Genomic Characterization of Antimicrobial Resistance Genes in *Pasteurella multocida* Isolates from East Nusa Tenggara and Lampung, Indonesia

Sri Suryatmiati Prihandani[1,2], I Wayan Teguh Wibawan[1], Susan Maphilindawati Noor[2], Fitrine Ekawasti[2], Aswin Rafif Khairullah[2], Hastuti Handayani S. Purba[3], Alif Rahman Rohim Puarada[4], Safika Safika[1]*

[1]Division of Medical Microbiology, School of Veterinary Medicine and Biomedical, IPB University, Bogor, West Java, Indonesia, [2]Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia, [3]Research Center for Genetic Engineering, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia, [4]Division of Clinic and Lab, Pethut Group, Tangerang, West Java, Indonesia.

*Corresponding author: safika@apps.ipb.ac.id

Abstract

Haemorrhagic Septicaemia (HS) is a devastating disease affecting cattle and water buffaloes in Indonesia, causing annual economic losses in livestock industries, particularly in Asia, Africa, and the Middle East. *Pasteurella multocida*, the causative agent of the disease, has shown increasing antimicrobial resistance, complicating treatment efforts. Therefore, this study aims to provide the first genomic analysis of *P. multocida* isolates from different Indonesian provinces, focusing on genes conferring resistance to beta-lactam antibiotics based on Whole Genome Sequencing (WGS) results. Genomic data can be used to confirm the results of phenotypic antibiotic resistance testing. *P. multocida* isolates analyzed in this study were sourced from the Indonesian Research Center for Veterinary Science (IRCVS) collection, which included 2 samples originating from different geographic locations within Indonesia. The samples were subjected to biochemical, molecular, and antimicrobial susceptibility testing. WGS was performed using Oxford Nanopore Technologies (ONT) with subsequent bioinformatics analysis for genome assembly and resistance profiling. Phenotypic analysis showed significant variations between the isolates. The NTT isolate showed resistance to Penicillin, while the Lampung 2952 remained susceptible in the disk antibiotic test. Genome sequencing revealed extensive resistance determinants, including β-lactamase genes (*bla*Z, *bla*R1) in the NTT isolate, correlating with its phenotypic resistance. The high-quality genome assemblies (N50: 17,225 bp for NTT; 12,662 bp for Lampung 2952) enabled comprehensive resistome characterization, identifying more than 22 resistance genes in each isolate, including novel variants not previously reported in Indonesian strains. Therefore, this study provides the first genomic and resistance analysis based on Whole Genomic Sequencing data of Indonesian *P. multocida* isolates. The results show the urgent need for enhanced surveillance and prudent antimicrobial use in livestock management.

Keywords: *Pasteurella multocida*, *haemorrhagic septicaemia*, whole genome sequencing, antimicrobial resistance, Indonesia

Received: May 5, 2025 **Revised:** June 3, 2025 **Accepted:** July 11, 2025

INTRODUCTION

Haemorrhagic Septicaemia (HS) caused by *Pasteurella multocida* serotypes B:2 (Asian) and E:2 (African) primarily affects cattle (*Bos indicus*) and water buffalo (*Bubalus bubalis*). HS is characterized by rapid onset and severe septicaemia, often within 2–3 days of clinical manifestation, depending on the disease stage (Horadagoda *et al*., 2001). Several studies have shown that its morbidity and mortality rates were 42.7% and 63.6%, respectively, with a financial loss of USD 952.50 per affected household. This also caused a loss of USD 373.00 per animal, representing a 66.1% reduction in animal's value (Kawasaki *et al*., 2015). In Indonesia, a total of 1,156 HS cases were documented in Aceh between 2006 and 2010 (Nurliana *et al*., 2019). Meanwhile, the disease occurs almost every year in Kupang Regency, East Nusa Tenggara, causing a decline in the livestock population (Berek *et al*., 2015).

According to previous studies, antimicrobial therapy remains useful for treating diseases caused by *P. multocida*, including HS. Antibiotics Penicillin, Tetracycline, or Chloramphenicol, are administered parenterally to treat this disease (Lestari *et al*., 2025). However, these treatments are only helpful in the early stages of HS.

The overuse and misuse of antibiotics have accelerated the development of antimicrobial resistance (AMR), complicating treatment and control measures. In a previous study have shown that *P. multocida* can acquire resistance genes through horizontal gene transfer (HGT), aggravating the AMR problem (Michael *et al*., 2012). In regions with high antimicrobial usage, inappropriate use has contributed to the emergence of multidrug-resistant strains (Sabsabi *et al*., 2021).

Whole Genome Sequencing (WGS) provides a powerful tool to explore the resistome of bacterial pathogens, offering detailed insights into their genetic makeup and resistance mechanisms (Cimmino and Rolain, 2016). Despite its utility, there remains a paucity of WGS-based studies focusing on *P. multocida* in Indonesia, where HS disease is a persistent issue. Therefore, this study aims to investigate the resistome of *P. multocida* isolates from different provinces in Indonesia to understand the AMR and genome properties using WGS. Understanding the AMR profiles is helpful in identifying resistance genes, improving treatment strategies, and guiding policy development for HS disease.

MATERIALS AND METHODS

Ethical Approval

The use of *P. multocida* as a candidate for HS vaccine development was approved by the National Study and Innovation Agency (Approval Number 059/KE.02/SK/03/2024).

Study Period and Location

This study was conducted at the Genomic Laboratory of the National Study and Innovation Agency Cibinong from June to December 2024.

Subculturing of *P. multocida* Isolates

The *P. multocida* isolates used in this study were obtained from the collection of the Indonesian Research Center for Veterinary Science (IRCVS), consisting of two isolates originating from 2 distinct geographical regions in Indonesia. One of the *P. multocida* isolates was obtained from East Nusa Tenggara, while the other originated from Lampung, Sumatra. Isolates were cultured and subcultured in Brain Heart Infusion broth (Oxoid) and 5% Sheep Blood Agar medium (Oxoid) at 37°C for 24 hours. Samples were identified by applying standard microbiological, biochemical methods using API® 20NE kit (BioMérieux, France) and then confirmed by PCR (OIE, 2021).

Species Gene Detection

DNA Extraction: DNA was extracted using the Qiagen QIAamp DNA Mini Kits, following the manufacturer's protocol. Subsequently, overnight cultures were centrifuged to collect bacterial pellets, which were then lysed using a buffer containing proteinase K. After lysis, DNA was purified through a spin-column method and eluted in nuclease-free water.

