2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(4): 882-890.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj89
PII: S232245682500089-15

Characterization of *Pasteurella multocida* Isolated from Layer Chickens Infected with Fowl Cholera in Sukabumi, Indonesia: Antibiotic Resistance and Virulence Gene Detection

Marsella Metta Arvia[1], Ryan Septa Kurnia[2], Christian Marco Hadi Nugroho[2], Muhammad Ade Putra[2], Safika[3], and Agustin Indrawati[3]*

[1]*School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia*
[2]*Indonesia Research and Diagnostic Unit, PT. Medika Satwa Laboratoris, Bogor, 16166, Indonesia*
[3]*Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia*

*Corresponding author's Email: indraseta@apps.ipb.ac.id

ORIGINAL ARTICLE
Received: September 30, 2025
Revised: October 27, 2025
Accepted: November 28, 2025
Published: December 25, 2025

ABSTRACT

Fowl cholera is a contagious bacterial disease in poultry caused by *Pasteurella multocida* (*P. multocida*), which presents a significant threat to layer chicken farming due to its economic impact and potential antibiotic resistance. This study aimed to characterize *P. multocida* isolates from a fowl cholera outbreak in Sukabumi by assessing antibiotic resistance profiles and detecting virulence-associated genes. Eight *P. multocida* bacterial isolates from organ sample were cultured on blood agar and subjected to antibiotic susceptibility testing using the Sensititre™ GN4F system. Genomic DNA was extracted and analyzed for 16 virulence genes through conventional polymerase chain reaction (PCR). Results showed that all isolates were generally susceptible to the tested antibiotics, except for isolate P.m1, which was confirmed resistant to levofloxacin and ciprofloxacin. Virulence gene detection revealed that most isolates carried nearly all virulence genes tested, particularly those encoding capsule (*capA*), iron acquisition proteins (*exbB*, *hgbB*, *fur*), fimbriae and adhesins (*fim4*, *fimA*, *pfhA*, *tadD*), outer membrane proteins (*oma87*, *plpB*), sialidases (*nanB*, *nanH*), and superoxide dismutases (*sodA*, *sodC*). Notably, no isolates harbored the *toxA* gene. The presence of extensive virulence factors despite general antibiotic susceptibility underscored the pathogen's potential to persist and cause disease, and highlighted the need for targeted surveillance and comprehensive control strategies in Indonesia.

Keywords: Antibiotic resistance, Characterization, Fowl cholera, *Pasteurella multocida*, Virulence gene

INTRODUCTION

The layer poultry farming sector has gained increased public attention due to the rising market demand for livestock products annually, driven by population growth and improved nutritional awareness (Maulana et al., 2017). However, the disease still represents a serious challenge in layer farms, causing economic impacts associated with lower egg yields and higher layer mortality (Faisal, 2019). One of the common diseases affecting layer farms is fowl cholera.

Fowl cholera is a contagious bacterial infection in avian species caused by *Pasteurella multocida* (*P. multocida*, Zainuddin, 2014). This gram-negative bacterium can colonize the mucosal surfaces of the upper respiratory tract, as well as the air sacs and lungs of chicken (Reuben et al., 2021). Transmission may occur through contact with infected layers, asymptomatic carriers, or contaminated equipment and environments. The clinical signs of fowl cholera include weakness, lethargy, anorexia, edema in the facial area, comb, and wattles (Zainuddin, 2014).

Treatment of *P. multocida* infections generally involves the use of antibiotics. Nonetheless, administration of antibiotics must be precise to prevent the emergence of antibiotic resistance (Furian et al., 2016). Antibiotic resistance poses a threat to animal health and well-being, increases production costs, and potentially facilitates the spread of resistance genes to other bacteria (Lobongona et al., 2019). Although antimicrobial resistance has been widely investigated in numerous poultry pathogens, information on the antibiotic resistance patterns of *P. multocida* in Asia remains limited (Sabsabi et al., 2021).

The pathogenesis of *P. multocida* infection arises from complex interactions between bacterial virulence genes and the host (Aski et al., 2016). Alongside comprehending pathogenesis, the identification of virulence genes advances the present understanding of the bacterium's epidemiology, immune evasion, and vaccine development. The identified virulence genes of *P. multocida* include capsule-based serotypes, iron metabolism proteins, toxins, fimbriae, adhesion factors, sialidase, and outer membrane proteins (Nguyen et al., 2023). Each virulence gene contributes a distinct role in enhancing the bacterium's ability to infect the host.

Understanding the antibiotic resistance profile of *P. multocida* is essential to ensure targeted and effective treatment while avoiding further resistance development. Likewise, identifying its virulence genes is important to determine the factors contributing to bacterial pathogenicity. These two parameters serve as a reference for developing appropriate control strategies for fowl cholera and preventing future outbreaks. The present study aimed to analyze the antibiotic

To cite this paper: Arvia MM, Kurnia RS, Nugroho CMH, Putra MA, Safika, and Indrawati A (2025). Characterization of *Pasteurella multocida* Isolated from Layer Chickens Infected with Fowl Cholera in Sukabumi, Indonesia: Antibiotic Resistance and Virulence Gene Detection. *World Vet. J.*, 15(4): 882-890. DOI: https://dx.doi.org/10.54203/scil.2025.wvj89

resistance and identify the virulence genes of *P. multocida* isolated from dead chickens in poultry during a fowl cholera outbreak in Sukabumi Regency, a major poultry production center in West Java Province, Indonesia.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was obtained from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Science, IPB University, Indonesia, under the approval number 121/SKE/X/2023.

Culture of *Pasteurella multocida* isolates

The *P. multocida* isolates used in this study were previously preserved as freeze-dried cultures and were revived prior to use. All eight isolates (P.m1–P.m8) originated from organ samples of dead chickens collected from poultry farms experiencing fowl cholera cases in Sukabumi between 2018 and 2022 (Table 1). The freeze-dried isolates were revived using 150 µL of Brain Heart Infusion (BHI) broth, streaked onto blood agar, and incubated overnight at 37°C. The isolates were tested biochemically for catalase, oxidase, and further verified by polymerase chain reaction (PCR; Mehmood et al., 2018).

