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Original article

Capsular typing and antimicrobial susceptibility of *Pasteurella multocida* from cattle in Afyonkarahisar, Turkey

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Abstract

Pasteurella multocida is primarily recognized as a respiratory pathogen in various animal species. However, sporadic cases of zoonotic infection in humans have also been reported. Understanding the distribution of capsular types is important for improving treatment strategies and developing preventive measures. This study aimed to determine the capsular type and antimicrobial susceptibility profiles of *P. multocida* isolates obtained from cattle. *P. multocida* isolates from 650 cattle samples were initially identified by conventional phenotypic methods and subsequently confirmed by PCR. Capsular typing of the isolates was performed using a slide agglutination test and PCR-based capsular genotyping. Of the 48 *P. multocida* strains typed using the slide agglutination test, 46 (95.8%) were classified as capsular type A, while 2 strains remained untypeable. Genotypic analysis confirmed all isolates as *P. multocida* via PCR targeting the *kmt1* and *omp* genes. PCR-based capsular genotyping revealed that all 48 isolates belonged to capsular type A. Accordingly, the highest nonsusceptibility rate was observed for OXY (19/48, 39.6%), followed by SXT (11/48, 22.9%), PEN (10/48, 20.8%) and AMC (4/48, 8.3%). Erythromycin was the least effective antimicrobial agent, with a susceptibility rate of 50.0% (24/48) and a resistance rate of 20.8% (10/48). Determining the capsular type and antimicrobial susceptibility profiles of *P. multocida* isolates may contribute to epidemiological studies and support the development of appropriate treatment strategies. However, further research is needed to elucidate the epidemiological and pathogenic characteristics of *P. multocida.*

Keywords: antimicrobial resistance, bovine respiratory disease, capsular typing, cattle, *Pasteurella multocida*, PCR

Introduction

Bovine respiratory disease (BRD) is one of the most serious health problems affecting cattle worldwide and is associated with substantial economic losses due to mortality, reduced weight gain, treatment costs and decreased productivity (Buczinski et al. 2021, Pratelli et al. 2021, Donlon et al. 2023). *Pasteurella multocida* is one of the major bacterial pathogens involved in BRD and is commonly found as a commensal organism in the upper respiratory tract of healthy cattle. It is regarded as one of the major bacterial pathogens involved in stress-related respiratory disease and shipping fever in cattle (Dabo et al. 2007, Quinn et al. 2011). In this context, capsular typing is an important epidemiological tool for understanding the distribution of *P. multocida* strains and their association with disease outbreaks. Furthermore, characterization of capsular type and antimicrobial susceptibility profiles may provide valuable information for disease control and treatment strategies (Harper et al. 2022, Gülaydin and Gürtürk 2020). Antimicrobial agents are widely used for the treatment and control of bovine respiratory disease. However, increasing antimicrobial resistance among respiratory bacterial pathogens has become a growing concern for animal health and treatment efficacy. Accordingly, monitoring the antimicrobial susceptibility profiles of *P. multocida* isolates is important for supporting effective therapeutic and disease-control strategies (Wang et al. 2018, Blakebrough-Hall et al. 2020, Andrés-Lasheras et al. 2021). Pasteurellosis occurs worldwide and affects a broad range of animal species. *P. multocida* causes acute or subacute pneumonia, hemorrhagic septicemia, meningoencephalitis and mastitis in cattle; pneumonia and mastitis in sheep; atrophic rhinitis and pneumonia in pigs; fowl cholera in poultry; and rhinitis and respiratory infections in laboratory animals (Quinn et al. 2011, WOAH 2021, Harper et al. 2022). Pasteurellosis is also recognized as a zoonotic disease. It has been reported that most *P. multocida* cases in humans occur as a result of being bitten or scratched by carnivores (Hitkova et al. 2024). On the other hand, *P. multocida* has been reported as an opportunistic human pathogen capable of causing sepsis, meningitis, pneumonia and skin and soft tissue infections even in the absence of direct animal bites, possibly through indirect environmental exposure or unrecognized animal contact (Zhu et al. 2024). *P. multocida* strains are classified into five capsular serogroups (A, B, D, E and F) and 16 lipopolysaccharide (LPS) serovars (formerly serotypes), according to the Heddleston classification system (Heddleston et al. 1972, Aktories et al. 2012). Various serological methods, including slide agglutination, indirect hemagglutination and agar gel immunodiffusion tests, have been used for strain typing (Aktories et al. 2012, Harper et al. 2022). In addition to the classical Heddleston serotyping scheme, eight distinct LPS biosynthesis loci (L1-L8) have been identified and are now used for molecular characterization of *P. multocida* strains (Harper and Boyce 2017). Pneumonic pasteurellosis is characterized by fibrinous or fibrinopurulent bronchopneumonia in cattle and enzootic pneumonia in young calves. Among the capsular type, type A is most frequently associated with bovine respiratory pasteurellosis and is commonly isolated from pneumonic cases (Garrity et al. 2004, Dabo et al. 2007, Harper et al. 2022). The aim of this study was to determine the capsular type and antimicrobial susceptibility profiles of *P. multocida* strains isolated from nasal swab, tracheal lavage and lung samples collected from cattle in Afyonkarahisar.