DNA Quantification and Purity Assessment: The concentration and purity of extracted DNA were measured using a NanoDrop 2000 Thermo Scientific. Furthermore, DNA purity was evaluated by measuring absorbance at 260 nm and 280 nm, and the A260/A280 ratio was used as an indicator of purity. A ratio between 1.8 and 2.0 was considered acceptable for downstream molecular applications. Accurate quantification with Qubit dsDNA HS Assay Kits (Thermo Scientific) and TapeStation 4150 Agilent were used in checking the quality of DNA integrity.

Polymerase Chain Reaction (PCR): PCR detected the specific gene of *P. multocida*, namely the *kmt* gene. A pair of standard primers KMT1T7: 5'-ATC-CGC-TAT-TTA-CCC-AGT-GG 3' and KMT1SP6: 5'-GCT-GTA-AAC-GAA-CTC GCC-AC-3' was used to identify the *kmt* gene (OIE, 2021). Subsequently, reactions were prepared in 25 µL volumes containing 12.5 µL of KAPA PCR master mix, 0.75 µL of each primer (forward and reverse), 0.75 µL of template

DNA, and nuclease-free water to reach the final volume. Thermal cycling conditions included initial denaturation at 95°C for 4 minutes, 30 cycles of denaturation at 98°C for 20 seconds, annealing at 68°C for 15 seconds, and extension at 72°C for 15 seconds. The final extension at 72°C for 3 minutes completed PCR amplification.

Gel Electrophoresis: PCR products were separated by agarose gel electrophoresis using a 1.5% agarose gel in 1× TBE buffer stained with RedSafe™ (iNtRON). A total of 80 mL TBE 1X, 1.35 grams agarose (Vivantis), and 4 µL RedSafe™ were boiled in the microwave for 2 minutes at medium-low heat. DNA products were loaded into gel wells alongside a 100 bp DNA ladder. Electrophoresis was conducted at 100 Volt, 115 mA for 45 minutes, and bands were visualized under UV transillumination.

Antibiotic Disk Diffusion Testing

This testing was conducted using the Kirby-Bauer Disc Diffusion Method. The test antibiotics used were Penicillin, Kanamycin, Gentamicin, Chloramphenicol, Tetracyclin, and Enrofloxacin, which were performed on a Mueller-Hinton agar plate (Oxoid).

Library Preparation and WGS

Genomic DNA samples were sent to Genetika Science, Jakarta, Indonesia for whole-genome sequencing and initial bioinformatics analysis. These samples were used as the input for library preparation by applying the Library Preparation Kit from Oxford Nanopore Technologies (ONT).

Bioinformatics Data Analysis

In this study, filtering was performed using Fitlong, while the quality of reads were evaluated with Nanoplot (De Coster *et al*., 2018). Reads correction and assembly were performed using Canu and Flye, respectively (Koren, 2017). Assembled genomes were annotated using Prokka (Seemann, 2014). The resistome was analyzed using the Comprehensive Antibiotic Resistance Database Resistance Gene Identifier (CARD RGI) (Alcock *et al*., 2020).

Data Analysis

The results of this study are presented descriptively in the form of tables and figures.

RESULTS AND DISCUSSION

The *P. multocida* isolates were part of the IRCVS collection, which originated from 2 geographically distinct Indonesian provinces, namely East Nusa Tenggara (NTT) and Lampung. *P. multocida* colonies in blood agar media were small, round, and moist with a smooth surface appearing mucoid or slightly shiny and grayish-white, showing non-hemolytic or slight beta-hemolysis on blood agar (Figure 1). Morphological characteristics matched previous studies, showing small, round, moist, and mucoid colonies (Desem *et al*., 2023). Furthermore, the observation of non-hemolytic or slight beta-hemolysis on blood agar further supported the typical traits of *P. multocida* (Wilson and Ho, 2013).

Biochemical identification was performed using the API 20NE system (bioMérieux, France) according to the manufacturer's instructions. In this study, both isolates were identified by the API® 20NE kit as *P. multocida* with a numerical profile of 3000004 (% ID = 96%). This numerical profile was consistent with the results of previous studies (Desem *et al*., 2023). The kit allowed for the identification of non-enteric Gram-negative bacteria through 20 different fermentation and enzymatic activity tests. The biochemical test results were consistent with previous reports that emphasized the reliability of the API 20NE system for *P. multocida* identification (Prihandani *et al*., 2022).

Species confirmation was achieved through PCR detection of the *kmt*1 gene. Figure 2 showed the electrophoresis of PCR amplification products using a UV transilluminator. The *kmt*1 gene was observed in both samples at 460 bp through PCR, which was a widely recognized method for confirming the presence of *P. multocida*. The *kmt*1 gene encoded a conserved outer membrane protein unique to *P. multocida*, making it a reliable species-specific marker. Amplification of a 460 bp fragment using primers KMT1SP6 and

KMT1T7 has been consistently reported in prior study (Simão *et al*., 2015).

The disk diffusion method was used to analyze antibiotic susceptibility, based on the measurement of inhibition zones on Mueller–Hinton agar, where clear areas showed suppressed bacterial growth. In this study, *P. multocida* NTT showed resistance to Penicillin (Figure 3) but was sensitive to Gentamicin, Kanamycin, Chloramphenicol, and Tetracycline. Meanwhile, isolate Lampung 2952 was susceptible to all 6 antibiotics as shown in Table 1.

The emergence of antibiotic resistance was influenced by multiple factors, one of which was the duration of antibiotic administration (Sabsabi *et al*., 2021). Furthermore, the observed resistance of the NTT isolates to Penicillin could be attributed to the production of β-lactamase enzymes, a common resistance mechanism among *P. multocida* strains that hydrolyzed the β-lactam ring (Rosenau *et al*., 1991), rendering the antibiotic ineffective (Jian *et al*., 2021). β-lactamase was an enzyme produced by various bacteria as a defense mechanism against β-lactam antibiotics, such as Penicillins, Cephalosporins, Carbapenems, and Monobactams (Abraham and Chain, 1988; Livermore, 2008; Jian *et al*., 2021).

