Archives of Razi Institute, Vol. 71, No. 3 (2016) 153-160

DOI: 10.22034/ari.2016.106920

Original Article

Isolation, identification, and monitoring of antibiotic resistance in *Pasteurella multocida* and *Mannheimia haemolytica* isolated from sheep in East Azerbaijan province, Iran

Khalili [1, *] I., Ghadimipour [2, 3], R., Ghaderi [4], R., Shokri [4], GH., Jabbari [4], A.R., Razmaraii [5], N., Ebrahimi [6], M.

1. Virus Biobank Laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran
2 Department of Parasite Vaccine Research and Production, Razi Vaccine and Serum Research Institute, Karaj, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
4. Department of Aerobic Bacterial Vaccines, Razi Vaccine and Serum Research Institute, Karaj, Iran
5. Department of Research and Development, Razi Vaccine and Serum Research Institute, Karaj, Iran
6. Technical Director of Veterinary Vaccines, Razi Vaccine and Serum Research Institute, Karaj, Iran

Received 01 October 2015; accepted 22 November 2015
Corresponding Author: I.khalili@rvsri.ac.ir

ABSTRACT

The present study was carried out in order to isolate, identify, and assess the antimicrobial susceptibility of the causative agent(s) of pneumonic pasteurellosis in sheep in East Azerbaijan province, northwest of Iran. Pneumonia was detected in 320 cases, and the affected lungs were sampled in the slaughterhouse. The samples were investigated bacteriologically for the isolation of two microorganisms from the *Pasteurellaceae* family. *Pasteurella multocida* was isolated from six (1.87%) samples, while none of the lung tissues were positive for *Mannheimia haemolytica*. After the isolation and detection of microorganisms via cultural and morphological tests, the bacteria were identified on the basis of biochemical criteria and polymerase chain reaction (PCR) technique. Antimicrobial susceptibility testing was performed on all *P. multocida* isolates, using broth microdilution method. Evaluation of the minimum inhibitory concentration (MIC) of eight antimicrobial agents against the tested isolates showed that all the organisms were resistant to amoxicillin and relatively susceptible to ceftiofur. In conclusion, *P. multocida* was introduced as the main cause of ovine pneumonic pasteurellosis in the studied district, and the outbreak frequency significantly varied in different seasons of the year ($P<0.05$).

Keywords: Isolation, *Pasteurella multocida*, *Mannheimia haemolytica*, Sheep, PCR, Antimicrobial resistance, Iran

Isolation, identification et contrôle de l'antibiorésistance des souches de Pasteurella multocida et Mannheimia haemolytica isolées à partir de moutons de l'Est Azarbaijan, Iran

Résumé: L'objectif de cette étude était d'isoler, d'identifier et d'évaluer la susceptibilité antimicrobienne des agents responsables de la pasteurellose pneumonique chez les moutons de l'Est Azerbaïdjan, situé au nord ouest de l'Iran. Trois cent vingt (320) cas de pneumonie ont été détectés et le prélèvement des poumons affectés a été mené dans un abattoir. Tous les échantillons ont été ensuite soumis à des analyses bactériologiques et l'incidence de deux microorganismes spécifiques appartenant à la famille Pasteurellaceae a été étudiée. Selon nos analyses, six prélèvements pulmonaires étaient contaminés par Pasteurella multocida (1,87%), alors qu'aucun échantillon positif à la souche Mannheimia haemolytica n'a été détecté. Après l'isolation et la détection des microorganismes par des testes de culture et d'observation morphologiques, les souches bactériennes ont été identifiées grâce à leurs propriétés biochimiques et la méthode de réaction en chaîne par polymérase (PCR). La susceptibilité antimicrobienne de tous les isolats de P. multocida a été évaluée par la méthode de microdilution du bouillon de culture. L'évaluation de la concentration inhibitrice minimum (MIC) de 8 agents antimicrobiens vis-à-vis des isolats identifiés a révélé une résistance à l' amoxicilline et une susceptibilité relative au ceftiofur. En conclusion, P. multocida se présente comme la cause majeure de pasteurellose pneumonique ovine dans la province de l'Est Azerbaïdjan et la fréquence cette épidémie varie de façon significative d'une saison à l'autre ($P<0.05$).