Materials and Methods

Ethics statement and experimental animals

The study was approved by the Animal Experiments Local Ethics Committee of Afyon Kocatepe University, Afyonkarahisar, Turkey (Approval No. 18-09; 04 May 2009). To generate hyperimmune sera against four standard *P. multocida* strains (A, B, D and E), 13 mature New Zealand rabbits weighing 3.5-4 kg were used. The animals were assigned to four distinct immunization groups, with two rabbits allocated to each specific strain (n=8 in total). For comparative baseline analysis, the remaining animals were divided into a placebo control group (n=4) receiving only sterile physiological saline solution (0.9% NaCl) and a completely untreated negative control group (n=1).

Sample collection

The study material comprised samples collected from 650 dairy cattle in Afyonkarahisar Province, Turkey, between October 2010 and October 2011. This included 300 lung tissue samples (46.2%) obtained from slaughterhouses, 200 nasal swabs (30.7%) and 150 tracheal lavage samples (23.1%) from farms. Lung tissue samples were transported in sterile containers, nasal swabs were collected using Stuart transport swabs (CM0111, Oxoid) and tracheal lavage samples were obtained in sterile syringes containing physiological saline solution. The samples were brought to the Laboratory of the Department of Microbiology, Faculty of Veterinary Medicine at Afyon Kocatepe University in a cold chain. The study population comprised 470 females (72.3%) and 180 males (27.7%). Regarding

Table 1. Demographic and clinical distribution of the study cattle population (*n*=650).

Variables	Subgroups	*n/N* (%)
Clinical Status	Healthy	600/650 (92.3)
	Diseased	50/650 (7.7)
Sex	Female	470/650 (72.3)
	Male	180/650 (27.7)
Age Groups	< 1 Year	150/650 (23.1)
	1-3 Years	320/650 (49.2)
	4-7+ Years	180/650 (27.7)
Total Samples		650/650 (100.0)

clinical status, 92.3% (600/650) of the animals were clinically healthy, whereas 7.7% (50/650) exhibited clinical signs of respiratory disease. With respect to age distribution, all tracheal lavage samples (150/150) were collected from calves younger than 1 year of age, whereas lung samples were obtained from cattle aged 1 year or older (Table 1). Furthermore, all lung samples (300/300) originated from clinically healthy animals without macroscopic lesions.

Isolation and identification

Lung, nasal swab and tracheal lavage samples were inoculated on 5% Columbia Blood Agar (CM0331, Oxoid) and incubated aerobically at 37°C for 24-48 h. Colony morphology and hemolytic characteristics of the bacterial growth on Columbia Blood Agar were examined. Colonies exhibiting Gram-negative coccobacillary morphology were subcultured on 5% Sheep Blood Agar (CM0055, Oxoid) to obtain pure cultures (Garrity et al. 2004, Quinn et al. 2011).

