



# Pathogenic and Antibiotic-Resistance Genes of *Pasteurella multocida* Isolated from Goats in the Mekong Delta, Vietnam

Thuong Thi Nguyen<sup>1</sup>, Vy Ly Phuong Nguyen<sup>2</sup>, Trung Thanh Truong<sup>3</sup>, Chi Thi Hanh Nguyen<sup>4</sup>, and Thuan Khanh Nguyen<sup>2\*</sup>

<sup>1</sup> Faculty of Animal Science and Veterinary Medicine, Nong Lam University Ho Chi Minh City, Region 6<sup>th</sup>, Linh Trung ward, Thu Duc City, Ho Chi Minh City 71308, Vietnam

<sup>2</sup> Faculty of Veterinary Medicine, College of Agriculture, Can Tho University, Campus II, 3/2 Street, Xuan Khanh ward, Ninh Kieu District, Can Tho City 90000, Vietnam

<sup>3</sup> Faculty of Animal Sciences, College of Agriculture, Can Tho University, Campus II, 3/2 Street, Xuan Khanh ward, Ninh Kieu District, Can Tho City 90000, Vietnam

<sup>4</sup> Faculty of Agriculture and Natural Resources, An Giang University - Vietnam National University HCMC, Ung Van Khiem Street, Dong Xuyen Ward, Long Xuyen City, An Giang Province 90100, Vietnam

\*Corresponding author's Email: [nkthuan@ctu.edu.vn](mailto:nkthuan@ctu.edu.vn)

## ABSTRACT

*Pasteurella multocida* (*P. multocida*) is one of the predominant pathogens that mostly cause respiratory diseases in domestic animals, such as goats. To determine *P. multocida* serotypes and the prevalence of pathogenic and antibiotic-resistance genes the PCR method was used. A total of 143 isolated *P. multocida* strains were collected from 289 healthy hybrid Boer-Saanen goats' nasal samples in the Mekong Delta, Vietnam, from March to June 2023. A total of 143 *P. multocida* strains, serotype B accounted for the highest proportion (51.05%), followed by serotype A (14.69%), and the lowest was serotype E (0.70%) while (39.86%) of strains could not be determined serotypes. Among the six virulence genes surveyed, the *sodA* gene (56.64%) had the highest presence, while the *ompH* gene (4.20%) had the lowest presence. Pathogenic genes were present mainly in serotypes A and B; *tbpA* was frequently detected in serotype A (66.67%), and *sodA* was commonly detected in serotype B (56.16%). There were 14 virulence gene combinations in 59/109 (54.13%) serotyped *P. multocida* strains, and the pattern of *sodA* + *tox*A + *tbpA* was prevalent at the highest rate (12.84%). Moreover, among the eight investigated antibiotic resistance genes, the *sulII* gene had the highest presence rate (74.13%), compared to the *tetA* gene with the lowest presence rate (13.29%). Gene *sulII* was mainly detected on strains belonging to serotypes A (80.95%), B (83.56%), and F (77.78%). A total of (77.98%) of serotyped *P. multocida* strains indicated multi-harbor from two to six antibiotic-resistance genes, and the most common pattern was *aadB* + *sulII* (10.09%). The prevalence of five pathogenic *P. multocida* serotypes harboring diverse antibiotic-resistance genes isolated from nasal samples could be a critical issue in treating and preventing the respiratory diseases caused by *P. multocida* in goats in the Mekong Delta.

**Keywords:** Antibiotic resistance, Goat, *Pasteurella multocida*, Pathogenicity, Mekong Delta

## INTRODUCTION

Respiratory disease caused by *Pasteurella multocida* is among the most common infections in ruminant animals. Goats and other small ruminants are at a moderate risk of contracting this pathogen due to exposure to physical stress or uncomfortable environmental conditions (Mohamed and Abdelsalam, 2008). *P. multocida* is more frequently associated with the outbreak of acute pneumonia and death of goats in all age groups than *Mannheimia haemolytica* is in previous reports (Falade, 2002). Respiratory diseases in goats cause economic losses arising from mortality and morbidity. The respiratory disease mortality rate caused by *P. multocida* is 10% or more (Smith and Sherman, 2009). Girma et al. (2023) reported 27,563 and 37,522 cases of sheep and goats with pneumonia, in Southern Ethiopia from 2016 to 2021. There were a few reports on *P. multocida* isolated from small ruminants in Vietnam. In a previous report, *P. multocida* was detected in healthy goats raised on medium-scale farms in Can Tho City, the Mekong Delta at 49.48% (Nguyen et al., 2024). These observations implied the significance of pneumonic pasteurellosis in small ruminants.

*Pasteurella multocida* is classified into five serotypes, A, B, D, E, and F, according to capsular antigen, and 16 serotypes according to lipopolysaccharide antigen. The capsular antigen is considered an essential form of virulence of *P. multocida*, which allows *P. multocida* to avoid innate host defense systems (Boyce et al., 2000). Each serotype has different circulation in different animals. Serotypes A and D are two serotypes that commonly appear in cases of pneumonia and pasteurellosis in goats (Rawat et al., 2009; Tabatabaei and Abdolahi, 2023). Besides, additional factors essential for the proliferation and maturation of *P. multocida* have been discovered. These encompass *P. multocida* toxin (PMT), fimbriae, adhesins, the capacity to metabolize sialic acid, outer membrane proteins, and hyaluronidases (Harper et al., 2006). Previous studies reported that the prevalence of some virulence-associated genes, including colonization

ORIGINAL ARTICLE  
Received: March 29, 2024  
Revised: April 25, 2024  
Accepted: May 18, 2024  
Published: June 25, 2024

factors (*ptfA*, *fimA*, *hsf2*), iron acquisition factors (*exbB*, *exbD*, *tonB*, *Fur*), superoxide dismutase (*sodA*, *sodC*), and outer membrane proteins (*ompA*, *ompH*, *oma87*, *plpB*), were frequently detected in the pig origin *P. multocida* isolates (May et al., 2001; Peng et al., 2016; 2018). Mombeni et al. (2021) surveyed virulence genes on *P. multocida* strains isolated from goats in Iran and showed that the *sodA* gene had the highest presence rate (100.00%), followed by *toxA*, *nanH*, and *ompH*, with the same ratio of 61.90%.