Mots clés: Isolation, Pasteurella multocida, Mannheimia haemolytica, Mouton, PCR, Résistance antimicrobienne, Iran

Introduction

Pasteurella is a genus of Gram-negative, fucltatively anaerobic bacteria, belonging to the *Pasteurellaceae* family. *Pasteurellaceae* are recognized as the largest subgroup of *Gammaproteobacteria,* with a wide range of physiological subtypes (Wilson and Ho, 2013). *P. multocida* is responsible for various diseases of economic significance in domestic animals, including hemorrhagic septicemia in cattle and buffaloes, pneumonic septicemic pasteurellosis in sheep and goats, atrophic rhinitis in pigs, snuffles in rabbits, and fowl cholera in poultry throughout the world. The outbreak of these diseases usually leads to high mortality rates and great economic loss for ruminant industries (Odugbo et al., 2006). On the other hand, *Mannheimia haemolytica* (formerly known as *P. haemolytica*) is the most common etiological agent of pneumonic pasteurellosis, septicemia, and mastitis and is considered as one of the most important pathogens in lambs, sheep, and goats (Wilson and Ho, 2013). Sheep and goats are considered as major sources of economic profit from domestic animals, especially in Middle East and Arab countries. These animals could compensate for the shortage of milk and meat supplies from cattle and buffaloes; besides, they are used as a source of wool, fiber, and hides. In fact, sheep rearing plays a multifaceted role in rural households by providing mutton, wool, manure, and hides (Dar et al., 2013). Pneumonia is among diseases causing considerable damage to the country's livestock industry every year. Pneumonia could occur due to bacterial pathogens, the most common of which include *P. multocida*, *M. haemolytica*, and *Mycoplasma ovipneumoniae* (Safaee et al., 2006). Pneumonic pasteurellosis is one of the most prevalent infections among sheep and goats in tropical and warm-temperate regions, causing weight loss and severe economic loss for livestock industries (Odugbo et al., 2006). *P. multocida*, as one of the main etiological agents of pneumonic pasteurellosis, leads to the production of a number of proteins and polysaccharides, which are thought to contribute to its virulence. With respect to surface polysaccharide capsules, *P. multocida* is differentiated into five major serogroups (i.e., A, B, D, E, and F) (Chung et al., 1998). Serogroups A and D of *P. multocida* have been recognized as incriminated agents of pneumonic pasteurellosis in sheep and goats (Zamri-Saad et al., 1996). These isolates synthesize a 145-kDa toxin, encoded by the chromosomal *toxA* gene. Overall, *toxA* proteins are essential virulence factors for the progression of pasteurellosis in sheep and goats, particularly those belonging to capsular serotypes A and D (Lichtensteiger et al., 1996). Pneumonic pasteurellosis is an endemic disease in Iran, reported in different regions such as West Azerbaijan, Mazandaran, Guilan, and Fars provinces (Tabatabaei et al., 2002; Tehrani et al., 2004; Shayegh et al., 2009). However, no information is available regarding this disease and its prevalence in East Azerbaijan province.

There are several methods for the isolation and identification of *Pasteurella* species, including bacterial culture techniques, differential biochemical tests, antiserum-based methods, and advanced molecular techniques such as ribotyping, restriction endonuclease analysis, and polymerase chain reaction (PCR). Some of these methods such as bacterial culturing are time-consuming and yield false negative results. Genotyping techniques for bacterial identification have been shown to be advantageous for overcoming some limitations of conventional culture-based, biochemical, and serological methods. Molecular typing allows for the direct detection of microorganisms from clinical samples or small amounts of cultured bacterial cells. Therefore, this method can dramatically improve the sensitivity of microorganism detection and decrease the time required for identification (Townsend et al., 1998). PCR by relying on primer sequences, designed to facilitate identification at any level of specificity (i.e., strain, species, genus, and domain levels), is considered as a particularly useful method. This method is both sensitive and specific and does not involve laboratory animals. With this background in mind, in this study, we aimed to isolate, identify, and evaluate the antimicrobial susceptibility of two microorganisms

from the *Pasteurellaceae* family in sheep via routine and PCR methods in East Azerbaijan, Iran.