Antibiotic resistance testing

Sensitivity to antibiotics assessed against 11 classes of antibiotics, including aminoglycosides, penicillins, β-lactamase inhibitor–penicillin combinations, carbapenems, fluoroquinolones, tetracyclines, cephalosporins, tigecycline, sulfonamides, monobactams, and nitrofurans. Antibiotic sensitivity was measured using the Minimum Inhibitory Concentration (MIC) method with the Sensititre™ GN4F system (Thermo Fisher, USA). Approximately 3–5 bacterial colonies were suspended in distilled water, and the bacterial concentration was adjusted to 0.5 McFarland standard, corresponding to an optical density (OD) of 0.08-0.1 at 600 nm, as measured using Genesys 10S UV-Vis spectrophotometer (Thermo Fisher, USA). Subsequently, 1 µL of the bacterial suspension was mixed with Mueller-Hinton Broth (MHB). A volume of 50 µL of the final suspension was dispensed into each well of the plate using a multichannel pipette. The plate was sealed and incubated at 34–36°C for 18–24 hours. The MIC results were manually interpreted by observing the presence or absence of bacterial growth, indicated by sediment at the bottom of each well. Genomic DNA from all *P. multocida* isolates was extracted using a modified boiling method as described by Sunartatie et al. (2024). Virulence gene detection was carried out through PCR. Each PCR reaction consisted of 5 µL of bacterial genomic DNA, 25 µL of MyTaq™ HS Red Mix master mix, 2 µL each of forward and reverse primers (Table 2) at a concentration of 10 µM, and 16 µL of nuclease-free water. The amplification was performed by SimpliAmp™ Thermal Cycler (Applied Biosystems, USA) under the following cycling conditions, including initial denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at 72°C for 10 seconds, repeated for 30 cycles. The PCR amplicons were analyzed by electrophoresis on a 1.5% agarose gel stained with 0.5 µg/mL ethidium bromide (EtBr). A 100 bp DNA ladder (VC 100 bp Plus DNA Ladder, Vivantis) was used as a molecular weight marker. The gel was electrophoresed at 120 V for 35 minutes (Indrawati et al., 2025).

Data analysis

Data obtained from the study were presented in tables and figures and analyzed descriptively. Antimicrobial susceptibility profiles were interpreted based on CLSI and EUCAST breakpoint criteria. The detection of virulence genes was reported as presence or absence for each isolate and displayed in a comparative table.

Table 1. *Pasteurella multocida* sample between 2018 and 2022 isolated from poultry farms in Sukabumi, West Java, Indonesia

No.	Isolate code	Date of isolation	Organ	Origin of the sample
1	P.m1	21/12/2018	Trachea	Cicurug, Sukabumi
2	P.m2	24/09/2019	Cardiac blood	Cicurug, Sukabumi
3	P.m3	25/09/2019	Liver	Cicurug, Sukabumi
4	P.m4	20/03/2020	Liver	Cicurug, Sukabumi
5	P.m5	23/12/2021	Tracheal	Cicurug, Sukabumi
6	P.m6	02/01/2022	Liver	Cicurug, Sukabumi
7	P.m7	01/08/2022	Lung	Cicurug, Sukabumi
8	P.m8	01/09/2022	Liver	Cicurug, Sukabumi

Table 2. Nucleotide sequence of *P. multocida* virulence gene primers

Genes	Targeted gene	Nucleotide Sequence (5'–3')	Amplicon size (bp)	Reference
CapA	*Serotyping A*	TGCCAAAATCGCAGTCAG TTGCCATCATTGTCAGTG	1044	Townsend et al. (2001)
ToxA	*Dermonecrotic toxin*	CTTAGATGAGCGACAAGG GAATGCCACACCTCTATAG	864	Tang et al. (2009)
TbpA	*Transferrin binding protein*	TGGTTGGAAACGGTAAAGC TAACGTGTACGGAAAAGCC	728	Townsend et al. (2001)
Fim4	*Type 4 fimbriae*	TGTGGAATTCAGCATTTTAGTGTGTC TCATGAATTCTTATGCGCAAAATCCTGCTGG	488	Tang et al. (2009)
SodC	*Superoxide dismutate*	AGTTAGTAGCGGGGTTGGCA TGGTGCTGGGTGATCATCATG	235	Ewers et al. (2006)
PfhA	*Filamentous hemagglutinin*	AGCTGATCAAGTGGTGAAC TGGTACATTGGTGAATGCTG	286	Atashpaz et al. (2009)
ExbB	*Iron acquisition*	TTGGCTTGTGATTGAACGC TGCAGGAATGGCGACTAAA	283	Tang et al. (2009)
HgbB	*Hemoglobin binding protein*	ACCGCGTTGGAATTATGATTG CATTGAGTACGGCTTGACAT	788	Ewers et al. (2006)
NanB	*Neuroaminidase*	GTCCTATAAAGTGACGCCGA ACAGCAAAGGAAGACTGTCC	554	Ewers et al. (2006)
TadD	*Putative non-spesific tight adherence protein D*	TCTACCCATTCTCAGCAAGGC ATCATTTCGGGCATTCACC	416	Tang et al. (2009)
PlpB	*Plasminogen-binding protein B*	TTTGGTGGTGCGTATGTCTTCT AGTCACTTTAGATTGTGCGTAG	282	Tang et al. (2009)
SodA	*Superoxide dismutate*	TACCAGAATTAGGCTACGC GAAACGGGTTGCTGCCGCT	361	Ewers et al. (2006)
Oma87	*Outer membrane protein 87*	ATGAAAAAACTTTTAATTGCGAGC TGACTTGCGCAGTTGCATAAC	948	Ewers et al. (2006)
FimA	*Fimbriae*	CCATCGGATCTAAACGACCTA AGTATTAGTTCCTGCGGGTG	866	Kim et al. (2019)
NanH	*Neuroaminidase*	GAATATTTGGGCGGCAACA TTCTCGCCCTGTCATCACT	360	Kim et al. (2019)
Fur	*Ferric uptake regulation protein*	GTTTACCGTGTATTAGACCA CATTACTACATTTGCCATAC	244	Kim et al. (2019)