On the other hand, antibiotic resistance is becoming a global concern as more and more multidrug-resistant bacteria appear. The cause is the overuse of antibiotics when treating animal diseases or using antibiotics as growth stimulants in animals (Martin et al., 2015). Kandimalla et al. (2022) revealed that *P. multocida* strains isolated from sheep and goats were susceptible to ceftriaxone, cefoperazone/sulbactam, ceftiofur, cloxacillin, ciprofloxacin, enrofloxacin, levofloxacin and tetracycline (100.00%) but were resistant to erythromycin (41.67%), gentamycin (66.67%). Nguyen et al. (2023) reported that *P. multocida* isolated from sheep in Central Vietnam was resistant to tetracycline (51.22%), ampicillin (53.66%), and erythromycin (65.85%). The frequent impact of antibiotics leads to mutations and the formation of genetic drug-resistance factors in bacteria. Surveying the presence of genes encoding drug-resistance factors in *P. multocida* gives a more general view of antibiotic resistance issues.

Diseases of domestic animals are still a massive issue in the Mekong Delta, Vietnam. There have been a few reports on the prevalence of pathogenic and antibiotic-resistance genes of *P. multocida* isolated from small ruminants; however, there were no reports in goats. This causes challenges in controlling and preventing diseases in goats. Therefore, this study aims to clarify the characteristic prevalence of *P. multocida* serotypes originating from goats and their pathogenicity and antibiotic resistance genes in the Mekong Delta, Vietnam.

## MATERIALS AND METHODS

### Ethical approval

The procedure for collecting fluid swab samples on goats was performed according to NAHMS (National Animal Health Monitoring System) guidelines (USDA, 2022), and *P. multocida* strains were isolated according to Vietnamese National Standard TCVN 8400-14:2011. In the author's previous study, samples were collected according to the guidelines outlined in the Helsinki Declaration and the animal welfare and safety procedures of Can Tho University, Vietnam.

### Identification of *Pasteurella multocida* serotypes

A total of 143 *P. multocida* strains were previously isolated from 289 nasal swabs of healthy hybrid Boer-Saanen meat and dairy goats at all ages in small-scale farms in the Mekong Delta, Vietnam, in 2023. *Pasteurella* spp. were isolated from nasal fluid samples in goats and isolated on blood agar with 5% of sheep blood according to Vietnamese National Standard TCVN 8400-14:2011. Then, *P. multocida* strains were identified using the PCR method to detect gene *Pm1231*. Those identified strains were kept in the Veterinary Food Hygiene Laboratory, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University to conduct this study.

*Pasteurella multocida* strains were subcultured on trypticase soy agar (TSA, Merck, Germany) at 37°C for 24 h to extract DNA. The DNA of *P. multocida* strains was extracted using the heat-shock method and stored at -20°C for the following experiments (Ahmed and Dabbool, 2017).

*Pasteurella multocida* serotypes were determined by performing PCR reactions with primers of genes encoding for each serotype, including *hyaD-hyaC* (serotype A), *bcvD* (serotype B), *dcbF* (serotype D), *ecbJ* (serotype E), and *fcvD* (serotype F). The PCR conditions and primer sequences followed the description of Townsend et al. (2001).

The PCR mixture for one reaction included Mastermix 2X (BIO25042, Boline, Meridian Bioscience, USA, 12.5 µl), forward primer (0.5 µl), reverse primer (0.5 µL), distilled water (9.5 µL), and DNA template (2.0 µL).

### Determination of pathogenic genes

This study determined six pathogenic genes encoded for capsular and lipopolysaccharide antigens, including *sodA*, *toxA*, *tbpA*, *ptfA*, *pflA*, and *ompH*. The PCR conditions and primer sequences followed the description of Doughty et al. (2000) and Ewers et al. (2006). Among genes, the annealing temperature was 55°C for *sodA*, *toxA*, *tbpA*, *ptfA*, and *ompH*, while it was 58°C for *pflA*.

The *P. multocida* strains, previously isolated from cattle in the Mekong Delta, were used as a control. The MyTaq Mix 2X (BIO25042, Boline, Meridian Bioscience, USA) was used in those experiments as described in the above method.

### Determination of antibiotic-resistance genes

The PCR assay was used to detect eight antibiotic-resistance genes representative of beta-lactam (*blaROB-1*, *blaOXA*), aminoglycoside (*aadB*, *strA*), tetracycline (*tetA*, *tetB*), sulfonamide (*sulII*), and macrolide (*mph*). The PCR conditions and primer sequences followed the description of [Randall et al. \(2004\)](#), [Saenz et al. \(2004\)](#), [Carattoli et al. \(2005\)](#), [Momtaz et al. \(2012\)](#), [Klima et al. \(2014\)](#), and [Abo-Elmagd et al. \(2023\)](#). The *P. multocida* strains, previously isolated from cattle in the Mekong Delta, were used as a positive control. The PCR procedure was conducted as described in the above experiment of detection of *P. multocida* serotypes.

### Statistical analysis

The difference in the prevalence of pathogenic and antibiotic-resistance genes in *P. multocida* isolated from goats was statistically analyzed at a significance rate of 95% using the Pearson Chi-square test in the Minitab 17.0 software.

## RESULTS

Of 143 *P. multocida* strains, serotype B was the most predominant serotype (51.05%), followed by serotype A (14.69%), serotype F (6.29%), serotype D (3.50%), and serotype E (0.70%,  $p < 0.05$ ). There were 39.86% of *P. multocida* strains, which could not determine serotypes in this study (Table 1).