Hyperimmune serum preparation and slide agglutination test

The reference strains of *P. multocida* subsp. *multocida* used in this study were ATCC 43137 (Carter's Group A), ATCC 43017 (Carter's Group B), ATCC 43019 (Carter's Group D) and ATCC 43020 (Carter's Group E), obtained from ATCC (Manassas, VA, USA). The standard strains were cultured in Brain Heart Infusion broth (110493, Merck) for 6-8 h and subsequently inoculated onto 5% Sheep Blood Agar (CM0055, Oxoid). Following incubation at 37°C for 18-24 h, the bacterial growth was harvested and adjusted to McFarland Standard No. 4 using physiological saline solution (0.9% NaCl) containing 0.3% formaldehyde. The prepared antigens were administered intravenously to the rabbits at 4-day intervals in ascending doses of 0.2, 0.5, 1.0, 1.5 and 2.0 mL. Seven days after the final immunization, 0.5 mL of a live bacterial suspension prepared using the same procedure, but without formaldehyde, was administered subcutaneously. Ten days later, blood samples were collected from the rabbits, their sera were extracted and the sera were kept at -20°C before subsequent capsular typing (Carter 1955, Namioka and Murata 1961, Carter 1962, WOAH 2021). For capsular typing of *P. multocida* strains, a colony from an 18-24 h blood agar culture was suspended in a drop of physiological saline. One drop of hyperimmune serum was then added and mixed thoroughly. Agglutination occurring within 30 s was recorded as a positive result (Namioka and Murata 1961, WOAH 2021).

Bacterial DNA extraction and PCR assays

To confirm the identity of the bacterial isolates previously characterized by classical methods, PCR assays were performed. Genomic DNA was extracted using a GeneJET Genomic DNA Purification Kit (K0721, Fermentas, Lithuania), following the manufacturer's instructions. Species-specific primers targeting *P. multocida* were used for the PCR analysis of strains isolated from cattle. For capsular type, capsule type-specific primers were used: CAPA (1044 bp), CAPB (760 bp), CAPD (657 bp) and CAPE (511 bp). Amplification and protocol optimization were performed in accordance with previously validated procedures, using positive controls as reference (Neumann et al. 1998, Townsend et al. 1998, Townsend et al. 2001) (Table 2). All PCR reaction mixtures were prepared under sterile conditions in a biosafety cabinet (ESCO Class II Type B2, USA).

PCR was carried out in a final reaction volume of 25 µl, comprising the following components: 1 µl of each primer (KMT1T7, PMOut, CAP A-B-D-E; 10 pmol), 2.5 µl of PCR buffer (10×; EP0402, Fermentas), 0.5 µl of dNTP mix (10 mM; R0192, Fermentas), 0.4 µl of Taq DNA polymerase (5 U/µl; EP0402, Fermentas), 2 µl of $MgCl_2$ (25 mM; EP0402, Fermentas),13.6 µl of sterile H_2O and 4 µl of template DNA.

Cycling conditions on a thermal cycler (Techne TC-plus, UK) were as follows: initial denaturation at 95°C for 4 min; followed by 30 cycles of denaturation

Table 2. Primers used for the identification and capsular typing of *Pasteurella multocida* isolates.

Capsular type	Target gene	Primer	Sequence (5′–3′)	Product size (bp)	Reference
–	*kmt1*	KMT1T7-KMT1SP6	F:ATCCGCTATTTACCCAGTGG R:GCTGTAAACGAACTCGCCAC	460	Townsend et al. 1998
–	*omp*	PMOut	F:AGGTGAAAGAGGTTATG R:TACCTAACTCAACCAAC	221	Neumann et al. 1998
A	*hyaD-hyaC*	CAPA	F:TGCCAAAATCGCAGTGAG R:TTGCCATCATTGTCAGTG	1044	Townsend et al. 2001
B	*bcbD*	CAPB	F:CATTTATCCAAGCTCCACC R:GCCCGAGAGTTTCAATCC	760	Townsend et al. 2001
D	*dcbF*	CAPD	F:TTACAAAAGAAAGACTAGGAGCCC R:CATCTACCCACTCAACCATATCAG	657	Townsend et al. 2001
E	*ecbJ*	CAPE	F:TCCGCAGAAAATTATTGACTC R:GCTTGCTGCTTGATTTTGTC	511	Townsend et al. 2001

Table 3. Antibiotic susceptibility profiles of the isolated *P. multocida* strains (*n*=48).

