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Molecular analysis of *Klebsiella pneumoniae* serotypes and virulence genes isolated from pneumonic Aceh cattle

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ABSTRACT

Background: *Klebsiella pneumoniae* is an important human and animal pathogen that causes a wide spectrum of infections.

Aim: This study attempted to isolate and characterize *K. pneumoniae* from the respiratory tract of Aceh cattle.

Methods: Pneumonic lung tissue and tracheal swabs were collected from a slaughterhouse in Banda Aceh city and the district of Aceh Besar. The isolates were identified using biochemical tests (indole, methyl red, Voges–Proskauer, sulfide indole motility, and citrate utilization) and polymerase chain reaction (PCR) to amplify the *rpo*B gene. The hypermucoviscous phenotype was determined by the string test. The presence of the capsule cluster genes *Mag*A, k2A, and *wzx*-K5 was used to characterize the capsular serotypes K1, K2, and K5, respectively.

Results: A total of 54 *K. Pneumoniae* isolates were obtained from 38 of 61 (62.3%) cattle. Twenty-two isolates (40.7%) were positive for the K1 genes, 16 isolates (29.6%) were positive for the K2 genes, and 16 isolates (29.6%) were negative for K1, K2, and K5 genes and classified into non-K1/K2/K5. The *rmp*A gene was detected in 62.5% and 63.6% of K2 and K1 isolates, respectively. *mrk*D and *ent* genes were detected in all isolates, and *ytbs* was detected in 72.7% and 75% of K1 and K2 isolates, respectively.

Conclusion: Numerous *K. pneumoniae* serotypes were involved in infecting Aceh cattle, and serotype K1 had the highest prevalence.

Keywords: Aceh cattle, Capsular serotype, *Klebsiella pneumoniae*, Pneumonic lung, Virulence genes.

Introduction

Klebsiella pneumoniae is an encapsulated Gram-negative bacterium that is widely distributed in the environment. *Klebsiella pneumoniae* is a common opportunistic pathogen that causes infections in humans and animals (Martin and Bachman, 2018; De Koster *et al.,* 2022). Typically, opportunistic *K. pneumoniae* mainly affects immunocompromised individuals or patients with pre-existing underlying diseases. *Klebsiella pneumoniae* is responsible for mastitis in cattle (Cheng *et al.,* 2018); urinary tract infections (UTI) in dogs and cats (Sartori *et al.,* 2019; Hayakawa Ito de Sousa *et al.,* 2021); and UTI, pneumonia, meningitis, and liver abscesses in humans (Jun, 2018; Choby *et al.,* 2020). *Klebsiella pneumoniae* possesses multiple virulence factors involved in the capability to initially colonize the mucosal surface. *Klebsiella pneumoniae* typically expresses capsular polysaccharides (CPS) and lipopolysaccharides (LPS), which are involved in mucosal colonization and the development of infections. Other *K. pneumoniae* virulence factors include capsules to avoid phagocytic killing, iron-scavenging systems (siderophores) for iron acquisition from the host, and fimbrial and non-fimbrial adhesions for host cell attachment (Paczosa and Mecsas, 2016; Riwu *et al.,* 2022).

There are two main pathotypes circulating worldwide: classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumoniae* (hvKp). hvKp is usually associated with a hypermucoviscous *K. pneumoniae* strain. The hvKp exhibits large amounts of mucopolysaccharide web and extraCPS to produce thick capsules (Walker and Miller, 2020). The emergent hvKp is associated with highly invasive infections and is largely resistant to the majority of antibiotics. *Klebsiella pneumoniae* capsular serotypes K1, K2, K4, and K5 have been reported to cause infection in humans and animals and have a high virulence rate in experimental infections in experimental

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mice (Sharma *et al.,* 2014; Jian-Li *et al.,* 2017; M'lan_ Britoh *et al.,* 2018; Xu *et al.,* 2022). These serotypes cause respiratory infections and septicemia more frequently than other serotypes (Jian-Li *et al.,* 2017).

On the other hand, *K. pneumoniae* causes pneumonia and mastitis with poor prognosis in cattle (Kot and Witeska, 2024). The invasion of hvKp in domestic animals poses a threat to livestock production and risk for public health, due to their potential role as reservoirs of multidrug-resistant *K. pneumoniae* strains (Cheng *et al.,* 2018; Effah *et al.,* 2020). Accurate pathogen detection and characterization are important for preventing controlling and managing infectious diseases in livestock, including those in Aceh cattle. However, studies related to this topic in local Indonesian cattle breeds, mainly Aceh cattle, have been poorly investigated. Aceh cattle are one of four native Indonesian cattle breeds (Aceh, Pesisir, Madura, and Bali cattle), as stipulated in the decision of the Indonesian Minister of Agriculture No: 2907/Kpts/OT/140/6/2011. The cattle have an original geographical distribution in Aceh Province and are the most widely cultivated by Aceh farmers. Therefore, this study aimed to explore the characteristics and prevalence of *K. pneumoniae* in Aceh cattle in Indonesia.