MATERIALS AND METHODS

Sampling. In total, 2550 sheep were inspected at Tabriz and Marand abattoirs in East Azerbaijan province during March 2013 and February 2014. After slaughtering the animals, samples were obtained from suspicious lungs with a sterile scalpel. A total of 320 lung tissue condemnations collected in situ showed consolidation, pleural fibrin deposition, pleurisy, and/or adhesion. The samples were transferred to the microbiology laboratory in the northwest branch of Razi Vaccine and Serum Research Institute in special sterile ice-filled containers. Considering the sensitivity of the organisms, the samples were immediately transferred to the laboratory.

Bacteriological assays. *P. multocida* and *M. haemolytica* were isolated, using techniques described in the literature (Songer and Post, 2004). Briefly, swabs were obtained from the depth of pneumonic lung tissues by scorching the lung surface with a hot spatula. Then, the samples were cultured on blood agar (5% fresh sheep blood) and incubated at 37 °C for 48 h. Also, two control cultures containing either *P. multocida* (ATCC 11039) or *M. haemolytica* (ATCC 55518) were used. Suspicious colonies from the initial cultures were transferred to the blood agar by conventional streaking techniques and were incubated at 37 °C for 48 h. In these pure cultures, *P. multocida* and *M. haemolytica* colonies could be more easily studied. At this stage, MacConkey agar medium was also used. The colonies in both culture media were studied in terms of shape, size, color, and hemolytic capacity. The colonies were also Gram-stained, and oxidase and catalase tests were applied on the colonies. Moreover, triple sugar iron (TSI), sulfide-indole motility (SIM), urea agar, nitrate, and citrate media, along with some other culture media containing sugars such as glucose, lactose, sucrose, mannitol, and trehalose, were used at 37 °C for 48 h under incubation conditions.

Molecular characterization. Preliminary identification of *P. multocida* and *M. haemolytica* isolates was carried out according to standard biochemical tests, as described in previous research (Songer and Post, 2004). The isolates were confirmed through PCR assay with specific primers (for amplification of *recN* and *lktA* genes) by adopting the methodology described in the literature (Ewers et al., 2006) with minor modifications. Briefly, the bacteria were initially grown on 5% sheep blood agar plates with incubation at 37 °C for 18-24 h. A single colony was inoculated into a test tube, containing 3 ml of brain-heart infusion (BHI) broth, and incubated at 37 °C for 18-24 h with minor rotations. The overnight BHI culture was centrifuged at 13,000×g for 5 min. The supernatant was discarded and the pellets were washed twice in phosphate-buffered saline (PBS) at 13,000×g for 5 min. The washed pellets were re-suspended in tris-ethylene diamine tetraacetate (EDTA) buffer (pH=7.3). The cells were extracted with phenol, which was previously saturated with tris-EDTA (pH=7.3), and centrifuged at 13,000×g for 10 min. The upper phase was collected and transferred to a clean 1.5 ml microtube. This phase was mixed with phenol/chloroform/isoamyl alcohol (24:24:1) and centrifuged at 13,000×g for 5 min. DNA was precipitated after the addition of sodium acetate and absolute ethanol. DNA was then washed with 70% ethanol, dried at room temperature, re-suspended in EDTA, and stored at -20 °C until further use. The absorbance of DNA was measured at 260 nm by a spectrophotometer. Identification of *P. multocida* and *M. haemolytica* was confirmed by PCR assay, using oligonucleotide primers, listed in Table 1. The genes indicated *P. multocida* and *M. haemolytica* among the isolates. PCR was performed in a total volume of 25 µL, containing 0.4 µM of each primer, 0.25 mM of deoxynucleotides (dNTPs), 1X Taq reaction buffer, 2.5 mM of $MgCl_2$, 1.25 U of Taq DNA polymerase, and 1 µL of the isolated DNA (all reagents from CinnaGen, Iran). DNA was amplified under the following conditions in a thermocycler (Mastercycler Gradient, Eppendorf, Germany): initial denaturation at 94 °C for

4 min, followed by 30 cycles of denaturation at 94 °C for 45 sec, annealing at 58°C for 45 sec, extension at 72 °C for 45 sec, and a final extension at 72 °C for 5 min. The amplified products were separated by electrophoresis on 1.0% agarose gel and visualized by ethidium bromide staining.