Antimicrobial Agent	Disk Content (µg)	This Study			S (mm)	I (mm)	R (mm)
		S *n/N* (%)	I *n/N* (%)	R *n/N* (%)			
AMC [1]	20/10 µg	44/48 (91.7)	-	-	≥ 27	-	-
ERY [1]	15 µg	24/48 (50.0)	14/48 (29.1)	10/48 (20.8)	≥ 27	25-26	≤ 24
OXY [1]	30 µg	29/48 (60.4)	-	-	≥ 23	-	-
PEN [1]	10 Units	38/48 (79.2)	-	-	≥ 25	-	-
SXT [1]	25 µg	37/48 (77.1)	-	-	≥ 24	-	-
ENR [2]	5 µg	42/48 (87.5)	4/48 (8.3)	2/48 (4.2)	≥ 21	17-20	≤ 16
FFN [2]	30 µg	46/48 (95.8)	0/48 (0.0)	2/48 (4.2)	≥ 19	15-18	≤ 14
XNL [2]	30 µg	45/48 (93.8)	3/48 (6.3)	0/48 (0.0)	≥ 21	18-20	≤ 17
TIL [2]	15 µg	42/48 (87.5)	3/48 (6.3)	3/48 (6.3)	≥ 14	11-13	≤ 10
TUL [2]	30 µg	43/48 (89.6)	3/48 (6.3)	2/48 (4.2)	≥ 18	15-17	≤ 14

[1] CLSI 2015a, [2] CLSI 2015b, S – susceptible, I – intermediate, R – resistant, AMC – Amoxicillin-clavulanate, ERY – erythromycin, OXY – oxytetracycline, PEN – penicillin, SXT – Trimethoprim-sulfamethoxazole, ENR – enrofloxacin, FFN – florfenicol, XNL – ceftiofur, TIL – tilmicosin, TUL – tulathromycin

at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 9 min. PCR amplicons were resolved by electrophoresis on a 2% agarose gel (101674PR, Basica Le Agarose) at 4 V/cm for 1 h, stained with ethidium bromide (17896, Thermo), visualized under UV illumination and photographed using a gel documentation system (Vilber Lourmat ETX-20M, France). DNA bands were evaluated by comparison with a DNA marker (SM0323, Fermentas) and with bands from positive (ATCC 43137, ATCC 43020, ATCC 43019, ATCC 43017) and negative controls (DEPC-treated water).

Antibiotic sensitivity test

Antimicrobial susceptibility of the isolates was determined using the Kirby–Bauer disk diffusion method. A bacterial suspension equivalent to a 0.5 McFarland standard was prepared from 16-18 h cultures grown on 5% Sheep Blood Agar (CM0055, Oxoid) and inoculated onto Mueller–Hinton agar (1.05437, Merck). The following antimicrobial disks were used: amoxicillin-clavulanic acid (20/10 µg, CT0223B), erythromycin (15 µg, CT0020B), oxytetracycline (30 µg, CT0054B), penicillin G (10 U, CT0043B), trimethoprim-sulfamethoxazole (1.25/23.75 µg, CT0052B), enrofloxacin (5 µg, CT0643B), florfenicol (30 µg, CT0735B), ceftiofur (30 µg, CT1751B), tilmicosin (15 µg, CT0793B) and tulathromycin (30 µg, Pfizer 256244). Following incubation at 37°C for 24 h, inhibition zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2015a, CLSI 2015b). The breakpoint values used for interpretation are presented in Table 3.

Table 4. Distribution of samples and isolation rates of *P. multocida* according to sample type and clinical status (*n*=650).

Sample Type	Total *N*	Healthy *n/N* (%)	Diseased *n/N* (%)	Age Group	Positive *n/N* (%)
Lung	300	300/300 (100.0)	0/300 (0.0)	≥ 1 year	22/300 (7.3)
Nasal Swab	200	170/200 (85.0)	30/200 (15.0)	1-3 years	10/200 (5.0)
Tracheal Lavage	150	130/150 (86.7)	20/150 (13.3)	< 1 year	16/150 (10.7)
Total	650	600/650 (92.3)	50/650 (7.7)	-	48/650 (7.4)

Statistical analyses

Statistical analyses were performed using SPSS 13.0 for Windows. Associations between the isolation of *P. multocida* and variables including sample type, clinical status, sex and age group were evaluated using the Pearson chi-square (χ^2) test. Fisher's exact test was applied when expected cell frequencies were low. A *p*-value <0.05 was considered statistically significant.