Of the six pathogenic genes examined (Table 2), *sodA* was present at the highest rate (56.64%), followed by *toxA* (45.45%), *tbpA* (30.77%), *ptfA* (10.49%), *pfhA* (4.90%), and *ompH* (4.20%,  $p < 0.05$ ). Most pathogenic genes were found in *P. multocida* strains belonging to serotypes A and B.

Of 109 serotyped *P. multocida* strains, gene *tbpA* was frequently detected in serotype A (66.67%), and *sodA* was commonly in serotype B (56.16%); however, only one strain belonging to serotype E harbored gene *sodA* (Table 3).

There were 59/109 (54.13%) serotyped *P. multocida* strains that harbored a combination of two to three pathogenic genes (Table 4). Among gene combinations, the *sodA* + *toxA* + *tbpA* pattern was the most common (12.84%), followed by *sodA* + *toxA* (11.93%).

Of the eight antibiotic-resistance genes examined (Table 5), gene *sulII* was detected at the highest rate (74.13%), followed by *aadB* (42.66%), *strA* (33.57%), *mph* (28.67%), *blaROB-1* (21.68%), *blaOXA* (17.48%), *tetB* (16.08%), and *tetA* (13.29%).

Moreover, *sulII* and *aadB* genes were also recorded at the highest rate in serotyped *P. multocida* strains belonging to all serotypes, followed by *mph* gene (Table 6). Besides, 85/109 (77.98%) serotyped *P. multocida* strains harbored combinations of two to six antibiotic-resistance genes (Table 7). Among gene patterns, the pattern of *aadB* + *sulII* was the most common (10.09%).

**Table 1.** Distribution of *Pasteurella multocida* serotypes isolated from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023 (n=143)

Serotype	Encoded gene	No. of positive strains	Percentage
A	<i>hyaD-hyaC</i>	21	14.69
B	<i>bcbD</i>	73	51.05
D	<i>dcbF</i>	5	3.50
E	<i>ecbJ</i>	1	0.70
F	<i>fcgD</i>	9	6.29
Untyped		57	39.86

Untyped: *P. multocida* strains were not determined in the serotypes (A, B, D, E, and F) using the specific primers in this study. No: Number

**Table 2.** Prevalence of pathogenic genes in *Pasteurella multocida* strains isolated from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023 (n=143)

Pathogenic gene	No. of positive strains	Percentage
<i>sodA</i>	81	56.64
<i>toxA</i>	65	45.45
<i>tbpA</i>	44	30.77
<i>ptfA</i>	15	10.49
<i>pfhA</i>	7	4.90
<i>ompH</i>	6	4.20

No: Number

**Table 3.** Distribution of pathogenic genes in serotyped *Pasteurella multocida* strains isolated from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023

Gene	No. of positive strains (%)	Serotype A (n = 21)	Serotype B (n = 73)	Serotype D (n = 5)	Serotype E (n = 1)	Serotype F (n = 9)	Total (n = 109)
<i>sodA</i>		10 (47.62)	41 (56.16)	2 (40.00)	1 (100.00)	5 (55.56)	59 (54.13)
<i>toxA</i>		9 (42.86)	36 (49.32)	2 (40.00)	0 (0.00)	4 (44.44)	51 (46.79)
<i>tbpA</i>		14 (66.67)	29 (39.73)	2 (40.00)	0 (0.00)	4 (44.44)	49 (44.95)
<i>ptfA</i>		1 (4.76)	9 (12.33)	1 (20.00)	0 (0.00)	1 (11.11)	12 (11.01)
<i>pfhA</i>		0 (0.00)	3 (4.11)	0 (0.00)	0 (0.00)	0 (0.00)	3 (2.75)
<i>ompH</i>		1 (4.76)	4 (5.48)	0 (0.00)	0 (0.00)	0 (0.00)	5 (4.59)

No: Number

**Table 4.** Pathogenic gene patterns of serotyped *Pasteurella multocida* strains from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023 (n=109)

No. of genes	Pattern	No. of strains	Percentage
2	<i>sodA</i> + <i>toxA</i>	13	11.93
	<i>sodA</i> + <i>tbpA</i>	5	4.59
	<i>sodA</i> + <i>ptfA</i>	4	3.67
	<i>sodA</i> + <i>pfhA</i>	1	0.92
	<i>toxA</i> + <i>pfhA</i>	1	0.92
	<i>toxA</i> + <i>tbpA</i>	10	9.17
	<i>toxA</i> + <i>ompH</i>	1	0.92
	<i>ptfA</i> + <i>tbpA</i>	1	0.92
	<i>ompH</i> + <i>tbpA</i>	1	0.92
3	<i>sodA</i> + <i>toxA</i> + <i>tbpA</i>	14	12.84
	<i>sodA</i> + <i>toxA</i> + <i>ptfA</i>	1	0.92
	<i>sodA</i> + <i>ptfA</i> + <i>tbpA</i>	3	2.75
	<i>sodA</i> + <i>toxA</i> + <i>ompH</i>	3	2.75
	<i>toxA</i> + <i>ptfA</i> + <i>tbpA</i>	1	0.92
Total		59	54.13

No: Number

**Table 5.** Prevalence of antibiotic-resistance genes in *Pasteurella multocida* strains isolated from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023 (n=143).