Antimicrobial susceptibility testing. Antimicrobial susceptibility of the isolates to eight antimicrobial agents was determined by broth microdilution method on Mueller-Hinton broth in a 96-well microplate, according to the procedures outlined in the Clinical and

Table 1. Sequences of oligonucleotides used for the detection of *multocida* and *M. haemolytica*

Primer	Gene	Primer sequence	Amplicon size (bp)
All pass (*P. Multocida*)	*recN*	PMF (5' CATTTGAATATTGAATTTACGCAA 3') PMR (5' GGATTTATCTGAGGTAAGCAC 3')	1320
All pass (*M. haemolytica*)	*lktA*	MHF (5' TGTGGATGCGTTTGAAGAAGG 3') MHR (5' ACTTGCTTTGAGGTGATCCGG 3')	1146

Table 2. Detection and identification of *P. multocida* and *M. haemolytica* isolates from 320 suspicious sheep lung samples by conventional culture-based tests and polymerase chain reaction (PCR) in East Azerbaijan province over a 12-month period (2013-2014)

Microorganism	Culture tests		PCR technique		
	Positive	Negative	Positive	Negative	Positive (total)
P. multocida	5	315	6	314	6
M. haemolytica	0	320	0	320	0

Table 3. Comparison of the relative frequency distribution of *P. multocida*-positive samples (in a total of 320 sheep lung samples) in different seasons during 2013-2014 in East Azerbaijan province

Animal	Season															
	Spring				Summer				Fall				Winter			
	PS	%		No. S	PS	%		No. S	PS	%		No. S	PS	%		No. S
		FSS	FT PS			FSS	FTPS			FSS	FTPS			FSS	FTPS	
Sheep	1	1.6	16.6	60	0	0.0	0.0	55	0	0.0	0.0	102	5	4.8	83.3	103

No. S= Number of samples, PS= Positive samples, FSS= From seasonal samples, FTPS= From the total of positive samples

Table 4. Minimum inhibitory concentrations (MICs) and interpretation criteria of the tested antimicrobial agents used against *P. multocida* according to CLSI M31-A3 document (2008)

Antimicrobial agent	Dilution range (µg/ml)	MIC (µg/ml)*		
		S	I	R
Gentamicin	0.125-256	≤4	8	≥16
Ceftiofur	0.125-256	≤2	4	≥8
Enrofloxacin	0.125-256	≤0.25	0.5-1	≥2
Amoxicillin	0.125-256	≤8	16	≥32
Tetracycline	0.125-256	≤4	8	≥16
Lincomycin**	0.125-256	≤2	4	≥8
Penicillin	0.125-256	≤1	2-16	≥16
Trimethoprim–sulfamethoxazole**	0.125-256	≤2	N/A	≥4

*S= Susceptible, I= Intermediate, R= Resistant
**Since no MIC was specifically determined for *P. multocida* in CLSI standards, MICs defined for other bacteria were used.

Table 5. Antimicrobial susceptibility testing of six *P. multocida* strains isolated from sheep during 2013-2014 in East Azerbaijan province

Antimicrobial agent	Number of isolates		
	Susceptible	Intermediate	Resistant
Gentamicin	1 (16.6%)	2 (33.3%)	3 (50.0%)
Ceftiofur	6 (100%)	0 (0.0%)	0 (0.0%)
Enrofloxacin	1(16.6%)	4 (66.6%)	1(16.6%)
Amoxicillin	0 (0.0%)	0 (0.0%)	6 (100%)
Tetracycline	1(16.6%)	5 (83.3%)	0 (0.0%)
Lincomycin	0 (0.0%)	3 (50.0%)	3 (50.0%)
Penicillin	2 (33.3%)	3 (50.0%)	1(16.6%)
Trimethoprim–sulfamethoxazole	2 (33.3%)	0 (0.0%)	4 (66.6%)