Results

Isolation rates of *P. multocida*

Among the 650 samples collected from cattle, *P. multocida* was isolated and identified from 48 samples (7.4%). The organism was recovered from 22 of 300 lung samples (7.3%), 10 of 200 nasal swab samples (5.0%) and 16 of 150 tracheal lavage samples (10.7%). No significant differences in isolation rates were observed according to sex or age group (p>0.05). None of the *P. multocida* isolates exhibited hemolysis on 5% Sheep Blood Agar. All isolates were positive for catalase, oxidase, indole, ornithine decarboxylase and nitrate reduction tests, whereas urease, citrate utilization, methyl red, Voges–Proskauer, H_2S production in TSI agar and motility tests were negative. No growth was observed on MacConkey agar and all isolates exhibited a fermentative metabolism in Hugh–Leifson medium. In carbohydrate fermentation tests, D-glucose, D-sucrose and D-mannitol were fermented by all isolates, whereas inositol, amygdalin, L-rhamnose, D-melibiose and L-arabinose were not fermented. Fermentation of D-sorbitol was variable among the isolates (Garrity et al. 2004, Quinn et al. 2011).

Demographic and clinical characteristics of the study population

Clinically healthy animals constituted the majority of the study population (92.3%) and this predominance was statistically significant (χ^2=465.38, p<0.001). Females accounted for 72.3% of the study population and were significantly more prevalent than males (χ^2=129.38, p<0.001). Likewise, cattle aged 1-3 years represented the predominant age group (49.2%), with the difference also being statistically significant (χ^2=75.99, p<0.001).

Association between *P. multocida* isolation and clinical status

A total of 38/600 (6.3%) isolates were recovered from clinically healthy animals, whereas 10/50 (20.0%) isolates were obtained from clinically diseased animals. The isolation rate was significantly higher among clinically diseased animals (χ^2=12.50, *df*=1, p<0.001).

The distribution of samples and *P. multocida* isolation rates according to sample type and clinical status are presented in Table 4. Although the highest isolation rate was observed in tracheal lavage samples (10.7%), followed by lung (7.3%) and nasal swab samples (5.0%), no significant association was detected between sample type and isolation rate (χ^2=4.28, *df*=2, p=0.117).

Capsular typing results of *P. multocida* isolates

Capsular typing of *Pasteurella multocida* strains was performed using the Slide Agglutination Test. Of the 48 *P. multocida* isolates, 46 were identified as capsular type A, whereas the remaining two isolates could not be typed.

In addition to conventional phenotypic methods, the identification of the *P. multocida* isolates was confirmed by PCR. Amplification products of 221 bp (PMOut) and 460 bp (KMT1T7-KMT1SP6) were detected in all 48 isolates, confirming their identification as *P. multocida* (Neumann et al. 1998, Townsend et al. 1998). All isolates yielded the 1044 bp PCR product specific for capsular type A. Accordingly, all 48 isolates were genetically classified as capsular type A.

Antimicrobial susceptibility profiles of *P. multocida* isolates

The antimicrobial susceptibility of 48 *P. multocida* isolates was interpreted according to the CLSI guidelines (CLSI 2015a, CLSI 2015b). The isolates exhibited high in vitro susceptibility to florfenicol (46/48, 95.8%), ceftiofur (45/48, 93.8%), tulathromycin (43/48, 89.6%) and enrofloxacin (42/48, 87.5%).

For antimicrobials with CLSI-defined susceptible-only breakpoints (AMC, OXY, PEN and SXT), isola-

Table 5. Phenotypic resistance patterns of bovine *P. multocida* isolates (*n*=48).

No. of Antimicrobial in Pattern	Antibiotics	*P.multocida* *n/N* (%)
0	No resistance detected	38/48 (79.2)
1	ERY	1/48 (2.1)
2	ERY, ENR	2/48 (4.2)
2	ERY, FFN	2/48 (4.2)
2	ERY, TUL	2/48 (4.2)
2	ERY, TIL	3/48 (6.3)

ERY – erythromycin, ENR – enrofloxacin, FFN – florfenicol, TUL – tulathromycin, TIL – tilmicosin

tes below the susceptibility threshold were classified as nonsusceptible (NS). Accordingly, the highest NS rate was observed for oxytetracycline (19/48, 39.6%), followed by Trimethoprim-sulfamethoxazole (11/48, 22.9%), penicillin (10/48, 20.8%) and Amoxicillin-clavulanate (4/48, 8.3%). Erythromycin was the least effective agent, with a susceptibility rate of 50.0% (24/48) and a resistance rate of 20.8% (10/48). The antimicrobial susceptibility profiles of the isolated *P. multocida* strains are given in Table 3.