Isolate NTT and isolate Lampung 2952 respectively showed genome size: 2.29 Mbp, 2.34 Mbp (Moustafa *et al*., 2015); N50: 17,225 bp, 12,662 bp; GC content 40.25% and 40.1% (Figure 4). Recent studies identified a natural discontinuity in average nucleotide identity (ANI) values within bacterial species, typically between 99.2% and 99.8%, with a midpoint of approximately 99.5% (Table 2). The genome features of *P. multocida* NTT Kpg_1 and Lampung 2952, compared to *P. multocida* strain Razi_Pm0001 serotype B (CP017961.1) and serotype A:3 (NZ_CP132898) from GenBank, was presented in Table 3.

Table 1. Results of antibiotic susceptibility testing

Bacteria	**Antibiotic Inhibition Zone (cm)**					
	P	**TE**	**C**	**CN**	**K**	**ENR**
NTT	0	2.2	4.09	2	2.3	3.4
Lampung 2952	2.8	2.95	3.75	1.25	1.2	3

(P) Penicillin, (K) Kanamycin, (TE) Tetracycline, (C) Chloramphenicol, (CN) Gentamicin, (ENR) Enrofloxacin.

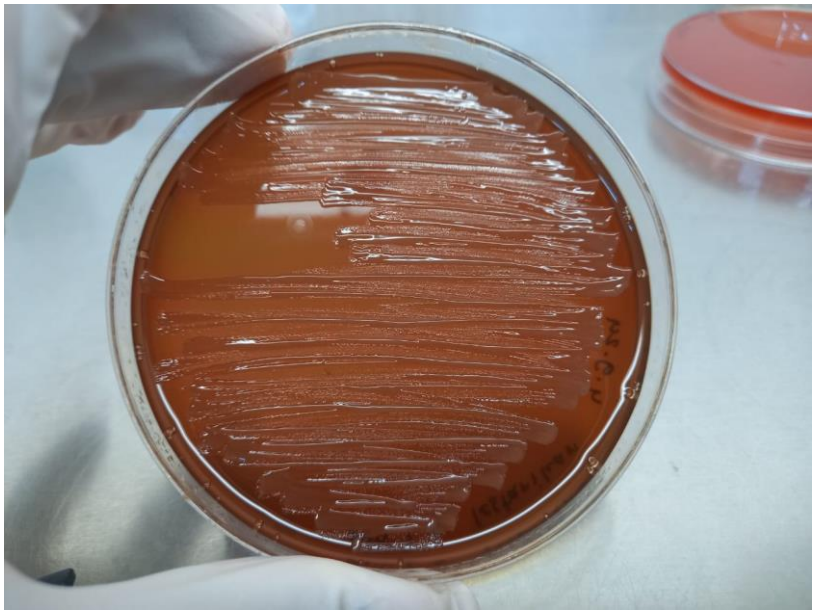

Figure 1. *P. multocida* colony growing on 5% Blood Agar Medium.

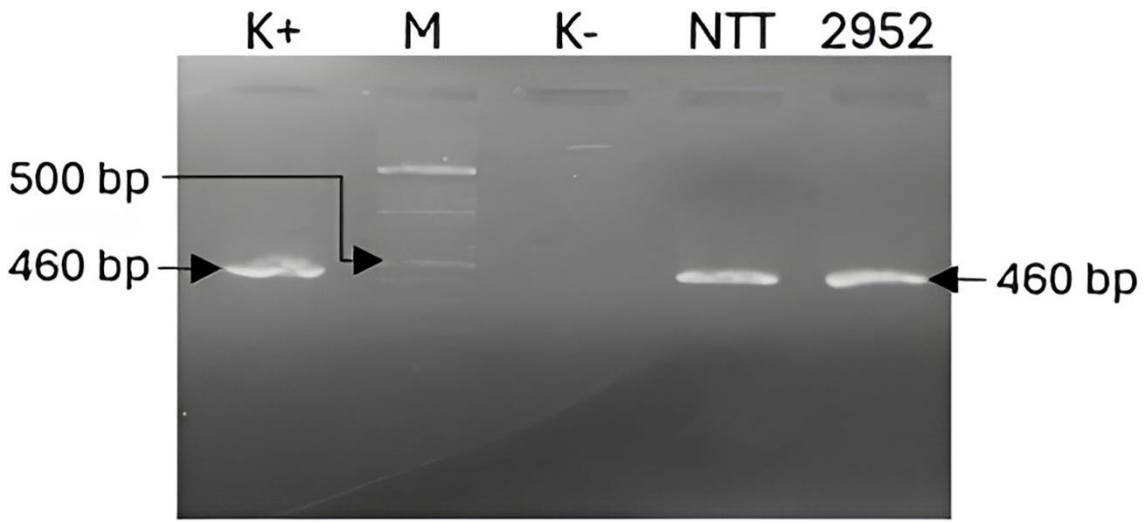

Figure 2. PCR results were visualized using agarose gel electrophoresis, showing a band at approximately 460 bp, which corresponds to the target *kmt* gene, specific for *P. multocida.*

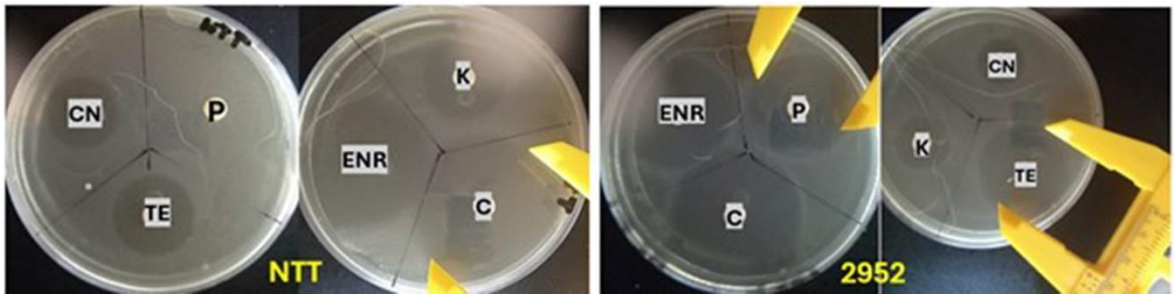

Figure 3. Antibiotic sensitivity testing. (P) Penicillin, (K) Kanamycin, (TE) Tetracycline, (C) Chloramphenicol, (CN) Gentamicin, (ENR) Enrofloxacin.