Laboratory Standards Institute (CLSI), document M31-A3 (2008). Moreover, broth microdilution assay was used to determine the minimum inhibitory concentration (MIC). First, two-fold serial dilutions of antimicrobial agents were prepared at a concentration range of 0.125-256 µg/ml in 50 µl of PBS. Two wells were considered as the controls with no antibiotics and microorganisms, respectively. Inoculations of the tested organisms were prepared from 18-h Mueller-Hinton broth cultures. Suspensions were adjusted to 0.5 McFarland nephelometer standard (10^8 cfu/ml) and then diluted to a final concentration of 5×10^5 cfu/ml. The plates were sealed and incubated at 35°C for 16-20 h. *Staphylococcus aureus* (ATCC 25923) was used as the quality control strain. The MICs were measured immediately before and 20 h after incubation. MIC was defined as the lowest concentration of antibiotics preventing the growth of microorganisms. MICs were calculated and the results were interpreted, according to the guidelines described in CLSI document M31-A3 (Table 4). The results were interpreted as resistant (R), intermediate (I), and susceptible (S). Antimicrobial agents including penicillin, enrofloxacin, gentamicin, ceftiofur, tetracycline, amoxicillin, lincomycin, and trimethoprim-sulfamethoxazole (Sigma, USA) were used in this study.

Statistical analysis. For statistical analysis, SPSS version 19.0 was used (SPSS Inc., Chicago, IL, USA). Descriptive statistics were computed to determine the proportion of isolated bacteria in different seasons and assess the proportion of isolates resistant to different antimicrobial agents. Chi-square test was adopted to evaluate the statistical difference between the proportions.

RESULTS

A total of 320 sheep lungs suspected of infection were studied over a 12-month period (2013-2014). The prevalence of *Pasteurella* species in the collected lung samples is presented in Table 2. The isolates, subjected to cultural and biochemical tests, were Gram-negative rods and non-hemolytic, round, grayish, smooth, and mucoid colonies on the blood agar; however, no growth was detected on MacConkey agar. The isolates were small, non-motile *Coccobacilli*, which were positive for indole, oxidase, and catalase, while being negative for urease. Nitrate-to-nitrite reduction was reported without citrate. Glucose was fermented without producing gas, trehalose, mannitol, or sucrose (unlike lactose). According to the biochemical and microbiological tests, five (1.56%) out of 320 samples were *P. multocida*-positive, while none were positive for *M. haemolytica.* The PCR amplification of different DNA samples showed amplified bands of identical size (1320 bp), using *recN*-specific primers, while six (1.87%) samples were identified as *P. multocida* (Figure 1).

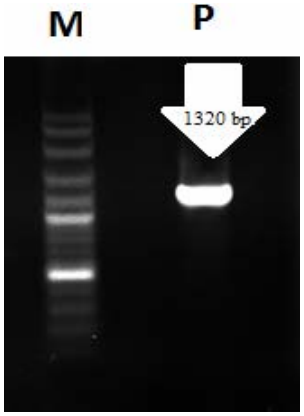

Figure 1. Electrophoresis of PCR products on 1.0% agarose gel, stained with ethidium bromide. Lane M: 100 bp ladder and lane P: 1320 bp *P. multocida*-specific band (*recN* primer).

Based on the present results, five (83.33%) positive samples were gathered in winter, while one (16.66%) positive sample was collected during spring (Table 3). Based on these findings, the outbreak frequency significantly varied in different seasons of the year ($P<0.05$). According to the present results, *P. multocida* was the main cause of ovine pneumonic pasteurellosis in the studied district. Antimicrobial resistance profiles of six *P. multocida* isolates in this study are displayed in Table 5. All the isolated organisms were resistant to amoxicillin and relatively susceptible to ceftiofur. Moreover, resistance to enrofloxacin, gentamicin, tetracycline, penicillin, lincomycin, and trimethoprim-sulfamethoxazole was observed at different frequencies.