Most isolates were pan-susceptible (38/48, 79.2%,) and no multidrug-resistant isolates were detected. The phenotypic resistance patterns of the isolates are presented in Table 5.

Discussion

Bovine respiratory disease is considered one of the leading causes of morbidity and mortality in both beef and dairy cattle worldwide. Although BRD may occur at all stages of production, its greatest economic impact is observed in young calves and recently weaned animals, where respiratory disease contributes substantially to reduced performance, treatment costs and mortality (USDA 2011, Chamorro and Palomares 2020). In the United States, respiratory disease accounted for 45.4% of calf mortality in 1996 and 58.9% in 2014, highlighting its continuing importance for cattle production systems (USDA 2014). In addition to production losses, BRD imposes a considerable economic burden on producers, with estimated costs of approximately $1,293 per calf death and $43.95 per treated calf (Ayvazoglu et al. 2019).

The etiology of BRD is multifactorial and involves complex interactions among bacterial and viral pathogens, host immunity, environmental conditions and management-related stress factors (Dabo et al. 2007). Among the bacterial agents associated with BRD, *P. multocida* is of particular importance because of its widespread distribution, broad host range and frequent involvement in respiratory disease. In a recent meta-analysis, Zhou et al. (2023) identified *P. multocida* as one of the principal bacterial pathogens associated with BRD and reported that capsular type A accounted for 91.67% of the isolates, whereas type D represented 8.33%. The same study also demonstrated frequent co-infections between *P. multocida* and other respiratory pathogens, emphasizing its important role in the multifactorial pathogenesis of BRD.

Studies conducted in Turkey have provided varying data in terms of the isolation rates of *P. multocida* in cattle. Kilic and Muz (2004), isolated *P. multocida* from 6% of 500 samples of lungs with pneumonia obtained from cattle slaughtered at a slaughterhouse in Elazıg. In another study, among 570 intratracheal swab samples collected from slaughterhouses in Aydin and İzmir, *P. multocida* was isolated and identified in 28 (4.9%) (Erbas and Kaya 2008). Onat et al. (2010) collected nasal swab samples from 47 clinically healthy cattle and identified *P. multocida* in 27 (57.4%) of the samples. Ulker et al. (2012), detected *P. multocida* by PCR in 3 (2.45%) of 122 lung samples obtained from cattle slaughtered at a slaughterhouse. Gülaydin and Gürtürk (2018) analyzed respiratory tract swab samples using culture and PCR methods and detected *P. multocida* in 31 (13.9%) of 222 healthy cattle and 19 (32.2%) of 59 clinically diseased cattle. Baykan et al. (2023), studied 152 lung samples collected from cattle, sheep and goats with pneumonia and reported that they isolated and identified *P. multocida* in nine (20%) of 45 cattle samples. In epidemiological studies conducted in different countries, *P. multocida* has been reported at varying prevalence rates in cattle with respiratory disease. Autio et al. (2007) investigated healthy and clinically diseased calves with respiratory signs in Finland and reported a 34% isolation rate of *P. multocida* from tracheobronchial lavage samples. Furthermore, the presence of *P. multocida* was significantly associated with clinical respiratory disease. Similarly, Shayegh et al. (2010) examined samples collected from both clinically healthy and diseased cattle in Iran and detected *P. multocida* in 10 (6.02%) of 166 nasal swab, lung and milk samples. Positive samples in that study

included nasal swabs obtained from clinically healthy cattle, lung tissue samples from pneumonic cattle and milk samples from mastitic cattle.

In this study, among 650 samples collected from cattle, *P. multocida* strains were identified in 48 (7.4%) and the isolation rate in this study was compatible and very similar to those reported in previous studies (Kilic and Muz 2004, Shayegh et al. 2010). On the other hand, in comparison to this rate, some other studies reported higher values (Autio et al. 2007, Onat et al. 2010, Gülaydin and Gürtürk 2018, Baykan et al. 2023), whereas others reported lower values (Erbas and Kaya 2008, Ulker et al. 2012). The prevalence of pneumonic pasteurellosis may vary among countries and regions. Such variations may be influenced by differences in animal health status, environmental and climatic conditions, management practices, stress factors and diagnostic methodologies used among studies (Panciera and Confer 2010).