RESULTS AND DISCUSSION

Antibiotic resistance

Antibiotics have been utilized in animal production for over five decades as therapeutic agents. Their ability to resist bacterial infections has contributed to increased average animal life expectancy, appropriate management of infectious diseases, reduced morbidity and mortality rates, and enhanced food safety. Their broad application in livestock health has also supported growth performance and productivity (Venkateswaran et al., 2023; Elbehiry and Marzouk, 2024). However, the misuse and overuse of antibiotics in these systems have accelerated the emergence of antimicrobial resistance, posing significant threats to both animal and public health (Alhassan et al., 2025). Antibiotic resistance denotes the ability of microorganisms to endure the inhibitory or bactericidal activity of antibiotic compounds (Abreu et al., 2023). Variations in Minimum Inhibitory Concentration (MIC) were observed among isolates for several antibiotics. The MIC is defined as the lowest concentration of an antibiotic compound, measured in µg/mL, that entirely inhibits the visible growth of a test microorganism *in vitro*. Analogous to the inhibition zone in qualitative methods, MIC values are used to determine the susceptibility or resistance category of a pathogen to a specific antibiotic. Elevated values correlate with an increased risk of treatment failure, regardless of the isolate's classification as susceptible to the tested antibiotic (Kowalska-Krochmal and Dudek-Wicher, 2021).

The antibiotic resistance profile of *P. multocida* isolates is presented in Table 3. Based on the analysis of sensitivity breakpoints for each antibiotic class, seven isolates were classified as sensitive to the tested antibiotics. Only one isolate, P.m1, exhibited resistance to two antibiotic classes (fluoroquinolones and tetracyclines). Fluoroquinolones are broad-spectrum antibiotics with high oral bioavailability and exert their bactericidal action by inhibiting bacterial topoisomerase II (gyrase) and topoisomerase IV (Redgrave et al., 2014; Hooper and Jacoby, 2015). Two fluoroquinolones, levofloxacin and ciprofloxacin, were evaluated in this study. According to EUCAST (2025), bacteria are considered susceptible to levofloxacin and ciprofloxacin if the MIC values are less than 2 µg/mL and less than 1 µg/mL, respectively. Isolate P.m1 was confirmed resistant to both levofloxacin and ciprofloxacin. Antibiotic resistance may arise from mutations in the target enzymes (Baggio and Ananda-Rajah, 2021).

Tetracyclines are broad-spectrum antibiotics that inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit, preventing aminoacyl-tRNA from attaching to the A-site of the ribosome (Manoharan et al., 2023). Two

tetracyclines, tetracycline and minocycline, were tested in the present study. According to CLSI M45 (2015), bacteria are deemed susceptible to tetracycline if the MIC is less than 4 µg/mL. One isolate was verified resistant to tetracycline. Resistance to tetracycline is often facilitated by efflux pump mechanisms (Simanjuntak et al., 2022). Nevertheless, all isolates remained sensitive to minocycline.

In addition to its resistance phenotype, P.m1 carried fewer virulence-associated genes (11/16) compared to the other isolates, which possessed approximately 13–14 virulence genes. This difference suggests that P.m1 does not possess a unique virulence genotype that would confer a selective advantage over the fully susceptible isolates. The reduced virulence gene content may indicate a lower intrinsic virulence potential. Previous studies have demonstrated that the acquisition of antimicrobial resistance can impose a fitness cost on bacteria, sometimes affecting growth rate, virulence factor expression, or overall pathogenic potential (Hernando-Amado et al., 2017). Therefore, although P.m1 is resistant to multiple antibiotic classes, it is unlikely to be more pathogenic or epidemiologically successful than isolates with a broader virulence gene repertoire. These findings indicate that multidrug resistance alone does not necessarily translate into higher virulence or increased transmission potential in poultry infections.

Table 3. Results of *P. multocida* antibiotic resistance isolated from infected chicken between 2018-2022 in Sukabumi, West Java, Indonesia

Abs	Antibiotics	MIC (µg/mL)								Sensitivity standards
		P.m1	P.m2	P.m3	P.m4	P.m5	P.m6	P.m7	P.m8	
AMI	Amikacin	<8	<8	<8	<8	<8	<8	<8	16	≤16
P/T4	Piperacillin / tazobactam constant 4	<8/4	<8/4	<8/4	<8/4	<8/4	<8/4	<8/4	<8/4	≤16/8
TGC	Tigecycline	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	≤ 2
TIM2	Ticarcilin / clavulanic acid constant 2	< 8/2	< 8/2	< 8/2	< 8/2	< 8/2	< 8/2	< 8/2	<8/2	≤16/2
LEVO	Levofloxacin	2	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 2
NIT	Nitrofurantoin	< 32	< 32	< 32	< 32	< 32	< 32	< 32	< 32	≤ 32
TET	Tetracycline	➢	< 4	< 4	< 4	< 4	< 4	< 4	< 4	≤ 4
DOR	Doripenem	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	≤ 0.5
MIN	Minocycline	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	≤ 1
ETP	Ertapenem	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	≤ 0.5
SXT	Trimethoprim / sulfamethoxazole	< 2/38	< 2/38	< 2/38	< 2/38	< 2/38	< 2/38	< 2/38	< 2/38	≤ 2/38
IMI	Imipenem	1	< 0.5	< 0.5	1	< 0.5	< 0.5	< 0.5	< 0.5	≤ 1
PIP	Piperacillin	< 16	< 16	< 16	< 16	< 16	< 16	< 16	< 16	≤ 16
MERO	Meropenem	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	≤ 1
GEN	Gentamicin	< 2	< 2	< 2	< 2	< 2	< 2	4	< 2	≤ 4
FAZ	Cefazolin	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	≤ 2
TOB	Tobramycin	< 2	< 2	< 2	< 2	< 2	< 2	4	< 2	≤ 4
TAZ	Ceftazidime	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	≤ 4
A/S2	Ampicillin / sulbactam 2:1 ratio	< 4/2	< 4/2	< 4/2	< 4/2	< 4/2	< 4/2	< 4/2	< 4/2	≤ 8/4
AZT	Aztreonam	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	≤ 1
AMP	Ampicillin	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	≤ 8
FEP	Cefepime	< 4	< 4	< 4	< 4	< 4	< 4	< 4	< 4	≤ 4
CIP	Ciprofloxacin	2	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	≤ 1
AXO	Ceftriaxone	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	≤ 1
POS	Positive control	+++	+++	+++	+++	+++	+++	+++	+++	