Antibiotic group	Gene	No. of positive strains	Percentage
Beta-lactam	<i>blaROB-1</i>	31	21.68
	<i>blaOXA</i>	25	17.48
Aminoglycoside	<i>aadB</i>	61	42.66
	<i>strA</i>	48	33.57
Tetracycline	<i>tetA</i>	19	13.29
	<i>tetB</i>	23	16.08
Sulfonamide	<i>sulII</i>	106	74.13
Macrolide	<i>mph</i>	41	28.67

No: Number

**Table 6.** Distribution of antibiotic-resistance genes in serotyped *Pasteurella multocida* strains from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023

Gene	No. of positive strains (%)	Serotype A (n = 21)	Serotype B (n = 73)	Serotype D (n = 5)	Serotype E (n = 1)	Serotype F (n = 9)	Total (n = 109)
<i>blaROB-1</i>		5 (23.81)	16 (21.92)	1 (20.00)	0 (0.00)	0 (0.00)	22 (20.18)
<i>blaOXA</i>		2 (9.52)	13 (17.81)	1 (20.00)	1 (100.00)	1 (11.11)	18 (16.51)
<i>aadB</i>		12 (57.14)	30 (41.10)	1 (20.00)	1 (100.00)	6 (66.67)	50 (45.87)
<i>strA</i>		8 (38.10)	27 (36.99)	2 (40.00)	1 (100.00)	1 (11.11)	39 (35.78)
<i>tetA</i>		3 (14.29)	11 (15.07)	0 (0.00)	0 (0.00)	1 (11.11)	15 (13.76)
<i>tetB</i>		6 (28.57)	14 (19.18)	2 (40.00)	0 (0.00)	0 (0.00)	22 (20.18)
<i>sulII</i>		17 (80.95)	61 (83.56)	1 (20.00)	0 (0.00)	7 (77.78)	86 (78.90)
<i>mph</i>		9 (42.86)	26 (35.62)	2 (40.00)	0 (0.00)	5 (55.56)	42 (38.53)

No: Number

**Table 7.** Multiple antibiotic-resistance gene patterns of serotyped *Pasteurella multocida* strains (on the strains harbored from two antibiotic-resistance genes) from hybrid Boer-Saanen goats in the Mekong Delta, Vietnam from March to June 2023 (n=109).

No. of genes	Gene patterns	No. of strains	Percentage
2	<i>blaROB-I + sulII</i>	1	0.92
	<i>blaROB-I + strA</i>	1	0.92
	<i>aadB+ sulII</i>	11	10.09
	<i>aadB+ mph</i>	3	2.75
	<i>strA + sulII</i>	2	1.83
	<i>strA + tetB</i>	1	0.92
	<i>strA + mph</i>	1	0.92
	<i>tetA + sulII</i>	2	1.83
	<i>sulII + mph</i>	1	0.92
3	<i>blaROB-I + tetA + sulII</i>	1	0.92
	<i>blaROB-I + strA + sulII</i>	3	2.75
	<i>blaROB-I + strA + tetB</i>	1	0.92
	<i>blaROB-I + sulII + tetB</i>	1	0.92
	<i>blaOXA + aadB+ sulII</i>	4	3.67
	<i>blaOXA + strA + sulII</i>	1	0.92
	<i>blaOXA + aadB+ mph</i>	3	2.75
	<i>blaOXA + sulII + tetB</i>	2	1.83
	<i>blaOXA + aadB+ strA</i>	1	0.92
	<i>aadB+ tetA + sulII</i>	1	0.92
	<i>aadB+ sulII + mph</i>	8	7.34
	<i>strA + tetB + mph</i>	1	0.92
	<i>strA + sulII + mph</i>	1	0.92
	<i>strA + tetA + sulII</i>	1	0.92
	<i>strA + sulII + tetB</i>	3	2.75
	<i>sulII + tetB + mph</i>	2	1.83
4	<i>blaROB-I + strA + tetB + mph</i>	1	0.92
	<i>blaROB-I + blaOXA + sulII + mph</i>	1	0.92
	<i>blaROB-I + aadB+ strA + sulII</i>	2	1.83
	<i>blaROB-I + strA + tetA + sulII</i>	3	2.75
	<i>blaROB-I + blaOXA + tetA + sulII</i>	1	0.92
	<i>blaOXA + aadB+ sulII + mph</i>	1	0.92
	<i>aadB+ strA + sulII + mph</i>	4	3.67
	<i>aadB+ sulII + tetB + mph</i>	2	1.83
	<i>aadB+ strA + sulII + tetB</i>	1	0.92
	<i>aadB+ tetA + sulII + mph</i>	2	1.83
	<i>strA + tetA + sulII + mph</i>	1	0.92
5	<i>blaROB-I + blaOXA + aadB+ sulII + mph</i>	1	0.92
	<i>blaROB-I + strA + sulII + tetB + mph</i>	3	2.75
	<i>blaROB-I + blaOXA + strA + tetA + sulII</i>	1	0.92
	<i>aadB+ strA + tetA + sulII + tetB</i>	1	0.92
	<i>aadB+ strA + sulII + tetB + mph</i>	2	1.83
6	<i>blaROB-I + aadB+ strA + sulII + tetB + mph</i>	1	0.92
<b>Total</b>		85	77.98

No: Number

## DISCUSSION

In this study, *P. multocida* serotypes A and B were more prevalent than the remaining serotypes. [Shayegh et al. \(2009\)](#) and [Mombeni et al. \(2021\)](#) indicated that *P. multocida* strains isolated from goats in Iran belonged mainly to two serotypes, A and D. Serotypes B and E are two serogroups reported to commonly cause hemorrhagic infections in ruminant carriers. Serogroup B was often found in the nasopharyngeal fluid of livestock in Southeast Asia, while serogroup E was more common in Africa ([Markey et al., 2013](#)). [Aski and Tabatabaei \(2016\)](#) recorded the prevalence of three serotypes, A, B, and D, in *P. multocida* strains isolated from healthy and clinically infected goats. *P. multocida* serotype B, specifically serotype B:2, was a common serotype detected in cases of infected cattle. However, 39.86% of *P. multocida* strains were not determined serotypes in this study. The reason could be due to the specific primers or the characteristic structure of capsular antigens in those *P. multocida* strains. Further study should be done to clarify and confirm the prevalence of diverse serotypes of *P. multocida* strains isolated from goats using other primers or serotyping methods.