Species-specific PCR assays targeting chromosomal gene regions of *P. multocida* enable rapid and accurate identification of the organism and are considered advantageous over conventional diagnostic methods (Townsend et al. 1998, Jamil et al. 2025). Molecular epidemiological studies conducted in different geographical regions have consistently demonstrated the predominance of capsular type A among bovine *P. multocida* isolates. Ewers et al. (2006) reported that 84 of 91 isolates (92.3%), obtained primarily from cattle with respiratory disease, belonged to capsular type A. Similarly, Guler et al. (2013) found that capsular type A accounted for 91.6% of bovine isolates, while Gülaydin and Gürtürk (2018) identified all isolates recovered from healthy and diseased cattle (53/53, 100%) as type A. Consistent with these findings, Calderón Bernal et al. (2023) reported that 168 of 170 BRD-associated isolates (98.8%) belonged to capsular type A. Likewise, a meta-analysis by Zhou et al. (2023) identified *P. multocida* as one of the major pathogens involved in bovine respiratory disease and showed that capsular type A accounted for 91.67% (22/24) of the isolates, whereas type D represented only 8.33% (2/24). More recently, Lachowicz-Wolak et al. (2025) also identified capsular type A as the predominant type among *P. multocida* isolates recovered from dairy calves with clinical signs of bovine respiratory disease.

Nevertheless, variations in capsular type distribution have been reported according to geographical region, host species and disease manifestation. Catry et al. (2005) found that all *P. multocida* isolates recovered from calves with fatal peritonitis belonged to capsular type F, whereas Shayegh et al. (2010) identified capsular types A, B and D among isolates recovered from cattle and buffaloes in Iran, with capsular type B being the predominant type (46.15%). Despite these variations, the available evidence indicates that capsular type A remains the principal capsular type associated with bovine respiratory disease.

Consistent with this observation, slide agglutination testing identified 46 of 48 isolates (95.8%) as capsular type A, whereas two isolates were non-typable. Species-specific PCR analyses further confirmed all isolates as *P. multocida* and capsular PCR classified all 48 isolates as type A. Weak antigen-antibody reactions or variations in capsular expression may occasionally result in non-typable isolates during slide agglutination testing. This may explain why two isolates in the present study were not typeable by slide agglutination but were subsequently identified as capsular type A by PCR, a method widely used for capsular typing (Dziva et al. 2008). These findings are consistent with previous molecular studies and further support the epidemiological importance of capsular type A in bovine respiratory infections in the study region. Nevertheless, reports describing the occurrence of other capsular types indicate that the distribution of *P. multocida* capsular types may vary among geographical regions and cattle populations. Such variations may reflect differences in geographical location, host species, management practices and disease patterns associated with specific capsular types (Harper et al. 2022).

Various antimicrobial agents are routinely used for the treatment and control of bovine respiratory disease in Turkey, which may contribute to the emergence of antimicrobial resistance among respiratory bacterial pathogens. In the present study, high susceptibility rates were observed for florfenicol (46/48, 95.8%), ceftiofur (45/48, 93.8%), tulathromycin (43/48, 89.6%) and enrofloxacin (42/48, 87.5%), whereas reduced susceptibility was mainly detected against erythromycin and oxytetracycline. These findings are generally consistent with previous reports from Turkey and other countries. Karadeniz Pütür et al. (2024) demonstrated high susceptibility of bovine *P. multocida* isolates to florfenicol, ceftiofur and enrofloxacin, while resistance to macrolides and tetracyclines was observed at variable levels. Similarly, Gülaydin and Gürtürk (2020) reported high susceptibility of respiratory *P. multocida* isolates to florfenicol and enrofloxacin in cattle from Turkey. Comparable results were also reported by Welsh et al. (2004), who identified high susceptibility rates to ceftiofur, enrofloxacin and florfenicol among bovine respiratory isolates. Likewise, Kudirkiene et al. (2021) reported high susceptibility of *P. multocida* isolates to β-lactams, tulathromycin, tilmicosin, aminoglycosides, florfenicol and chlortetracycline, while resistance was primarily associated with tylosin. In contrast, nonsusceptibility to oxytetracycline was detected in 39.6% (19/48)