Abs: Abbreviation; MIC: Minimum inhibitory concentration; P.m: *Pasteurella multocida* isolates refer to Table 1; +++: The appearance of a cell sediment indicates bacterial growth

Detection of virulence genes

The pathogenicity of *Pasteurella multocida* is largely determined by the assortment of virulence-associated genes and factors it carries (Effendi et al., 2022). Each gene group contributes a distinct role in enhancing the bacterium's ability to infect the host. In this study, 16 virulence genes were detected and classified into seven functional groups, including capsule, toxins, iron acquisition proteins, fimbriae and adhesins, sialidases, outer membrane proteins (OMPs), and superoxide dismutases (SODs). Genes involved in iron acquisition (*exbB, exbD, tonB*), OMPs (*ompA, oma87*), adhesins/fimbriae (*fimA*), heme-binding (*hgbA*), and sialidase (*nanB*) were present in over 90% of sheep isolates (Nguyen et al., 2023). Toxin gene *toxA*, however, was detected only in specific serotypes, such as serotype D in sheep, highlighting serotype-dependent variation (Nguyen et al., 2023). In rabbit-derived isolates, fimbrial and adhesin genes (*fimA, pfhA, ptfA*) and *toxA* were present, and superoxide dismutase gene *sodC* was detected in all strains; additionally, *pfhA* correlated with capsular type, indicating its role in bacterial persistence (D'Amico et al., 2024; Wang et al., 2024). Collectively, these virulence genes contribute to essential processes, including iron uptake, adhesion to host tissues,

immune evasion through capsule and OMPs, sialic acid modification, toxin-mediated damage, and protection against oxidative stress, supporting the infection and survival of *P. multocida* in the host.

Detection findings revealed that 100% of isolates harbored the genes *capA*, *fim4*, *sodC*, *exbB*, *nanB*, *plpB*, *sodA*, *oma87*, and *fim*A; 87.5% carried *hgbB* and *fur*; 62.5% were positive for *pfhA*, *tadD*, and *nanH*; and 50% for *tbpA*. The *toxA* gene was absent in all isolates (Table 4). Among the isolates, the antibiotic-resistant strain P.m1 exhibited a slightly lower number of positive virulence genes (11/16) compared with most other isolates (13-14/16). Notably, P.m1 lacked *pfhA, tadD, tbpA, hgbB, fur,* and *nanH,* which are associated with adhesion, iron acquisition, and sialidase activity. However, it retained all core genes (*capA, fim4, sodC, exbB, nanB, plpB, sodA, oma87,* and *fimA*) that are essential for bacterial survival, adhesion, immune evasion, and oxidative stress resistance. These findings suggest that although P.m1 carries antimicrobial resistance, it maintains the fundamental virulence determinants necessary for host infection. The combination of resistance and retained core virulence genes indicates that resistant isolates like P.m1 can still pose significant pathogenic threats, highlighting the importance of considering both resistance and virulence profiles in epidemiological and clinical assessments of *P. multocida*.

The capsule is a polysaccharide structure that contributes to desiccation resistance, anti-phagocytic activity, and interacts with the complement system. *Pasteurella multocida* is categorized into five capsular types (A, B, D, E, and F) according to the presence of distinct capsule antigens. The findings of this study align with those of Furian et al. (2014), who identified capsular type A as the most prevalent in avian cholera cases, detecting *capA* in 49 of 54 isolates via multiplex PCR. The result of this study aligns with Shivachandra et al. (2006), who reported that 92 out of 94 isolates carried the *capA* gene.

The *toxA* gene encodes a mitogenic toxin that stimulates cell proliferation by interacting with heterotrimeric G proteins and modifying the alpha subunit through deamidation. This mechanism leads to cytoskeletal rearrangement, cellular proliferation, differentiation, and survival (Kubatzky, 2022). In contrast with previous studies by Sahragard et al. (2012), which reported high *toxA* prevalence in isolates from nasal and tonsil swabs of sheep and goats this present study did not detect *toxA* in any isolate. This absence can be explained by the capsular type of the isolates analyzed, which all belong to capsular type A. Notably, *toxA* is primarily associated with serogroup D strains in ruminants, including sheep and goats, and is rarely found in type A isolates or poultry-derived strains (Harper et al., 2006). Therefore, the lack of *toxA* is consistent with known serotype-specific distribution patterns in *P. multocida*, because *toxA* is sparsely distributed and associated with a prophage in only some serogroup A and D strains (Siddaramappa et al., 2021). The absence of *toxA* in capsular type A isolates aligns with these observations. Importantly, even in the absence of *toxA*, other virulence determinants such as *oma87, plpB, exbB, sodA, sodC,* and *nanB* remain present and may drive infection in type A isolates, demonstrating that pathogenicity is not solely dependent on the toxin gene.