Furthermore, three genes, *sodA*, *toxA*, and *tbpA*, were more commonly detected in *P. multocida* strains. The *sodA* gene was frequently detected in *P. multocida* isolated from poultry, pigs, and rabbits in previous studies ([Furian et al., 2015](#); [Li et al., 2018](#); [Mahrous et al., 2022](#)). Therefore, it is difficult to determine whether *sodA* is a characteristic gene in *P. multocida* strains isolated from goats or was related to serotypes A and B. According to [Rimac et al. \(2017\)](#), the *tbpA* gene is closely related to *P. multocida* strains isolated from ruminants with pneumonia and sepsis. [Katsuda et al. \(2013\)](#) also indicated a close relationship between serotype A strains and the *tbpA* gene. Research by [Nguyen et al. \(2023\)](#) showed that the *tbpA* gene was detected in serotypes A, B, and D strains in clinical pneumonic pasteurellosis sheep in central Vietnam at 48.78%, 7.32%, and 21.95%, respectively. The above results suggested that there might be a relationship between serotypes A and B and the *tbpA* gene, especially in diseased animals. On the other hand, [Pullinger et al. \(2003\)](#) reported that gene *toxA* can be transferred horizontally, and the *toxA* gene was determined to be concerning serotypes A and D strains ([Furian et al., 2015](#)). [Cid et al. \(2019\)](#) reported that the *toxA* gene encodes the *P. multocida* toxin (PMT), which is a dermonecrotic protein in the virulence factor of capsular type D.

*Pasturella multocida* causes progressive atrophic rhinitis in pigs and is significantly found in ovine pneumonia isolates in Spain. Besides, the detection of the *toxA* gene could serve as a reliable indicator of the toxigenic fitness of *P. multocida* ([Cid et al., 2019](#)). Thus, the high presence of the *toxA* gene in *P. multocida* strains isolated from goats showed that those *P. multocida* strains harboring PMT toxin could cause pasteurellosis in goats in the Mekong Delta, Vietnam.

Of serotyped strains, three genotypes with high prevalence rates included *sodA* + *toxA* + *tbpA*, *sodA* + *toxA*, and *toxA* + *tbpA*, with rates of 12.84%, 11.93%, and 9.17% respectively. The *toxA* was present in most of the common patterns. [Pullinger et al. \(2003\)](#) showed that the *toxA* gene was encoded in the genome of a latent bacteriophage to allow the *toxA* gene to circulate easily among *P. multocida* strains of many different serotypes. [Bernal et al. \(2023\)](#) recorded that all *P. multocida* strains isolated from cows with respiratory disease carried more than two virulence genes. The appearance of serotypes carrying diverse virulent genes makes it difficult to prevent and control diseases in goats in the Mekong Delta.

Among antibiotic-resistance genes, gene *sulIII* had the highest presence rate (74.13%). Gene *sulIII* was not generally considered part of a separate genetic element; it was found on large conjugative plasmids and was associated with resistance to other antibiotics ([Bean et al., 2009](#); [Wu et al., 2010](#)). Antibiotic-resistant genes could be silent resistance genes that are not frequently exhibited or exhibited at low levels, even when exposed to antibiotics. They are nonessential residues and do not play an essential role in the bacterial life cycle ([Stasiak et al., 2021](#)). However, antibiotic-resistance genes *blaROB-1*, *blaOXA*, *tetA*, and *tetB* had a relatively low presence rate in *P. multocida* strains in this study. In another report, [Babetsa et al. \(2012\)](#) indicated that *P. multocida* strains isolated from bovine, ovine, caprine, and swine pneumonic lungs were resistant to tetracycline in Greece. Among those tetracycline-resistance *P. multocida* strains, 72.22% of strains carried the *tetH* gene, and 22.22% of strains carried the *tetB* gene, while *tetA* and *tetM* were not found. The current study results showed a difference in the prevalence of antibiotic-resistance genes in *P. multocida* isolated from goats in the Mekong Delta. Thus, further research should be conducted to clarify the diverse prevalences of antibiotic-resistance genes in *P. multocida* isolated from goats in this region.

Of serotyped *P. multocida* strains, the *sulIII* had the highest presence rate in serotypes A, B, and F. The gene *blaROB-1* and *tetB* genes were not found in serotype F strains. Most antibiotic-resistance genes are present in the bacterial genome through random gene transfer using mobile genetic elements ([Bennett, 2008](#)). Besides, genes *tetA* and *tetB* are two representatives of genetic factors for tetracycline resistance through the formation of ABC (ATP-binding cassette superfamily) transporters ([Reynolds et al., 2016](#)). [Rendueles et al. \(2018\)](#) revealed that capsular antigens were related to antibiotic resistance genes, especially genes related to antibiotic efflux pumps. Previous studies mainly focused on the relationship between serotypes and virulent genes; however, a few studies have shown the relationship between



serotypes and antibiotic-resistance genes in *P. multocida* (Harper et al., 2006; Katsuda et al., 2013; Cid et al., 2019; Nguyen et al., 2023). Thus, the current study results seemed to be the first report on the prevalence of antibiotic-resistance genes in *P. multocida* serotypes isolated from healthy goats in the Mekong Delta, Vietnam.

Moreover, 77.98% *P. multocida* strains isolated from goats in the Mekong Delta showed several antibiotic-resistance gene patterns. Among 42 antibiotic-resistance gene combinations of *P. multocida* strains, the most common patterns across serotypes were *aadB* + *sulII* (10.09%) and *aadB* + *sulII* + *mph* (7.34%) in this study. The *aadB* gene is usually associated with type 1 integron or plasmids, and the *sulII* gene is mainly located on small non-conjugative plasmids or large multiresistant plasmids that can be horizontally transferred (Antunes et al., 2005; Naderi et al., 2023). The common prevalence of *aadB* and *sulII* genes in several antibiotic-resistance gene patterns in serotypes showed that *aadB* and *sulII* might be a typical resistance gene cluster in *P. multocida* isolated from goats in the Mekong Delta, Vietnam. In addition, the diversity of antibiotic-resistance gene patterns in those *P. multocida* strains exhibited the high ability of multidrug resistance of those strains. It could cause difficulty in treating and preventing the respiratory diseases caused by *P. multocida* in goats in this region.