DISCUSSION

Isolation of *P. multocida* from sheep and goats has been the subject of extensive research, particularly in Southeast Asia. On many occasions, *P. multocida* has been isolated in both healthy and diseased sheep and goats. A theory suggesting the infection of susceptible hosts subsequent to the dissemination of resting pathogens from the respiratory tract of convalescents (as well as non-clinical carriers) and rigors of nature has been widely accepted (Kumar et al., 2004). However, different *P. multocida* isolation rates have been reported in these domestic animals. According to the present study, out of 320 sheep lung samples, six (1.87%) were *P. multocida*-positive, while none were positive for *M. haemolytica*. These isolates were identified by both conventional tests and PCR amplification technique (Table 2). Therefore, *P. multocida* was not a highly prevalent agent among sheep in this research. The frequency of these organisms reported in some previous studies differs from the present results. In this regard, based on a study by Hawari et al. (2008), *P. multocida* was a high frequently isolated organism (31.7%) from the pneumonic lungs of infected sheep. Moreover, according to a study by Demissie et al. (2014), the total isolation rate of *M. haemolytica* and *P. multocida* from pneumonic sheep and goats was 28% and 2.2%, respectively. In contrast with the present findings, Azizi et al. (2013) detected *P. multocida* in 24.5% of sheep lung samples in Kurdistan province, Iran. Similarly, other studies identified *P. multocida* in 16.6% of lung samples gathered from pneumonic sheep and goats in Fars province, Iran. Based on a study by Tehrani et al. (2004), *M. haemolytica* biotype A was isolated from 316 (16%) out of 1988 sheep lung tissues during May 1998 and April 1999 in Urmia, Iran. In congruence with our findings, Shayegh et al. (2009) reported that 3.3% of nasal swabs from suspected goats were positive for *P. multocida* in West Azerbaijan province. Additionally, Ebrahimi et al. (2010) showed that only 0.6% of the studied pneumonic sheep lesions in Kashan abattoirs contained *P. multocida*. The researchers concluded that pneumonic pasteurellosis due to *P. multocida* is not of great significance in Kashan province. Similarly, in the current research, among 320 studied sheep lung samples, only six (1.87%) contained *P. multocida* in East Azerbaijan province (Table 2). The present results indicated that five (83.33%) positive samples were taken during winter (December 2013 and January-February 2014), while one (16.66%) positive sample was gathered in spring (April 2013) (Table 3). The evaluation of the prevalence of infection among sheep in four seasons of the year revealed the highest relative frequency of *Pasteurella* infection in winter. Infections with *P. multocida* are commonly managed by broad-spectrum antimicrobials (Lion et al., 2006; Brogden et al., 2007). However, previous studies have reported the resistance of *P. multocida* isolates to a large number of antimicrobial agents (Davies et al., 2004; Arashima and Kumasaka, 2005). In the current study, all *P. multocida* isolates were more susceptible to ceftiofur and resistant to amoxicillin. Moreover, the present results showed that penicillin, enrofloxacin, and tetracycline exhibited moderate activities against the tested isolates. In total, the isolated organisms were resistant to gentamicin, lincomycin, and trimethoprim-sulfamethoxazole. Occurrence of antimicrobial resistance varies in different countries and regions. In line with the present study, Guler et al. (2013) in Turkey showed that all *P. multocida* isolates were susceptible to ceftiofur, enrofloxacin, penicillin, florfenicol, and trimethoprim-sulfamethoxazole. In consistent with the present results, Jabeen et al. (2013) in Pakistan showed that *P. multocida* was resistant to augmentin and co-trimoxazole, relatively susceptible to amoxicillin and aztreonam, and sensitive to gentamicin and ceftiofur. Similarly, Ferreira et al. (2012) in a study performed in Brazil showed that *P. multocida* strains were mostly resistant to sulfonamides and co-trimoxazole (28.3%), followed by penicillin (10.9%), amoxicillin (6.5%), and erythromycin (4.3%). Based on the findings, all the tested strains were sensitive to ceftiofur, florfenicol, norfloxacin, enrofloxacin, ciprofloxacin, tetracycline, and doxycycline. Moreover, according to a study by