Iron plays a vital role in DNA and protein biosynthesis, biofilm formation, redox activity, and electron transport and is required for bacterial growth and metabolism. During host-pathogen interactions, iron facilitates bacterial proliferation, adhesion, and expression of virulence factors (Shen et al., 2025). These findings correspond with Ewers et al. (2006), who identified *tbpA* in 70% of ruminant isolates but not in those from poultry, felines, or canines. The absence of *tbpA* in some isolates may be due to its incompatibility with the iron sources present in the avian host. The high detection rate of *hgbB* suggests that heme serves as a primary iron source for *P. multocida*. Other iron acquisition and virulence genes, including *fur, tonB, exbB, exbD, hgbA,* and *hgbB1/2,* have been frequently identified in avian isolates, highlighting their importance in bacterial survival and pathogenicity (Farahani et al., 2019).

Fimbriae and adhesin genes are critical for the initial stages of pathogenesis by facilitating attachment to host surfaces and colonization of the upper respiratory tract (Gross 2006; Haghnazari et al., 2017). These findings are consistent with Tawor et al. (2024), who reported *pfhA* and *tadD* in 55% and 60% of isolates, respectively, obtained from lung samples and nasal swabs of cattle, sheep, goats, and pet birds with respiratory symptoms. D'Amico et al. (2024) similarly detected *pfhA, tadD, fim4, and fimA* in 76.27%, 59.32%, and 92.23% respectively, also 69.49% of isolates possessed *capA*. Despite variations in prevalence among studies, these adhesin-related genes are commonly distributed among *P. multocida* isolates from diverse host species, highlighting their potential role in colonization and pathogenesis.

Sialidases allow *P. multocida* to hydrolyze sialic acids for nutrient acquisition, enabling the bacterium to use sialic acid as a carbon source, facilitating its growth and persistence on mucosal surfaces (Mizan et al., 2000). In this study, the detection of *nanB* and *nanH* aligns with previous reports. Gharibi et al. (2017) documented the occurrence of *nanB* and *nanH* in 63.6% and 81.8% of isolates, respectively, obtained from nasopharyngeal and nasal swab samples collected from slaughtered cattle and buffaloes. Nugroho et al. (2022) further demonstrated the functional significance of *nanB*, showing its toxicity and hemolytic activity using chicken and rabbit red blood cells, while also reporting variable detection rates of these genes, although the causes of this variability remain ambiguous. Additional evidence across species has been reported. Lachowicz-Wolak et al. (2025) detected *nanH* in isolates recovered from deep nasal swabs

and lung/bronchi swabs of calves with respiratory disease, while Ziagham et al. (2024) identified both *nanB* and *nanH* in isolates obtained from the oral cavity of various cat breeds. Although detection rates vary among studies and host species, these findings collectively suggest that sialidase genes are widely distributed in *P. multocida* and may play an important role in host colonization and pathogenicity.

Outer membrane proteins function as selective barriers that inhibit the entry of toxic compounds and are crucial for nutritional uptake, molecular transport, and interactions with the host environment (Hatfaludi et al., 2010). In this study, all isolates carried *oma87*, consistent with Furian et al. (2016), who detected *oma87* in 100% of poultry isolates obtained from the liver or heart of chickens and turkeys and from the lung of pigs. Additional reports further support its prevalence. In goats from Sichuan, *oma87* was found in 100% of isolates (Li et al., 2025), and in a Vietnamese study on porcine *P. multocida*, *oma87* was also universally present (Vu-Khac et al., 2020). Aski et al. (2016) detected *oma87* in 100% of isolates obtained from several clinically healthy and diseased animals (bovine, sheep, goat, poultry, dog, and cat). The widespread distribution of *oma87* across diverse host species underscores its high conservation and likely essential role in the pathogenic potential of *P. multocida*.

In order to shield bacteria from oxidative stress, which may harm cellular components, including DNA, proteins, and lipids, superoxide dismutases catalyze the conversion of reactive oxygen species (ROS) generated by neutrophils and macrophages into hydrogen peroxide and oxygen (Verma et al., 2013). These genes are widely distributed among *P. multocida* isolates. Furian et al. (2016) reported that *sodA* and *sodC* were present in 96% of poultry isolates obtained from the liver or heart of chickens and turkeys and from the lungs of pigs. Similarly, Aski et al. (2016) detected both genes in 100% of isolates collected from nasal and lung swabs of clinically healthy and diseased bovine, sheep, goats, poultry, dogs, and cats. The consistently high detection rates across studies highlight the essential role of these oxidative-stress defense genes in the survival of *P. multocida* within the host environment.

Based on the 16 virulence gene detections, it is evident that all genes except *toxA* were present in the *P. multocida* isolates tested. The existence of these virulence genes supports the bacterium's ability to infect hosts and survive under host defense conditions, even when isolates remain susceptible to the majority of tested antibiotics.

Table 4. The virulence gene of *P. multocida* isolated from infected chickens between 2018-2022 in Sukabumi, West Java, Indonesia

Genes	Targeted gene	Isolates								Total of positive	Percentage
		P.m1	P.m2	P.m3	P.m4	P.m5	P.m6	P.m7	P.m8		
CapA	*Serotyping A*	+	+	+	+	+	+	+	+	8/8	100
ToxA	*Dermonecrotic toxin*	-	-	-	-	-	-	-	-	0/8	0
TbpA	*Transferrin binding protein*	-	+	+	+	-	+	-	-	4/8	50
Fim4	*Type 4 fimbriae*	+	+	+	+	+	+	+	+	8/8	100
SodC	*Superoxide dismutate*	+	+	+	+	+	+	+	+	8/8	100
PfhA	*Filametous hemagglutinin*	-	+	+	-	+	-	+	+	5/8	62.5
ExbB	*Iron acquisition*	+	+	+	+	+	+	+	+	8/8	100
HgbB	*Hemoglobin binding protein*	+	+	+	+	+	+	-	+	7/8	87.5
NanB	*Neuraminidase*	+	+	+	+	+	+	+	+	8/8	100
TadD	*Putative nonspesific tight adherence protein D*	-	+	+	-	-	+	+	+	5/8	62.5
PlpB	*Lipoprotein B*	+	+	+	+	+	+	+	+	8/8	100
SodA	*Superoxide dismutate*	+	+	+	+	+	+	+	+	8/8	100
Oma87	*Outer membrane protein 87*	+	+	+	+	+	+	+	+	8/8	100
FimA	*Fimbriae*	+	+	+	+	+	+	+	+	8/8	100
NanH	*Neuroaminidase*	+	-	-	+	+	+	+	-	5/8	62.5
Fur	*Ferric uptake regulation protein*	-	+	+	+	+	+	+	+	7/8	87.5
Total of positive genes		11/16	14/16	14/16	13/16	13/16	14/16	13/16	13/16		