## CONCLUSION

This study showed that five pathogenic *P. multocida* serotypes were detected in healthy hybrid Boer-Saanen goats in the Mekong Delta, Vietnam. The results indicated that *P. multocida* serotypes A and B were the dominant serotypes in goats in the Mekong Delta. Moreover, those *P. multocida* strains harbored diverse pathogenic genes and antibiotic-resistance genes with several gene patterns. The pathogenic gene (*sodA*) and the antibiotic-resistance gene (*aadB*) were the most detected in all *P. multocida* serotypes in this study. In addition, the pathogenic gene pattern of *sodA* + *toxA* + *tbpA* and the antibiotic-resistance gene pattern of *aadB* + *sulII* were frequently prevalent in those *P. multocida* strains. It revealed that *P. multocida* strains isolated from goats were potential pathogens causing severe diseases in goats in the Mekong Delta. Therefore, the control of pathogenic and antibiotic-resistant *P. multocida* is essential to prevent and treat pasteurellosis in goats.

## DECLARATION

### Acknowledgments

We thank the farmers and co-authors for completing this study.

### Conflicts of Interests

The authors declare that we do not have any conflicts of interest.

### Funding

This study is funded in part by the Can Tho University, Code: T2023-149.

### Authors' contributions

Thuan K. Nguyen, Thuong T. Nguyen, and Trung T. Truong conceptualized, designed, and supervised the research. Thuan K. Nguyen and Thuong T. Nguyen critically reviewed the study. Thuan K. Nguyen, Thuong T. Nguyen, Chi T.H. Nguyen, Vy L.P. Nguyen, and Trung T. Truong collected samples and conducted experiments. Chi T.H. Nguyen and Vy L.P. Nguyen analyzed and interpreted the data generated. All authors revised and approved the submitted manuscript.

### Competing interests

The authors declare that they have no conflict of interest.

### Availability of data and materials

The authors of this article confirm that all data supporting the findings of this research are available upon reasonable request.

### Ethical considerations

The authors considered farmers' ethical concerns and consent before conducting the study. This article was written originally without any copy from data of published articles and books.