Khamesipour et al. (2014), all *P. multocida* bovine isolates were susceptible to ciprofloxacin, cotrimoxazole, doxycycline, enrofloxacin, nitrofurantoin, and tetracycline. Pneumonia occurs in all breeds of sheep and goats at all ages in different countries. The climatic conditions play a major role in respiratory problems in different areas. Factors such as crowding, dust, humid weather, and stress can increase the prevalence of such conditions (Weiser et al., 2003). In fact, pneumonic pasteurellosis is one of the most prevalent infections among sheep and goats in tropical and warm-temperate regions, causing weight loss and severe economic loss to livestock industries (Odugbo et al., 2006). The results of the present study provide information on the isolation and identification of agents responsible for pasteurella pneumonia by using both conventional and molecular methods in East Azerbaijan province, Iran. *P. multocida* was recognized as the main cause of ovine pneumonic pasteurellosis in the studied district, and the outbreak frequency significantly differed in various seasons of the year ($P<0.05$). However, in this research, *P. multocida* was not a highly prevalent agent among sheep (1.87%). Based on the present results, all the isolated organisms were resistant to amoxicillin and relatively susceptible to ceftiofur. Also, these organisms were resistant to gentamicin, lincomycin, and trimethoprim-sulfamethoxazole. Resistance to these antimicrobial agents is likely to be related to their widespread and indiscriminate use. Based on the present results, local veterinarians and manufacturers should pay particular attention to the use of antibiotics for the treatment of pneumonia among sheep. Moreover, the obtained results highlight the growing risk posed by the dissemination of *P. multocida* strains resistant to most antimicrobials, which could complicate the future treatment of pneumonia in these animals.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This study was financially supported by Razi Vaccine and Serum Research Institute and Education and Research Deputy of Jihad Agriculture Ministry.

Acknowledgments

The authors wish to thank their colleagues at the Department of Research and Development of Razi Vaccine and Serum Research Institute and the expert team at the Microbiology Department, especially Dr. Ahmad Reza Jabbari and Dr. Gholamreza Shokri for their expertise, understanding, and patience.

References

Arashima, Y., Kumasaka, K., 2005. Pasteurellosis as zoonosis. Intern Med 44, 692-693.

Azizi, S., Korani, F.S., Oryan, A., 2013. Pneumonia in slaughtered sheep in south-western Iran: pathological characteristics and aerobic bacterial aetiology. Vet Ital 49, 109-118.

Brogden, K.A., Nordholm, G., Ackermann, M., 2007. Antimicrobial activity of cathelicidins BMAP28, SMAP28, SMAP29, and PMAP23 against Pasteurella multocida is more broad-spectrum than host species specific. Vet Microbiol 119, 76-81.

Chung, J.Y., Zhang, Y., Adler, B., 1998. The capsule biosynthetic locus of Pasteurella multocida A:1. FEMS Microbiol Lett 166, 289-296.

Dar, L.M., Darzi, M.M., Mir, M.S., Kamil, S.A., Rashid, A., Abdullah, S., 2013. Prevalence of lung affections in sheep in northern temperate regions of India: A postmortem study. Small Ruminant Research 110, 57-61.

Davies, R.L., MacCorquodale, R., Reilly, S., 2004. Characterisation of bovine strains of Pasteurella multocida and comparison with isolates of avian, ovine and porcine origin. Vet Microbiol 99, 145-158.

Demissie, T., Dawo, F., Sisay, T., 2014. Biochemical and antigenic characterization of Mannheimia, Pasteurella and Mycoplasma species from naturally infected pneumonic sheep and goats, Bishoftu, Ethiopia. African Journal of Basic & Applied Science 6, 198-204.

Ebrahimi, A., Halajifar, M., Ataei, S., Lotfalian, S., 2010. Investigation of Pasteurella multocida in sheep pneumonic lesions of Kashan abattoir. Journal of Animal Health and Disease 4, 29-32.

Ewers, C., Lubke-Becker, A., Bethe, A., Kiebling, S., Filter, M., Wieler, L.H., 2006. Virulence genotype of Pasteurella multocida strains isolated from different hosts with various disease status. Vet Microbiol 114, 304-317.

Ferreira, T.S., Felizardo, M.R., Senade Gobbi, D.D., Gomes, C.R., Nogueira Filsner, P.H., Moreno, M., 2012. Virulence genes and antimicrobial resistance profiles of Pasteurella multocida strains isolated from rabbits in Brazil. Science World Journal, 1-6.