Table 2. Overview of the recent bovine *P. multocida* genome assembly statistic

Assembly Statistic	***P. multocida* NTT**	***P. multocida* Lampung 2952**
Number of contigs	1	1
Total length (Mbp)	2.29	2.35
Number of reads	139,998	261,742
N50/L50	115853/8Kb	118284/7Kb
Total %GC	40.25	40.1
N50 length (bp)	17,225.0	12,662.0
BUSCO score (%)	99.8	99.2
Mean read length	10,183.36 kb	10,776.78 kb

CARD RGI analysis tools in Proksee were used to detect antibiotic resistance genes in both *P. multocida* WGS results. The number of antibiotic resistance genes detected in *P. multocida* NTT and 2952 was remarkably high. Some of these genes were presented sequentially in Tables 4 and 5. The result showed a circular map as Figure 5 and Figure 6 for *P. multocida* NTT and Lampung 2952 respectively. Specifically, the focus on β-lactamase genes showed the presence of the Ambler class A β-lactamase encoding PC1 beta-lactamase (*bla*Z) and the others (*bla*R1, *bla*B, and *bla*C). The detection of *bla*Z was particularly significant, as it was known to confer resistance to β-lactam antibiotics, including Penicillins. This result correlated with the observed Penicillin resistance in disc diffusion testing, suggesting that the *bla*Z gene played an essential role in resistance mechanism of *P. multocida* NTT. The detection

Table 3. Genome features of *P. multocida* NTT Kpg_1 and Lampung 2952 genome compared to *P. multocida* strain Razi_Pm0001 serotype B (CP017961.1) and serotype A:3 (NZ_CP132898)

Species	Accession Number	Genome size Mbp	GC%	No. genes	Average Nucleotide Identity	
					A:3 NZ_CP132898	B CP017961.1
P. multocida NTT	NA	2.29	40.25	1,755	98.44	99.95
P. multocida Lampung 2952	NA	2.34	40.10	1,816	98.41	98.41
P. multocida Razi	CP017961	2.36	40.40	1,879	98.32	100
P. multocida A:3	NZ_CP132898	2.62	40.50	2,646	100	98.32

of *bla*Z and *bla*B-32 β-lactamase genes suggests that this strain has acquired mechanisms to hydrolyze β-lactam antibiotics, a phenomenon that had been previously described in multidrug-resistant *P. multocida* isolates (Michael *et al*., 2012).

The presence of the *bla*Z gene in *P. multocida* NTT showed potential acquisition through HGT or intrinsic resistance mechanisms, possibly facilitated by mobile genetic elements such as plasmids, transposons, or integrative conjugative elements (ICEs). This could contribute to the dissemination of β-lactam resistance. The gene responsible for β-lactam resistance in *P. multocida* in Spain is *bla*ROB-1 (Millan *et al*., 2009; Jahnen *et al*., 2025). *P. multocida* utilizes the coexistence and horizontal spread of small plasmids as a strategy to develop multidrug resistance. The detection of β-lactamase-producing *P. multocida* strains emphasized the need for targeted antibiotic management strategies. This study result was consistent with previous reports by Rahimzadeh *et al.* (2024) where *bla*Z-mediated resistance was identified in various bacterial species, contributing to the global AMR crisis. The absence of resistance to Gentamicin, Kanamycin, Chloramphenicol, Enrofloxacin, and Tetracycline suggested that resistance mechanisms in *P. multocida* NTT may be selective rather than broad-spectrum. Further investigation is needed to determine whether *P. multocida* NTT acquired *bla*Z through plasmids or chromosomal integration. Phenotypic testing showed that *P. multocida* NTT was resistant to Penicillin using disc antibiotics, while CARD RGI analysis showed a wider range of resistance genes.

The difference in resistance genes was influenced by local antibiotic usage patterns in Lampung 2952 and NTT. Variations in antibiotic exposure could drive the selection of different resistance genes. Farming practices, veterinary treatment policies, and livestock management also contributed to variations in AMR profiles.

This identification of β-lactamase genes, including *blaZ*, emphasized the importance of implementing targeted antimicrobial stewardship efforts.

Table 4. List of identified resistance genes in *P. multocida* NTT

Resistance Gene	Drug Class	Resistance Mechanism	AMR Gene Family
*bla*Z	Penam	Antibiotic inactivation	*Bla*Z beta-lactamase
*bla*B-32	Carbapenem; Penam	Antibiotic inactivation	*Bla*B beta-lactamase
Vgae	Lincosamide antibiotic; Streptogramin antibiotic; Streptogramin A antibiotic; Pleuromutilin antibiotic	antibiotic target protection	vga-type ABC-F protein
CRP-1	Carbapenem	Antibiotic inactivation	CRP beta-lactamase
*leu*O	Nucleoside antibiotic; disinfecting agents and antiseptics	antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
Tet(Q)	Tetracycline antibiotic	antibiotic target protection	Tetracycline-resistant ribosomal protection protein
*vga*B	Streptogramin antibiotic; Streptogramin A antibiotic; Pleuromutilin antibiotic	antibiotic target protection	vga-type ABC-F protein
Tet(35)	Tetracycline antibiotic	antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump
*tlr*C	Macrolide antibiotic; Lincosamide antibiotic	antibiotic target protection	Miscellaneous ABC-F subfamily ATP-binding cassette ribosomal protection proteins
*Erm*D	Macrolide antibiotic; Lincosamide antibiotic; Streptogramin antibiotic; Streptogramin A antibiotic; Streptogramin B antibiotic	antibiotic targ*et al*teration	Erm 23S ribosomal RNA methyltransferase
*ade*C	Glycylcycline; Tetracycline antibiotic	antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
*chr*B	Macrolide antibiotic; Lincosamide antibiotic	antibiotic targ*et al*teration	Antibiotic targ*et al*teration
*mac*B	Macrolide antibiotic	Antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump
DHA-28	Cephalosporin cephamycin	Antibiotic inactivation	DHA beta-lactamase
ANT(4')	Aminoglycoside antibiotic	Antibiotic inactivation	ANT(4')
BcIII	Cephalosporin; Penem	Antibiotic inactivation	Class A Bacillus cereus Bc beta-lactamase