P.m: *Pasteurella multocida* isolates

CONCLUSION

Antibiotic resistance investigations revealed that all *P. multocida* isolates were generally susceptible to the tested antibiotics, with the exception of isolate P.m1, which was confirmed to exhibit resistance to levofloxacin, ciprofloxacin, and tetracycline. Virulence gene detections showed that 100% of isolates were positive for *capA*, *fim4*, *sodC*, *exbB*, *nanB*, *plpB*, *sodA*, *oma87*, and *fimA*; 87.5% were positive for *hgbB* and *fur*; 62.5% for *pfhA*, *tadD*, and *nanH*; and 50% for *tbpA*. The *toxA* gene was not detected in any of the isolates. These findings indicated that, despite the isolates being mostly susceptible to antibiotics, the presence of multiple virulence genes suggests that *P. multocida* retains the potential to induce disease. Future studies should focus on monitoring virulence and resistance gene profiles in local isolates,

assessing vaccine efficacy under field conditions, and implementing effective preventive and management strategies to reduce the impact of fowl cholera in poultry farms.

DECLARATIONS

Acknowledgments

The authors acknowledge IPB University and PT Medika Satwa Laboratoris for their support and cooperation during the study. The authors would like to thank Lusianawati Widjaja for her valuable assistance in improving the clarity of the manuscript and for providing constructive input during the response-to-reviewers process.

Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

Marsella Metta Arvia, Ryan Septa Kurnia, Christian Marco Hadi Nugroho, Muhammad Ade Putra, Safika, and Agustin Indrawati conceived, designed, collected and analyzed data, and wrote the manuscript. Ryan Septa Kurnia, Christian Marco Hadi Nugroho, and Agustin Indrawati designed, supervised the study, and reviewed the manuscript. All authors read and approved the final manuscript for publication.

Competing interests

The authors declared that there are no competing interests.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors. During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely to improve grammar, language clarity, and overall readability. The tool was not used to generate scientific content, perform data analysis, or contribute to the conclusions. All AI-assisted outputs were thoroughly reviewed, verified, and edited by the authors, who take full responsibility for the integrity and accuracy of the final manuscript.

Availability of data and materials

The additional data from the present study can be provided on request from the corresponding author.

REFERENCES

Abreu R, Semedo-Lemsaddek T, Cunha E, Tavares L, and Oliveira M (2023). Antimicrobial drug resistance in poultry production: current status and innovative strategies for bacterial control. Microorganisms, 11(4): 953. DOI: https://www.doi.org/10.3390/microorganisms11040953

Alhassan MY, Kabara MK, Ahmad AA, Abdulsalam J, and Habib HI (2025). Revisiting antibiotic stewardship: Veterinary contributions to combating antimicrobial resistance globally. Bulletin of the National Research Centre, 49(1): 25. DOI: https://www.doi.org/10.1186/s42269-025-01317-3

Atashpaz S, Shayegh J, and Hejazi MS (2009). Rapid virulence typing of *Pasteurella multocida* by multiplex PCR. Research in Veterinary Science, 87(3): 355-357. DOI: https://www.doi.org/10.1016/j.rvsc.2009.04.004

Aski HS and Tabatabaei M (2016). Occurence 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

Baggio D and Ananda-Rajah MR (2021). Fluoroquinolone antibiotics and adverse events. Australian Prescriber, 44(5): 161-164. DOI: https://www.doi.org/10.18773/austprescr.2021.035

D'Amico F, Messina D, Casalino G, Schiavitto M, Bove A, Romito D, D'onghia FP, Camarda A, and Circella E (2024). Characterisation of *Pasteurella multocida* strains from different lesions in rabbits. Animals, 14(11): 1569. DOI: https://www.doi.org/10.3390/ani14111569

Effendi MH, Faridah HD, Wibisono FM, Wibisono FJ, Nisa N, Fatimah, and Ugbo EN (2022). Detection of virulence factor encoding genes on *Escherichia coli* isolated from broiler chicken in Blitar District, Indonesia. Biodiversitas, 23(7): 3437. DOI: https://www.doi.org/10.13057/biodivd230716

Elbehiry A and Marzouk E (2025). From farm to fork: Antimicrobial-resistant bacterial pathogens in livestock production and the food chain. Veterinary Sciences, 12(9): 862. DOI: https://www.doi.org/10.3390/vetsci12090862

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

Farahani MF, Esmaelizad M, and Jabbari AR (2019). Investigation of iron uptake and virulence gene factors (*fur, tonB, exbD, exbB, hgbA, hgbB1, hgbB2 and tbpA*) among isolates of *Pasteurella multocida* from Iran. Iranian Journal of Microbiology, 11(3): 191-197. DOI: https://www.doi.org/10.18502/ijm.v11i3.1314

Faisal ZA (2019). Web-based expert system for diagnosing layer chicken diseases using the case-based reasoning method. Jati, 3(2): 126-132. DOI: https://www.doi.org/10.36040/jati.v3i2.882

Furian TQ, Borges KA, Pilatti RM, Almeida C, Nascimento VP, Salle CTP, Moraes HL, and De S (2014). Identification of the capsule type of *Pasteurella multocida* isolates from cases of fowl cholera by multiplex PCR and comparison with phenotypic methods. Brazilian Journal of Poultry Science, 16(2): 31-36. DOI: https://www.doi.org/10.1590/1516-635z160231-36