Guler, L., Gunduz, K., Sarishahin, A.S., 2013. Capsular typing and antimicrobial susceptibility of Pasteurella multocida isolated from different hosts. Kafkas University Veterinary Fakultesi Dergisi 19, 843-849.

Hawari, A.D., Hassawi, D.S., Sweiss, M., 2008. Isolation and Identification of Mannheimia haemolytica and Pasteurella multocida in Sheep and Goats using Biochemical Tests and Random Amplified Polymorphic DNA (RAPD) Analysis. Journal of Biological Sciences 8, 1251-1254.

Jabeen, A., Khattak, M., Munir, S., Jamal, Q., Hussain, M., 2013. Antibiotic susceptibility and molecular analysis of bacterial pathogen Pasteurella multocida isolated from cattle. Journal of Applied Pharmaceutic Science 3, 106-110.

Khamesipour, F., Momtaz, H., Azhdary Mamoreh, M., 2014. Occurrence Of Virulence Factors And Antimicrobial Resistance In Pasteurella Multocida Strains Isolated From Slaughter Cattle In Iran. Frontiers in Microbiology 5.

Kumar, A.A., Shivachandra, S.B., Biswas, A., Singh, V.P., Singh, V.P., Srivastava, S.K., 2004. Prevalent serotypes of Pasteurella multocida isolated from different animal and avian species in India. Vet Res Commun 28, 657-667.

Lichtensteiger, C.A., Steenbergen, S.M., Lee, R.M., Polson, D.D., Vimr, E.R., 1996. Direct PCR analysis for toxigenic Pasteurella multocida. J Clin Microbiol 34, 3035-3039.

Lion, C., Conroy, M.C., Carpentier, A.M., Lozniewski, A., 2006. Antimicrobial susceptibilities of Pasteurella strains isolated from humans. Int J Antimicrob Agents 27, 290-293.

Odugbo, M.O., Odama, L.E., Umoh, J.U., Lamorde, A.G., 2006. Pasteurella multocida pneumonic infection in sheep:

Prevalence, clinical and pathological studies. Small Ruminant Research 66, 273-277.

Safaee, S., Weiser, G.C., Cassirer, E.F., Ramey, R.R., Kelley, S.T., 2006. Microbial diversity in bighorn sheep revealed by culture-independent methods. J Wildl Dis 42, 545-555.

Shayegh, J., Dolgari Sharaf, J., Mikaili, P., Namvar, H., 2009. Pheno- and genotyping of Pasteurella Multocida isolated from goat in Iran. African Journal of Biotechnology 8, 3707-3710.

Songer, J.G., Post, K.W., 2004. Veterinary Microbiology - Elsevieron VitalSource: Bacterial and Fungal Agents of Animal Disease, Elsevier Health Sciences.

Tabatabaei, M., Liu, Z., Finucane, A., Parton, R., Coote, J., 2002. Protective immunity conferred by attenuated aroA derivatives of Pasteurella multocida B:2 strains in a mouse model of hemorrhagic septicemia. Infect Immun 70, 3355-3362.

Tehrani, A.A., Ras, M.B., Niazy, H., 2004. Isolation and identification of Pasteurella Haemolytica biotype A from sheep in Uremia, Iran. Iranian Journal of Veterinary Research 5, 105-109.

Townsend, K.M., Frost, A.J., Lee, C.W., Papadimitriou, J.M., Dawkins, H.J., 1998. Development of PCR assays for species- and type-specific identification of Pasteurella multocida isolates. J Clin Microbiol 36, 1096-1100.

Weiser, G.C., DeLong, W.J., Paz, J.L., Shafii, B., Price, W.J., Ward, A.C., 2003. Characterization of Pasteurella multocida associated with pneumonia in bighorn sheep. J Wildl Dis 39, 536-544.

Wilson, B.A., Ho, M., 2013. Pasteurella multocida: from zoonosis to cellular microbiology. Clin Microbiol Rev 26, 631-655.

Zamri-Saad, M., Effendy, A.W.M., Maswati, M.A., Salim, N., Sheikh-Omar, A.R., 1996. The goat as a model for studies of pneumonic pasteurellosis caused by Pasteurella multocida. British Veterinary Journal 152, 453-458.