## REFERENCES

- Abo-Elmagd EE, Sabala RF, Abd-Elghany SM, Jackson CR, Ramadan H, Imre K, Morar A, Herman V, and Salla KI (2023).  $\beta$ -Lactamase producing *Escherichia coli* encoding blaCTX-M and blaCMY genes in chicken carcasses from Egypt. *Foods*, 12(3): 598. DOI: <https://www.doi.org/10.3390/foods12030598>
- Ahmed OB and Dablood SA (2017). Quality improvement of the DNA extracted by boiling method in gram negative bacteria. *International Journal of Bioassays*, 6(4): 5347-5349. DOI: <https://www.doi.org/10.21746/IJBIO.2017.04.004>
- Antunes P, Machado J, Sousa JC, and Peixe L (2005). Dissemination of sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) in Portuguese *Salmonella enterica* strains and relation with integrons. *Antimicrobial Agents and Chemotherapy*, 49(2): 836-839. DOI: <https://www.doi.org/10.1128/aac.49.2.836-839.2005>
- Aski HS and Tabatabaei M (2016). Occurrence of virulence-associated genes in *Pasteurella multocida* isolates obtained from different hosts. *Microbial Pathogenesis*, 96: 52-57. DOI: <https://www.doi.org/10.1016/j.micpath.2016.04.008>
- Babetsa M, Sandalakis V, Vougidou C, Zdragas A, Sivropoulou A, Psaroulaki A, and Ekateriniadou LV (2012). Tetracycline resistance genes in *Pasteurella multocida* isolates from bovine, ovine, caprine and swine pneumonic lungs originated from different Greek prefectures. *African Journal of Microbiology Research*, 6(17): 3917-3923. DOI: <https://www.doi.org/10.5897/AJMR12.208>
- Bean DC, Livermore DM, and Hall LMC (2009). Plasmids imparting sulfonamide resistance in *Escherichia coli*: Implications for persistence. *Antimicrobial Agents and Chemotherapy*, 53(3): 1088-1093. DOI: <https://www.doi.org/10.1128/aac.00800-08>
- Bennett PM (2008). Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology*, 153(S1): 347-357. DOI: <https://www.doi.org/10.1038/sj.bjp.0707607>
- Bernal JMC, Fernandez A, Arnal JL, Tejero CS, Fernandez-Garayzabal JF, Vela AI, and Cid D (2023). Molecular epidemiology of *Pasteurella multocida* associated with bovine respiratory disease outbreaks. *Animals*, 13(1): 75. DOI: <https://www.doi.org/10.3390/ani13010075>
- Boyce JD, Chung JY, and Adler B (2000). *Pasteurella multocida* capsule: Composition, function and genetics. *Journal of Biotechnology*, 83(1-2): 153-160. DOI: [https://www.doi.org/10.1016/s0168-1656\(00\)00309-6](https://www.doi.org/10.1016/s0168-1656(00)00309-6)
- Carattoli A, Bertini A, Villa L, Fallo V, Hopkins KL, and Threlfall EJ (2005). Identification of plasmids by PCR-based replicon typing. *Journal of Microbiological Methods*, 63(3): 219-228. DOI: <https://www.doi.org/10.1016/j.mimet.2005.03.018>
- Cid D, García-Alvarez A, Domínguez L, Fernández-Garayzábal JF, and Vela AI (2019). *Pasteurella multocida* isolates associated with ovine pneumonia are toxigenic. *Veterinary Microbiology*, 232: 70-73. DOI: <https://www.doi.org/10.1016/j.vetmic.2019.04.006>
- Doughty SW, Ruffolo CG, and Adler B (2000). The type 4 fimbrial subunit gene of *Pasteurella multocida*. *Veterinary Microbiology*, 72(1-2): 79-90. DOI: [https://www.doi.org/10.1016/s0378-1135\(99\)00189-3](https://www.doi.org/10.1016/s0378-1135(99)00189-3)
- Ewers C, Lubke-Becker A, Bethe A, Kiebling S, Filter M, and Wieler LH (2006). Virulence genotype of *Pasteurella multocida* strains isolated from different hosts with various disease status. *Veterinary Microbiology*, 114(3-4): 304-317. DOI: <https://www.doi.org/10.1016/j.vetmic.2005.12.012>
- Falade S (2002). Further *Pasteurella* isolates from the Republic of Zambia. *Tropical Veterinarian*, 20(3): 130-131. DOI: <https://www.doi.org/10.4314/tv.v20i3.4491>
- Furian TQ, Borges KA, Pilatti RM, Laviniki V, Rocha SLS, Almeida CN, Nascimento VP, Salle CTP, and Moraes HLS (2015). Virulence genes and antimicrobial resistance of *Pasteurella multocida* isolated from poultry and swine. *Veterinary Microbiology*, 47(1): 210-216. DOI: <https://www.doi.org/10.1016/2fj.bjm.2015.11.014>
- Girma S, Getachew L, Beyene A, Tegegne DT, Tesgera T, Debelo M, Debano J, Teshome D, Abdisa K, Wirtu A et al. (2023). Identification of serotypes of *Mannheimia haemolytica* and *Pasteurella multocida* from pneumonic cases of sheep and goats and their antimicrobial sensitivity profiles in Borana and Arsi zones, Ethiopia. *Scientific Reports*, 13(1): 9008. DOI: <https://www.doi.org/10.1038/s41598-023-36026-2>
- Harper M, Boyce JD, and Adler B (2006). *Pasteurella multocida* pathogenesis: 125 years after Pasteur. *FEMS Microbiology Letters*, 265(1): 1-10. DOI: <https://www.doi.org/10.1111/j.1574-6968.2006.00442.x>
- Kandimalla K, Awati B, Ram VKS, Putty K, Lunavat G, Choudapur MK, Karate A, Patil NA, and Bhoyar RG (2022). Antibiotic sensitivity testing of *Pasteurella multocida* strains isolated from small ruminants. *The Pharma Innovation Journal*, 11(8): 1927-1929. Available at: <https://www.thepharmajournal.com/archives/2022/vol11issue8/PartX/11-8-239-267.pdf>
- Katsuda K, Hoshinoo K, Ueno Y, Kohmoto M, and Mikami O (2013). Virulence genes and antimicrobial susceptibility in *Pasteurella multocida* isolates from calves. *Veterinary Microbiology*, 167(3-4): 737-741. DOI: <https://www.doi.org/10.1016/j.vetmic.2013.09.029>
- Klima CL, Zaheer R, Cook SR, Booker CW, Hendrick S, Alexander TW, and McAllister TA (2014). Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. *Journal of Clinical Microbiology*, 52(2): 438-448. DOI: <https://www.doi.org/10.1128/jcm.02485-13>
- Li Z, Cheng F, Lan S, Guo J, Liu W, Li X, Luo Z, Zhang M, Wu M, and Shi Y (2018). Investigation of genetic diversity and epidemiological characteristics of *Pasteurella multocida* isolates from poultry in Southwest China by population structure, multi-locus sequence typing and virulence-associated gene profile analysis. *Journal of Veterinary Science*, 80(6): 921-929. DOI: <https://www.doi.org/10.1292/jvms.18-0049>
- Mahrous EH, Al-Azeem MWA, Wasel FA, and Younis W (2022). Molecular detection and characterization of *Pasteurella multocida* isolated from rabbits. *Journal of Animal Health and Production*, 10(1): 1-9. DOI: <http://www.doi.org/10.17582/journal.jahp/2022/10.1.1.9>
- Markey B, Leonard F, Archambault M, Cullinane A, and Maguire D (2013). *Clinical veterinary microbiology*, 2nd Edition. Elsevier Ltd., pp. 307-316. Available at: <https://vetbooks.ir/clinical-veterinary-microbiology-2nd-edition/>
- Martin MJ, Thottathil SE, and Newman TB (2015). Antibiotics overuse in animal agriculture: A call to action for health care providers. *American Journal of Public Health*, 105(12): 2409-2410. DOI: <https://www.doi.org/10.2105/AJPH.2015.302870>
- May BJ, Zhang Q, Li LL, Paustian ML, Whittam TS, and Kapur V (2001). Complete genomic sequence of *Pasteurella multocida*, Pm70. *Proceedings of the National Academy of Sciences of the United States of America*, 98(6): 3460-3465. DOI: <https://www.doi.org/10.1073/pnas.051634598>
- Mohamed RA and Abdelsalam EB (2008). A review on pneumonic pasteurellosis (respiratory manheimiosis) with emphasis on pathogenesis, virulence mechanism and predisposing factors. *Bulgarian Journal of Veterinary Medicine*, 11(3): 139-160. Available at: <http://www.uni-sz.bg/bjvm/vol11-no3-01.pdf>
- Mombeni EG, Gharibi D, Ghorbanpoor M, Jabbari AR, and Cid D (2021). Toxigenic and non-toxigenic *Pasteurella multocida* genotypes, based on capsular, LPS, and virulence profile typing, associated with pneumonic pasteurellosis in Iran. *Veterinary Microbiology*, 257: 109077. DOI: <https://www.doi.org/10.1016/j.vetmic.2021.109077>



