats was reported to be susceptible to ceftiofur and florfenicol.

In the study, 11 (22%) of 50 isolates were resistant at least one antimicrobial agent. Among these resistant isolates, majority (10/11) were from cattle 24 strains and only 1 was from sheep 20 strains. This may be related to more antimicrobial use in cattle populations than in sheep populations, generally. While 10 of all bovine strains were serogroup A, one sheep strain was untypable. Resistant strains were not cumulated in any specific year.

In conclusion, results of the study provide information about capsular types of *P. multocida* isolates mainly from pneumonic cattle and sheep by using PCR method in Turkey. In cattle, majority (33/36) of isolates was capsular type A and 8.3% was untypable. In sheep and goat, capsular type D was predominant (13/36) and 55.5% was untypable. In general, antimicrobial resistance development to commonly used antimicrobial agents for respiratory diseases was not high among *P. multocida* isolates.

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