*Lpe*B	Macrolide antibiotic	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
TEM-7	Monobactam; Cephalosporin; Penam; Penem	Antibiotic inactivation	TEM beta-lactamase
*qac*A	Fluoroquinolone antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
OXA-905	Carbapenem; Cephalosporin; Penam	Antibiotic inactivation	OXA beta-lactamase
*Nmc*R	Carbapenem; Cephalosporin; Cephamycin; Penam	Antibiotic inactivation	*Nmc*A beta-lactamase
Tet(X6)	Tetracycline antibiotic	Antibiotic inactivation	Tetracycline inactivation enzyme

Table 5. List of identified resistance genes in *P. multocida* 2952

Resistance Gene	Drug Class	Resistance Mechanism	AMR Gene Family
PFM-4	Carbapenem	Antibiotic inactivation	PFM beta-lactamase
*Tae*A	Pleuromutilin antibiotic	Antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump
vanY gene in vanG cluster	Glycopeptide antibiotic	antibiotic targ*et al*teration	*van*Y; glycopeptide resistance gene cluster
Erm(46)	Macrolide antibiotic; Lincosamide antibiotic; Streptogramin antibiotic	antibiotic targ*et al*teration	Erm 23S ribosomal RNA methyltransferase
ECV-1	Carbapenem	Antibiotic inactivation	ECV beta-lactamase
IMP-94	Carbapenem; Cephalosporin; Cephamycin; Penam; Penem	Antibiotic inactivation	IMP beta-lactamase
*efp*A	Rifamycin antibiotic; Isoniazid-like antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
*Isa*A	Lincosamide antibiotic; Streptogramin antibiotic; Streptogramin A antibiotic; Streptogramin B antibiotic; Pleuromutilin antibiotic	Antibiotic target protection	lsa-type ABC-F protein
LEN-26	Penam; Penem	Antibiotic inactivation	LEN beta-lactamase
ICR-Mc	Peptide antibiotic	Antibiotic targ*et al*teration	Intrinsic colistin resistant phosphoethanolamine transferase
PEDO-3	Carbapenem	Antibiotic inactivation	Subclass B1 PEDO beta-lactamase
*arn*A	Peptide antibiotic	Antibiotic targ*et al*teration	pmr phosphoethanolamine transferase

cmlA8	Phenicol antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
*mds*A	Monobactam; Carbapenem; Cephalosporin; Cephamycin; Penam; Phenicol antibiotic; Penem	Antibiotic efflux	Resistance-nodulation-cell division (RND) antibiotic efflux pump
SCO-1	Cephalosporin; Penam; Penem	Antibiotic inactivation	SCO beta-lactamase
ADC-100	Cephalosporin	Antibiotic inactivation	ADC beta-lactamases classification pending for carbapenemase activity
tet(41)	Tetracycline antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
EBR-5	Carbapenem; Cephalosporin; Penam	Antibiotic inactivation	EBR beta-lactamase
AAC(6')-lak	Aminoglycoside antibiotic	Antibiotic inactivation	AAC(6')
*bcr*A	Peptide antibiotic	Antibiotic efflux	ATP-binding cassette (ABC) antibiotic efflux pump
tet(E)	Tetracycline antibiotic	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump
CPS-1	Carbapenem	Antibiotic inactivation	CPS beta-lactamase

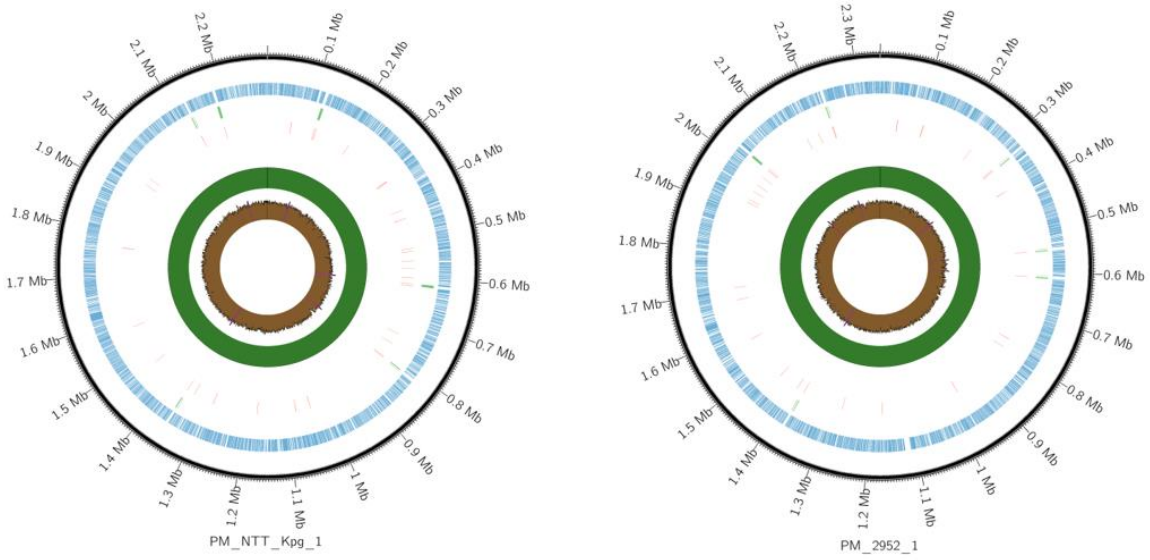

Figure 4. Visualization ONT sequencing result of *P. multocida* NTT and Lampung 2952. Figure Legend (from outer): contig (blue), genes (grey), pseudogenes (blue), CDS (black), rRNA (green), tRNA (purple), depth (depth > 50 = green; depth < 50 = red), GC content (GC content > 50% = purple; GC content < 50% = brown).