Furian TQ, Borges KA, Laviniki V, Rocha SLDS, Almeida CND, Nascimento VPD, Salle CTP, and Moraes HLDS (2016). Virulence genes and

antimicrobial resistance of *Pasteurella multocida* isolated from poultry and swine. Brazilian Journal of Microbiology, 47(1): 210-216. DOI: https://www.doi.org/10.1016/j.bjm.2015.11.014

Gharibi D, Hajikolaei MRH, Ghorbanpour M, and Barzegar SK (2017). Virulence gene profiles of *Pasteurella multocida* strains isolated from cattle and buffalo. Veterinarski Arhiv, 87: 677-690. DOI: https://www.doi.org/10.24099/vet.arhiv.160727

Gross L (2006). Bacterial fimbriae designed to stay with the flow. PLoS Biology, 4(9): e314. DOI: https://www.doi.org/10.1371/journal.pbio.0040314

Haghnazari S, Jabbari A, and Tadayon K (2017). Prevalence of adhesion virulence factor genes, antibiogram, and pathogenicity of avian *Pasteurella multocida* isolate from Iran. Archives of Razi Institute, 72(2): 83-91. DOI: https://www.doi.org/10.22092/ari.2017.109843

Harper M, Boyce JD, and Adler B (2006). *Pasteurella multocida* pathogenesis: 125 after pasteur. FEMS Microbiology Letters, 265(1): 1-10. DOI: https://www.doi.org/10.1111/j.1574-6968.2006.00442.x

Hatfaludi T, Al-hasani K, Boyce JD, and Adler B (2010). Outer membrane proteins of *Pasteurella multocida*. Veterinary Microbiology, 144(1-2): 1-17. DOI: https://www.doi.org/10.1016/j.vetmic.2010.01.027

Hernando-Amado S, Sanz-García F, Blanco P, and Martínez JL (2017). Fitness costs associated with the acquisition of antibiotic resistance. Essays in Biochemistry, 61(1): 37-48. DOI: https://www.doi.org/10.1042/EBC20160057

Hooper DC and Jacoby GA (2015). Mechanisms of drug resistance: quinolone resistance. Annals of the New York Academy of Sciences, 1354(1): 12-31. DOI: https://www.doi.org/10.1111/nyas.12830

Indrawati A, Safika S, Nugroho CMH, Putra MA, Silaen OSM, Krisnamurti DGB, Sanam MUE, and Kurnia RS (2025). Development and evaluation of a bivalent oil-adjuvanted vaccine against necrotic enteritis and avian colibacillosis in poultry. Advances in Animal and Veterinary Sciences, 13(6): 1378-1386. DOI: https://www.doi.org/10.17582/journal.aavs/2025/13.6.1378.1386

Kim J, Kim JW, Oh SI, So BJ, Kim WI, and Kim HY (2019). Characterisation of *Pasteurella multocida* isolates from pigs with pneumonia in Korea. BMC Veterinary Research, 15:119. DOI: https://www.doi.org/10.1186/s12917-019-1861-5

Kowalska-Krochmal B, and Dudek-Wicher R (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens, 10(2): 165. DOI: https://www.doi.org/10.3390/pathogens10020165

Kubatzky KF (2022). *Pasteurella multocida* toxin–lessons learned from a mitogenic toxin. Frontiers in Immunology, 13: 1058905. DOI: https://www.doi.org/10.3389/fimmu.2022.1058905

Lachowicz-Wolak A, Chmielina A, Przychodniak I, Karwańska M, Siedlecka M, Klimowicz-Bodys M, Dyba K, and Rypuła K (2025). Antimicrobial-resistance and virulence-associated genes of *Pasteurella multocida* and *Mannheimia haemolytica* isolated from Polish dairy calves with symptoms of bovine respiratory disease. Microorganisms, 13(3): 491. DOI: https://www.doi.org/10.3390/microorganisms13030491

Li S, Gu Q, Li B, Abi K, and Yang F (2025). High prevalence of virulence genes and multi-drug resistance in *Pasteurella multocida* from goats in Sichuan, China.The Veterinary Journal, 311: 106344. DOI: https://www.doi.org/10.1016/j.tvjl.2025.106344

Lobongona MLMNB, Afiff U, and Rotinsulu DA (2019). Antimicrobial effectiveness against *Pasteurella multocida* and *Mannheimia haemolytica* isolated from cattle suspected of having bovine respiratory disease complex. ARSHI Veteriner Letters, 3(2): 39-40. DOI: https://www.doi.org/10.29244/avl.3.2.39-40

Manoharan D, Srinivasan S, Vignesh NR, Senthilvel A (2023). Tetracyclines: The old, the new and the improved–A short review. Biomedical and Pharmacology Journal, 16(3): 1441-1450. DOI: https://www.doi.org/10.13005/bpj/2722

Maulana FH, Prasetyo E, and Sarenggat W (2017). Income analysis of layer chicken farming at Sumur Banger farm, Tersono Subdistrict, Batang Regency. Mediagro, 13(2): 1-12. DOI: https://www.doi.org/10.31942/mediagro.v13i2.2155

Mehmood MD, Zia S, Javed F, Gul M, Ashraf M, and Anwar H (2018). Physiochemical factors *in vitro* growth of *Pasteurella multocida*. African Journal of Microbiology Research, 12(11): 269-274. DOI: https://www.doi.org/10.5897/AJMR2017.8625

Mizan S, Henk L, Stallings A, Maier M, and Lee MD (2000). Coning and characterization of sialidases with 2-6 and 2-3 sialyl lactose specificity from *Pasteurella multocida*. Journal of Bacteriology, 182(24): 6874-6883. https://www.doi.org/10.1128/jb.182.24.6874-6883.2000