- Momtaf H, Rahimi E, and Moshkelani S (2012). Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinarni Medicina*, 57(4): 193-197. DOI: <https://www.doi.org/10.17221/5916-VETMED>
- Naderi G, Talebi M, Gheybizada R, Seifi A, Ghourchian S, Rahbar M, Abdollahi A, Naseri A, Eslami P, and Douraghi M (2023). Mobile genetic elements carrying aminoglycoside resistance genes in *Acinetobacter baumannii* isolates belonging to global clone 2. *Frontiers in Microbiology*, 14: 1172861. DOI: <https://www.doi.org/10.3389/fmicb.2023.1172861>
- Nguyen PV, Le CT, Nguyen XH, Nguyen TM, and Nguyen KCT (2023). First study on capsular serotypes and virulence factors of *Pasteurella multocida* isolates from Phan Rang sheep in Vietnam. *Veterinary World*, 16(2): 281-290. DOI: <https://www.doi.org/10.14202/vetworld.2023.281-290>
- Nguyen VLP, Truong NTK, Tran LP, Truong TT, and Nguyen TK (2024). Prevalence and antimicrobial susceptibility of *Pasteurella multocida* isolated from goats in Can Tho city. *The Journal of Agriculture and Development*, 23(1): 14-24. DOI: <https://www.doi.org/10.52997/jad.1.02.2024>
- Peng Z, Wang H, Liang W, Chen Y, Tang X, Chen H, and Wu B (2018). A capsule/lipopolysaccharide/MLST genotype D/L6/ST11 of *Pasteurella multocida* is likely to be strongly associated with swine respiratory disease in China. *Archives of Microbiology*, 200(1): 107-118. DOI: <https://www.doi.org/10.1007/s00203-017-1421-y>
- Peng, Z, Liang W, and Wu B (2016). Molecular typing methods for *Pasteurella multocida*- A review. *Acta Microbiologica Sinica*, 56(10): 1521-1529. Available at: <https://europepmc.org/article/med/29741340>
- Pullinger GD, Bevir T, and Lax AJ (2003). The *Pasteurella multocida* toxin is encoded within a lysogenic bacteriophage. *Molecular Microbiology*, 51(1): 255-269. DOI: <https://www.doi.org/10.1046/j.1365-2958.2003.03829.x>
- Randall LP, Cooles SW, Osborn MK, Piddock LJV, and Woodward MJ (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy*, 53(2): 208-216. DOI: <https://www.doi.org/10.1093/jac/dkh070>
- Rawat N, Gilhare VR, Kushwaha KK, Hattimare DD, Khan FF, Shende RK, and Jolhe DK (2009). Isolation and molecular characterization of *Mannheimia haemolytica* and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. *Veterinary World*, 12(2): 331-336. DOI: <https://www.doi.org/10.14202/vetworld.2019.331-336>
- Rendueles O, Sousa JAM, Bernheim A, Touchon M, and Rocha EPC (2018). Genetic exchanges are more frequent in bacteria encoding capsules. *PLoS Genetics*, 14(12): e1007862. DOI: <https://www.doi.org/10.1371/journal.pgen.1007862>
- Reynolds LJ, Roberts AP, and Anjum MF (2016). Efflux in the oral metagenome: The discovery of a novel tetracycline and tigecycline ABC transporter. *Frontiers in Microbiology*, 7: 1923. DOI: <https://www.doi.org/10.3389/fmicb.2016.01923>
- Rimac R, Luna L, Hurtado R, Rosadio R, and Maturrano L (2017). Detection and genetic characterization of *Pasteurella multocida* from alpaca (*Vicugna pacos*) pneumonia cases. *Tropical Animal Health and Production*, 49: 1325-1328. DOI: <https://www.doi.org/10.1007/s11250-017-1309-5>
- Saenz Y, Brinas L, Dominguez E, Ruiz J, Zarazaga M, Vila J, and Torres C (2004). Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrobial Agents and Chemotherapy*, 48(10): 3996-4001. DOI: <https://www.doi.org/10.1128/aac.48.10.3996-4001.2004>
- Shayegh J, Sharaf JD, Mikaili P, and Namvar H (2009). Pheno- and genotyping of *Pasteurella multocida* isolated from goat in Iran. *African Journal of Biotechnology*, 8(16): 3707-3710. Available at: <https://www.ajol.info/index.php/ajb/article/view/61921>
- Smith MC and Sherman DM (2009). *Goat medicine*, 3rd Edition. John Wiley & Sons Press., USA, pp. 389-433. Available at: <https://vetbooks.ir/goat-medicine-3rd-edition/>
- Stasiak M, Mackiw E, Kowalska J, Kucharek K, and Postupolski J (2021). Silent genes: Antimicrobial resistance and antibiotic production. *Polish Journal of Microbiology*, 70(4): 421-429. DOI: <https://www.doi.org/10.33073/2Fpjm-2021-040>
- Tabatabaei M and Abdolahi F (2023). Molecular evaluation of sheep and goats isolates of *Pasteurella multocida* and their antibiotic resistance. *Veterinary Research Forum*, 14(9): 481-487. DOI: <https://www.doi.org/10.30466/vrf.2022.556438.3524>
- Townsend KM, Boyce JD, Chung JY, Frost AJ, and Adler B (2001). Genetic organization of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology*, 39(3): 924-929. DOI: <https://www.doi.org/10.1128/jcm.39.3.924-929.2001>
- United States department of agriculture (USDA) (2022). NAHMS goat 2019 blood & swab sample collection records. Animal and plant health inspection service. Fort Collins., Colorado, pp. 1-6. Available at: <https://www.aphis.usda.gov/sites/default/files/blood-and-swab-cer.pdf>
- Wu S, Dalsgaard A, Hammerum AM, Porsbo LJ, and Jensen LB (2010). Prevalence and characterization of plasmids carrying sulfonamide resistance genes among *Escherichia coli* from pigs, pig carcasses and human. *Acta Veterinaria Scandinavica*, 52(1): 47. DOI: <https://www.doi.org/10.1186/1751-0147-52-47>

**Publisher's note:** Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access:** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.