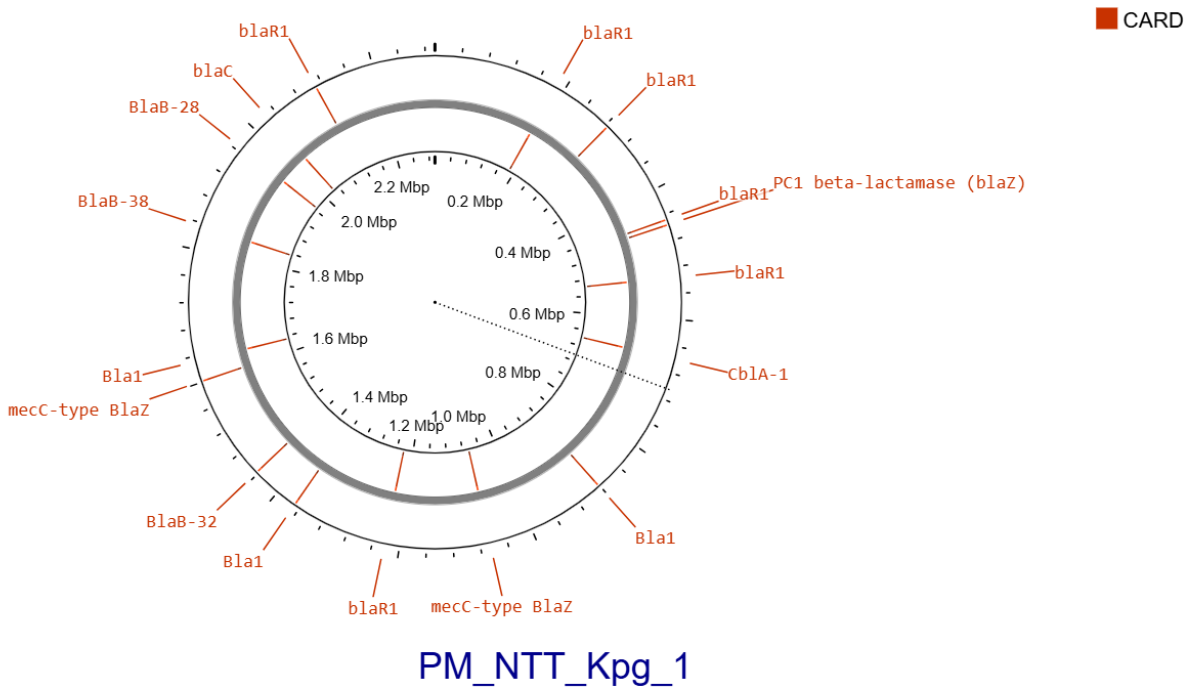

Figure 5. CARD RGI Analysis Map of β-lactamase genes detected in *P. multocida* NTT.

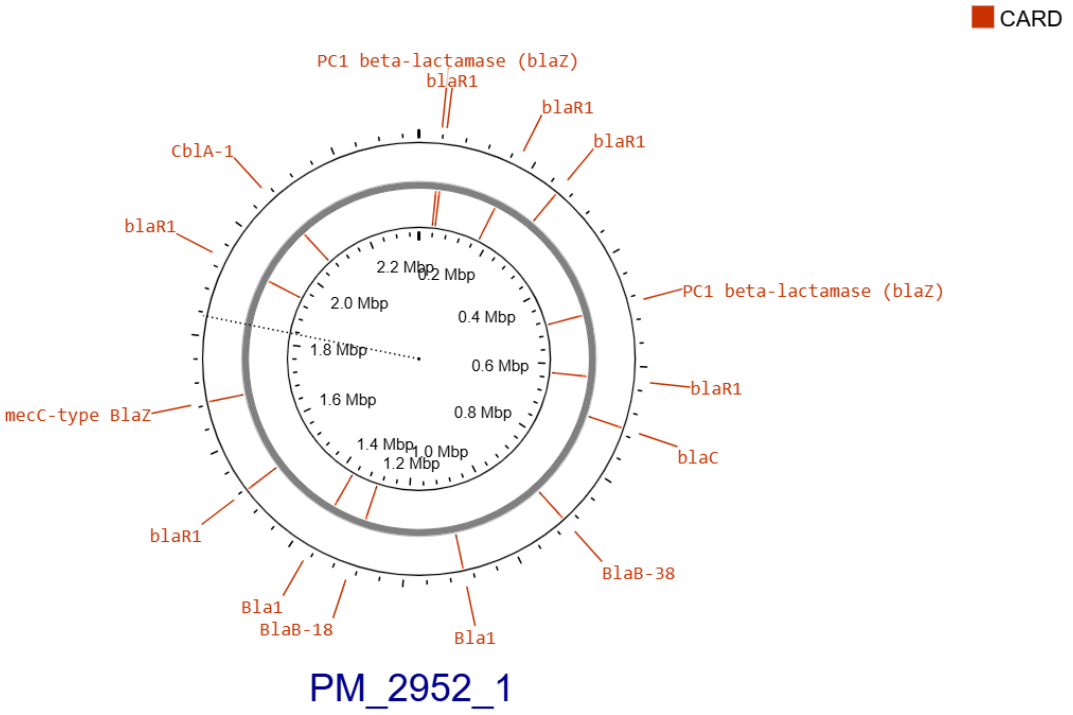

Figure 6. CARD RGI Analysis Map of β-lactamase genes detected in *P. multocida* Lampung 2952.

The emergence of β-lactamase-producing *P. multocida* strains called for stronger surveillance and responsible antibiotic use. Ongoing monitoring of resistance genes in both veterinary and clinical contexts was essential to curb the spread of multidrug-resistant isolates. Furthermore, rational antibiotic use in livestock management must be emphasized to reduce the selective pressure that caused resistance.

Whole-genome analysis showed insertions, deletions, or mutations in ARG-related regions (Gomes *et al.*, 2021). Differences in chromosomal versus plasmid-borne genes affected the detection of resistance genes by

CARD IRG. Some resistance genes could be present but not expressed due to regulatory mechanisms or gene silencing.

The differences in phenotypic (disk diffusion test) and genotypic resistance (CARD IRG prediction) emphasized the complexity of AMR in *P. multocida*. While CARD IRG detected the presence of ARGs, actual phenotypic resistance could be influenced by factors such as gene expression regulation, mutations affecting functional protein production, efflux pump activity, or enzymatic degradation of antibiotics (Jian *et al*., 2021; Korotetskiy *et al*., 2023; Gauba and Rahman, 2023). WGS and transcriptomic studies further clarified whether detected resistance genes were actively expressed in these isolates (He *et al*., 2021; Fikri *et al*., 2022).

P. multocida Lampung 2952 showed resistance to a broader range of antibiotics, with CARD IRG identifying genes linked to resistance against Monobactams, Carbapenem, Cephalosporin, Cephamycin, Rifamycin, Lincosamide, and Tetracycline resistance genes. The presence of Rifamycin resistance genes in Lampung 2952 but not in NTT suggested that this isolate had acquired additional resistance determinants, potentially through HGT, including plasmid acquisition or transposon integration. Furthermore, the absence of streptogramin and macrolide resistance genes in Lampung 2952, which were detected in NTT, emphasized genetic diversity between these isolates and suggested strain-specific resistance mechanisms.