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

Nugroho CMH, Kurnia RS, Tarigan S, Silaen OSM, Triwidyaningtyas S, Wibawan IWT, Natalia L, Takdir AK, and Soebandrio A (2022). Screening and purification of *nanB* sialidase from *Pasteurella multocida* with activity in hydrolyzing sialic acid Neu5Acα(2-6)Gal and Neu5Acα(2-3)Gal. Scientific Reports, 12: 9425. DOI: https://www.doi.org/10.1038/s41598-022-13635-x

Redgrave LS, Sutton SB, Webber MA, and Piddock LJV (2014). Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. Trends in Microbiology, 22(8): 438-445. DOI: https://www.doi.org/10.1016/j.tim.2014.04.007

Reuben RC, Sarkar SL, Ibnat H, Setu MAA, Roy PC, and Jahid IK (2021). Novel multi-strain probiotics reduces *Pasteurella multocida* induced fowl cholera mortality in broilers. Scientific Reports, 11: 8885. DOI: https://www.doi.org/10.1038/s41598-021-88299-0

Sabsabi MA, Zakaria Z, Abu J, and Faiz NM (2021). Molecular characterisation and antibiotic sensitivity profile of *Pasteurella multocida* isolated from poultry farms in Malaysia. Austral Journal of Veterinary Sciences, 53(2). DOI: https://www.doi.org/10.4067/S0719-81322021000200121

Sahragard I, Tahamtan Y, Valadan M, Hyati M, Moazeni F, and Shirazi Z (2012). Development of rapid PCR method for simultaneous identification of species, specific capsular type, and toxigenicity of *Pasteurella* sp. isolates. Comparative Clinical Pathology, 21: 1333-1336. DOI: https://www.doi.org/10.1007/s00580-011-1291-7

Shen X, Guan L, Zhang J, Xue Y, Si L, and Zhao Z (2025). Study in the iron uptake mechanism of *Pasteurella multocida*. Veterinary Research, 56: 41. DOI: https://www.doi.org/10.1186/s13567-025-01469-0

Shivachandra SB, Kumar AA, Gautam R, Singh VP, Saxena MK, and Srivastava SK (2006). Identification of avian strains of *Pasteurella multocida* in India by conventional and PCR assays. The Veterinary Journal, 172(3): 561-564. https://www.doi.org/10.016/j.tvjl.2005.05.007

Siddaramappa S (2021). Comparative genomics of the *Pasteurella multocida* toxin. Genome, 64(7): 679-692. DOI: https://www.doi.org/10.1139/gen-2020-0176

Simanjuntak HA, Simanjuntak H, Maimunah S, Rahmiati R, and Situmorang TS (2022). Diameter zona hambat antibiotik amoxicillin dan tetracycline terhadap *Escherichia coli*. Herbal Medicine Journal, 5(2): 55-59. DOI: https://www.doi.org/10.58996/hmj.v5i2.52

Sunartatie T, Safika S, Abhirama HR, Kurnia RS, Putra MA, Nugroho CMH, Mayasari NLPI, and Indrawati A (2024). Identification and antibiotic resistance of *Pasteurella multocida* isolated from infected layer chickens in West Java, Indonesia. Journal of World's Poultry Research, 14(3):

282-290. DOI: https://www.doi.org/10.36380/jwpr.2024.29

Tang X, Zhao Z, Hu J, Wu B, Cai X, He Q, and Chen H (2009). Isolation, antimicrobial resistance, and virulence genes of *Pasteurella multocida* strains from swine in China. Journal of Clinical Microbiology, 47(4): 951-958. DOI: https://www.doi.org/10.1128/jcm.02029-08

Tawor AB, Erganis O, Balevi A, Kebabcioglu C, and Marangoz H (2024). The correlation between *ptfA*, *pfhA*, and *tadD* fimbriae genes and biofilm formation in *Pasteurella multocida* virulence and multidrug-resistant nature. The Microbe, 4: 100135. DOI: https://www.doi.org/10.1016/j.microb.2024.100135

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

Venkateswaran N, Swetschinski LR, Fastl C, Bari CD, Criscuolo NG, Mulchandani R, Zhao C, Meštrović T, Ikuta KS, Martins SB et al. (2024). Using priorities between human and livestock bacterial antimicrobial resistance (AMR) to identify data gaps in livestock AMR surveillance. BMC Infectious Diseases, 24(1): 1027. DOI: https://www.doi.org/10.1186/s12879-024-09847-3

Verma S, Sharma M, Katoch S, Verma L, Kumar S, Dogra V, Chahota R, Dhar P, and Singh G (2013). Profiling of virulence associated genes of *Pasteurella multocida* isolated from cattle. Veterinary Research Communication, 37: 83-89. DOI: https://www.doi.org/10.1007/s11259-012-9539-5

Vu-Khac H, Trinh TTH, Nguyen TTG, Nguyen XT, and Nguyen TT (2020). Prevalence of virulence factor, antibiotic resistance, and serotype genes of *Pasteurella multocida* strains isolated from pigs in Vietnam. Veterinary World, 13(5): 896-904. DOI: https://www.doi.org/10.14202/vetworld.2020.896-904

Wang J, Sun S, Chen D, Gao C, Sang L, and Xie X (2024). Pathogenic and genomic characterization of rabbit-sourced *Pasteurella multocida* serogroup F isolates recovered from dead rabbits with respiratory disease. Microbiology Spectrum, 12(4): e03654-23. DOI: https://www.doi.org/10.1128/spectrum.03654-23

Zainuddin (2014). Pathological case study of fowl cholera in broiler chicken farming among local communities in Banda Aceh. Jurnal Medik Veteriner, 8(1): 5659. DOI: https://www.doi.org/10.21157/j.med.vet.v81.3337

Ziagham A, Gharibi D, Mosallanejad B, and Avizeh R (2024). Molecular characterization of *Pasteurella multocida* from cats and antibiotic sensitivity of the isolates. Veterinary Medicine and Science, 10(3): e1424. DOI: https://www.doi.org/10.1002/vms3.1424