of the isolates, while nonsusceptibility rates for penicillin G and trimethoprim-sulfamethoxazole were 20.8% (10/48) and 22.9% (11/48), respectively. In addition, erythromycin exhibited the lowest susceptibility rate among the antimicrobials tested. Similar patterns of reduced susceptibility, particularly against tetracyclines and macrolides, have been reported previously in bovine respiratory *P. multocida* isolates (Welsh et al. 2004, Hendriksen et al. 2008, Karadeniz Pütür et al. 2024). These differences in susceptibility profiles may be associated with regional antimicrobial usage practices and the long-term use of specific antimicrobial classes in cattle production.

A comparison with recent international data further highlights the favorable susceptibility profile observed in the present study. Lachowicz-Wolak et al. (2025) reported an oxytetracycline susceptibility rate of only 18.8% among *P. multocida* isolates recovered from Polish dairy calves, whereas the corresponding rate in the present study was 60.4% (29/48). More importantly, no multidrug-resistant (MDR) isolates were detected in the current study (0/48), in contrast to the 27.1% MDR rate reported in the Polish study. Nevertheless, both studies consistently identified florfenicol and enrofloxacin as highly effective antimicrobial agents for the treatment of bovine respiratory disease.

The unnecessary and inappropriate use of antimicrobial agents in food-producing animals contributes to the emergence and dissemination of antimicrobial resistance and represents an important challenge within the One Health framework (Hernando-Amado et al. 2019). In addition, changes in antimicrobial usage patterns have been documented in cattle production systems, including reduced use of tetracyclines and increased use of macrolides for respiratory disease management (USDA 2014). More recently, the European Medicines Agency (EMA 2023) reported a marked decrease in veterinary antimicrobial sales across Europe between 2010 and 2022, reflecting the growing emphasis on prudent antimicrobial use and antimicrobial stewardship. Although the relatively low levels of nonsusceptibility observed in the present study suggest that antimicrobial resistance among bovine respiratory *P. multocida* strains was at a manageable level, a key limitation is that these isolates were collected between 2010 and 2011. Evolving antimicrobial use practices and antibiotic selection pressures since that period may have altered the subsequent resistance profiles. Consequently, these data highlight the need for contemporary studies to fully evaluate the current epidemiological status and resistance trends.

Limitations

One limitation of the present study is that the isolates were collected from cattle in a single geographical region, which may limit the generalizability of the findings to other cattle populations. Furthermore, a key limitation is that the isolates were collected between 2010 and 2011. Given the highly dynamic nature of antimicrobial resistance, the presented susceptibility results may not fully reflect the current epidemiological situation and should therefore be interpreted with caution. In addition, although capsular typing was performed using both slide agglutination and PCR methods, further molecular characterization, including lipopolysaccharide (LPS) typing and detailed analysis of virulence-associated genes, was not undertaken. Therefore, future studies involving contemporary isolates from different geographical regions and incorporating comprehensive molecular epidemiological approaches are needed to improve our understanding of the population structure, virulence characteristics and antimicrobial resistance profiles of bovine *P. multocida* strains.

Conclusion

In conclusion, *P. multocida* isolates obtained from cattle in the Afyonkarahisar region were successfully identified and capsularly typed using conventional and molecular methods, with capsular type A identified as the predominant type. Additionally, the susceptibility profiles indicated that antimicrobial resistance among these strains was at a manageable level during the study period, characterized by generally high susceptibility to several commonly used agents alongside reduced susceptibility to erythromycin and oxytetracycline. Despite the temporal limitations of the sampled isolates, these findings highlight the baseline importance of continuous regional surveillance and rational antimicrobial use for the effective management of bovine respiratory infections and may serve as a potential reference point for future epidemiological insights.

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Author Declarations

Ethics approval

The study was approved by the Animal Experiments Local Ethics Committee of Afyon Kocatepe University, Afyonkarahisar, Turkey (Approval No. 18-09; 04 May 2009).

Use of generative artificial intelligence

The authors declare that no generative artificial intelligence tools were used in the preparation of this manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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