CARD IRG analysis detected differences in gene resistance of *P. multocida,* which exhibited genetic diversity across different geographic regions. Variations in antimicrobial resistance genes (ARGs) could arise due to differences in genetic background, HGT, or evolutionary adaptation (Jian *et al*., 2021). Comparative genomics studies showed that different strains of the same species could carry distinct resistance determinants due to differences in mobile genetic elements, such as plasmids or transposons. The difference in resistance genes was influenced by local antibiotic usage patterns in Lampung and NTT. Variations in antibiotic exposure could drive the selection of different resistance genes. Furthermore, farming practices, veterinary treatment policies, and livestock management also contributed to variations in AMR profiles. ARGs could spread through HGT mechanisms, including conjugation, transformation, and transduction (Jian *et al*., 2021; Michael *et al*., 2012). The presence or absence of certain resistance genes could be due to differences in the acquisition of plasmids, integrons, or prophages. Some *P. multocida* strains were reported by Kutzer *et al.* (2021) to acquire resistance genes from other bacterial species in mixed microbial communities, particularly in livestock environments. Whole-genome analysis showed insertions, deletions, or mutations in ARG-related regions (Zhu *et al*., 2019). Differences in chromosomal versus plasmid-borne genes affected the detection of resistance genes by CARD IRG. Some resistance genes could be present but not expressed due to regulatory mechanisms or gene silencing.

The variation in ARGs between *P. multocida* NTT and Lampung 2952 showed the role of genetic background, antibiotic selection pressure, and HGT in shaping resistance profiles. These results emphasized the need for both phenotypic and genotypic resistance testing to accurately assess antibiotic resistance in veterinary and clinical settings, particularly for guiding effective antimicrobial treatment strategies. The emergence of multidrug-resistant strains showed the need for judicious antibiotic use in veterinary and agricultural settings to prevent the spread of resistant pathogens (Matheou *et al*., 2025). Future studies must focus on elucidating the mechanisms underlying resistance gene acquisition and the potential impact of these genes on the virulence and pathogenicity of *P. multocida*.

CONCLUSION

This study provided the first comprehensive genomic and resistome analysis of Indonesian *P. multocida* isolates from 2 different geographical regions in Indonesia focusing on beta-lactam antibiotics. These results emphasized the need for both phenotypic and genotypic resistance testing to accurately assess antibiotic resistance to

minimize the potential risk to livestock and humans.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Genomics Laboratory and the National Research and Innovation Agency for their assistance in conducting this research.

AUTHORS' CONTRIBUTIONS

SSP conceived, designed, and coordinated the study. IWTW, SS, and SMN developed the data collection tools and supervised field sampling and data acquisition. SS contributed to validation, supervision, and formal analysis. ARRP performed data analysis, developed analytical tools, and assisted in validation. ARK and HHSP carried out data analysis, interpretation, and contributed to manuscript preparation. HHSP and FE provided reagents and materials. All authors read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Abraham, E. P., & Chain, E. (1988). An enzyme from bacteria able to destroy penicillin. 1940. *Reviews of Infectious Diseases*, 10(4), 677–678.

Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., Faltyn, M., Hernandez-Koutoucheva, A., Sharma, A. N., Bordeleau, E., Pawlowski, A. C., Zubyk, H. L., Dooley, D., Griffiths, E., Maguire, F., Winsor, G. L., Beiko, R. G., Brinkman, F. S. L., Hsiao, W. W. L., Domselaar, G. V., & McArthur, A. G. (2020). CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Research*, 48(D1), D517–D525.

Berek, H. S. D., Nugroho, W. S., & Wahyuni, A. E. T. H. (2015). Protectivity Against Septicemia Epizootica of Cows in Kupang District. *Jurnal Veteriner*, 16(2), 167–173.

Cimmino, T., & Rolain, J. M. (2016). Whole genome sequencing for deciphering the resistome of Chryseobacterium indologenes, an emerging multidrug-resistant bacterium isolated from a cystic fibrosis patient in Marseille, France. *New Microbes and New Infections*, 12(1), 35–42.

De Coster, W., D'Hert, S., Schultz, D. T., Cruts, M., & Van Broeckhoven, C. (2018). NanoPack: Visualizing and processing long-read sequencing data. *Bioinformatics*, 34(15), 2666–2669.

Desem, M. I., Handharyani, E., Setiyono, A., Safika, S., Subekti, D. T., & Ekawasti, F. (2023). Morphology, Biochemical, and Molecular Characterization of *Pasteurella multocida* Causing Hemorrhagic Septicemia in Indonesia. *Veterinary Medicine International*, 2023(1), 7778707.

Fikri, F., Wardhana, D. K., Purnomo, A., Khairani, S., Chhetri, S., & Purnama, M. T. E. (2022). Aerolysin gene characterization and antimicrobial resistance profile of *Aeromonas hydrophila* isolated from milkfish (*Chanos chanos*) in Gresik, Indonesia. *Veterinary World*, 15(7), 1759–1764.

Gauba, A., & Rahman, K. M. (2023). Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. *Antibiotics (Basel)*, 12(11), 1590.

Gomes, L. C., Campino, S., Marinho, C. R. F., Clark, T. G., & Phelan, J. E. (2021). Whole genome sequencing reveals large deletions and other loss of function mutations in Mycobacterium tuberculosis drug resistance genes. *Microbial Genomics*, 7(12), 000724.

He, F., Zhao, Z., Wu, X., Duan, L., Li, N., Fang, R., Li, P., & Peng, Y. (2021). Transcriptomic Analysis of High- and Low-Virulence

Bovine *Pasteurella multocida* in vitro and in vivo. *Frontiers in Veterinary Science*, 8(1), 616774.

Horadagoda, N. U., Hodgson, J. C., Moon, G. M., Wijewardana, T. G., & Eckersall, P. D. (2001). Role of endotoxin in the pathogenesis of haemorrhagic septicaemia in the buffalo. *Microbial Pathogenesis*, 30(3), 171–178.

Jahnen, J., Hanke, D., Kadlec, K., Schwarz, S., & Krüger-Haker, H. (2025). Antimicrobial Resistance in *Pasteurella multocida* Isolates from Bovine Mastitis Can Be Associated with Multidrug-Resistance-Mediating Integrative and Conjugative Elements (ICEs). *Antibiotics*, 14(2), 153.

Jian, Z., Zeng, L., Xu, T., Sun, S., Yan, S., Yang, L., Huang, Y., Jia, J., & Dou, T. (2021). Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of Basic Microbiology*, 61(12), 1049–1070.

Kawasaki, M., Young, J. R., Suon, S., Bush, R. D., & Windsor, P. A. (2015). The Socioeconomic Impacts of Clinically Diagnosed Haemorrhagic Septicaemia on Smallholder Large Ruminant Farmers in Cambodia. *Transboundary and Emerging Diseases*, 62(5), 535–548.

Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., & Phillippy, A. M. (2017). Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Research*, 27(5), 722–736.

Korotetskiy, I. S., Shilov, S. V., Kuznetsova, T., Kerimzhanova, B., Korotetskaya, N., Ivanova, L., Zubenko, N., Parenova, R., & Reva, O. N. (2023). Analysis of Whole-Genome Sequences of Pathogenic Gram-Positive and Gram-Negative Isolates from the Same Hospital Environment to Investigate Common Evolutionary Trends Associated with Horizontal Gene Exchange, Mutations and DNA Methylation Patterning. *Microorganisms*, 11(2), 323.

Kutzer, P., Szentiks, C. A., Bock, S., Fritsch, G., Magyar, T., Schulze, C., Semmler, T., & Ewers, C. (2021). Re-Emergence and Spread of Haemorrhagic Septicaemia in Germany: The Wolf as a Vector? *Microorganisms*, 9(9), 1999.

Lestari, T. D., Khairullah, A. R., Damayanti, R., Mulyati, S., Rimayanti, R., Hernawati, T., Utama, S., Wardhani, B. W. K., Wibowo, S., Kurniasih, D. A. A., Ma'ruf, I. F., Moses, I. B., Lisnanti, E. F., Ahmad, R. Z., Fauziah, I., Amalia, N., Fauzia, K. A., & Kusala, M. K. J. (2025). Hemorrhagic septicemia: A major threat to livestock health. *Open Veterinary Journal*, 15(2), 519–532.

Livermore, D. M. (2008). Defining an extended-spectrum beta-lactamase. *Clinical Microbiology and Infection*, 14(Suppl 1), 3–10.

Matheou, A., Abousetta, A., Pascoe, A. P., Papakostopoulos, D., Charalambous, L., Panagi, S., Panagiotou, S., Yiallouris, A., Filippou, C., & Johnson, E. O. (2025). Antibiotic Use in Livestock Farming: A Driver of Multidrug Resistance? *Microorganisms*, 13(4), 779.

Michael, G. B., Kadlec, K., Sweeney, M. T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Murray, R. W., Watts, J. L., & Schwarz, S. (2012). ICEPmu1, an integrative conjugative element (ICE) of *Pasteurella multocida*: Analysis of the regions that comprise 12 antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, 67(1), 84–90.

Millan, A. S., Escudero, J. A., Gutierrez, B., Hidalgo, L., Garcia, N., Llagostera, M., Dominguez, L., & Gonzalez-Zorn, B. (2009). Multiresistance in *Pasteurella multocida* is mediated by coexistence of small plasmids. *Antimicrobial Agents and Chemotherapy*, 53(8), 3399–3404.

Moustafa, A. M., Seemann, T., Gladman, S., Adler, B., Harper, M., Boyce, J. D., & Bennett, M. D. (2015). Comparative Genomic Analysis of Asian Haemorrhagic Septicaemia-Associated Strains of *Pasteurella multocida* Identifies More than 90 Haemorrhagic Septicaemia-Specific Genes. *PLoS One*, 10(7), e0130296.

Nurliana, Karmil, T., Ferasyi, T., Darmawi, Razali, Sugito, Rastina, & Ismail. (2019). Identification of Risk Factors and Seroprevalence For Septicemia Epizootica (SE) Endemic Case Of Buffaloes in Aceh Barat, Aceh. *Proceedings of the Conference of the International Society for Economics and Social Sciences of Animal Health - South East Asia 2019 (ISESSAH-SEA 2019)*. 19(1), 106–110.

OIE. (2021). Haemorrhagic septicaemia (*Pasteurella multocida*) serotypes 6:B and 6:E. Mannual, 1–17.

Prihandani, S. S., Noor, S. M., Wibawan, I. W. T., Safika, Purba, H. H. S., Sumirah, & Kusumawati, A. (2022). Polymerase chain reaction to confirm biochemically characterization method of *Pasteurella multocida* isolate from fatal cases of Septicaemia epizootica in Nusa Tenggara Timur. *IOP Conference Series: Earth and Environmental Science*, 976(1), 012046.

Rahimzadeh, G., Ghezljai, N., Rezai, S., Rezai, R., Navaiefar, M. R., Rezai, M. S., Moradi, M., & Shakeri, M. (2024). Updating the Antimicrobial Resistance Pattern among Critical Priority Pathogens in the Intensive Care Unit in Northern Iran Post COVID-19 Pandemic. *Advanced Biomedical Research*, 13(1), 123.

Rosenau, A., Labigne, A., Escande, F., Courcoux, P., & Philippon, A. (1991). Plasmid-mediated ROB-1 beta-lactamase in *Pasteurella multocida* from a human specimen. *Antimicrobial Agents and Chemotherapy*, 35(11), 2419–2422.

Sabsabi, M. A., Zakaria, Z., Abu, J., & Faiz, N. M. (2021). Molecular characterisation and antibiotic sensitivity profile of *Pasteurella multocida* isolated from poultry farms in Malaysia. *Austral Journal of Veterinary Sciences*, 53(2), 121–126.

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*, 30(14), 2068–2069.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, 31(19), 3210–3212.

Wilson, B. A., & Ho, M. (2013). *Pasteurella multocida*: From Zoonosis to cellular microbiology. *Clinical Microbiology Reviews*, 26(3), 631–655.

Zhu, D., He, J., Yang, Z., Wang, M., Jia, R., Chen, S., Liu, M., Zhao, X., Yang, Q., Wu, Y., Zhang, S., Liu, Y., Zhang, L., Yu, Y., You, Y., Chen, X., & Cheng, A. (2019). Comparative analysis reveals the Genomic Islands in *Pasteurella multocida* population genetics: on Symbiosis and adaptability. *BMC Genomics*, 20(1), 63.

***