Materials and Methods

Sample collection

A total of 61 pneumonic lung tissues and tracheal swabs were collected from Aceh cattle. The samples were collected from slaughterhouses in Banda Aceh city and Aceh Besar districts, Indonesia. The tracheal swab was then cultured in a sterile Brain Heart Infusion Broth (BHIB) (Oxoid, UK) as an enrichment medium, while pneumonic lung tissue was crushed with a sterile pestle before culturing in BHIB for 24 hours at 37°C. Each sample was cultured on MacConkey agar (Oxoid, UK) and Blood agar media (Oxoid, UK) and incubated at 37°C for 24 hours.

Biochemical identification

Biochemical tests were performed to determine the phenotypic characteristics of the bacterial isolates. The principal tests used for this purpose are catalase, indole, urease, methyl-red, Voges–Proskauer (VP), citrate utilization, sulfide indole motility, hydrogen sulfide production on TSI agar, and carbohydrate fermentation test (glucose, lactose, sucrose, and mannitol).

PCR-based identification

Suspected colonies were identified by detecting the *rpoB* gene (F-CAACGGTGTGGTTACTGACG and R-TCTACGAAGTGGCCGTTTTC), which is 108 base pairs in size. We extracted nucleic acids from each colony using the boiling method according to Chen *et al.* (2011) and Li *et al.* (2022). The polymerase chain reaction (PCR) was carried out in a total volume of 25 µl consisting of template DNA (1 µl), 0.6µl of each primer (10 µM), 12.5 µl master mix MyTaq HS Mix Red (Bioline), and 10.3 µl dH_2O. Amplification was performed in a Bio-Rad thermal cycler (BioRad Laboratories, Inc., Hercules, CA, USA) using the following amplification conditions: initial denaturation at 95°C for 3 minutes; 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 90 seconds, extension at 72°C for 90 seconds; and a final extension at 72°C for 10 minutes. The PCR products were separated by 1.5% (wt/v) agarose gel electrophoresis for 30 minutes at 120 V and visualized using a UV transilluminator.

Hypermucoviscous virulent phenotype detection (string test)

A string test was used to identify the hypermucoviscous phenotype of *K. pneumoniae*. The hvKP phenotype strains were defined by the formation of a mucoid string >5 mm in length when bacterial colonies on the blood agar plate are stretched vertically by an inoculation loop (Chang and Ong 2022; Yan *et al.,* 2024).

Capsular serotype and gene virulence amplification

A multiplex PCR assay was designed to detect *Mag*A, k2A, and *wzx*-K5 genes for K1, K2, and K5 serotypes, respectively, and virulence factors (*rmp*A, *mrk*D, *ent*, and *ytbs*) gene, using specific primers (Table 1). The thermal profile for the multiplex PCR reaction was as follows: initial denaturation at 95°C for 3 minutes, followed by 30 cycles of denaturation at 95°C for 90 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes. The amplicons were separated at 120 V for 30 minutes in a 1.5% (wt/v) agarose gel containing ethidium bromide.

Ethical approval

The animal ethics committee of the Animal Care and Use Committee of Research and Community Service, Bogor Agricultural University, approved the present study, with ethical approval number: 144/KEH/SKE/VII/2019.

Results

Phenotypic characterization of K. pneumoniae

A total of 54 *K. pneumoniae* isolates were identified in this study, originating from pneumonic lung tissue and tracheal swabs of 38 Aceh cattle. Sixteen cattle were infected with different isolates in the lungs and trachea. Based on the phenotypic characteristics, *K. pneumoniae* was observed to be rounded, measuring 4–5 mm, and mucoid, producing pink discoloration on MacConkey agar (Fig. 1a), and whitish-gray colonies with γ-hemolysis on Blood agar (Fig. 1b). The cells were found to be Gram-negative bacilli or coccobacilli, non-spore forming, and surrounded by a thick capsule. The isolates were positive for catalase, urease production, citrate utilization, and VP. The isolates tested negative for methyl red, indole production, motility test, and H_2S production. The *rpo*B analysis confirmed the isolates as *K. pneumoniae* (Fig. 2).

Hypermucoviscosity and serotypes of K. pneumoniae isolates

A string test-positive *K. pneumoniae* was defined as a hypermucoviscous colony. In this study, 75.9% (41/54)

Table 1. Primer set to detect the virulence factor of *K. pneumoniae.*

Primer	DNA sequence (5'to 3')	Target gene	Size (bp)	References
*Mag*A_for	GGTGCTCTTTACATCATTGC	Serotype K1	1,283	Fang *et al.* (2004)
*Mag*A_rev	GCAATGGCCATTTGCGTTAG			
k2A_for	CAACCATGGTGGTCGATTAG	Serotype K2	531	Yu *et al.* (2007)
k2A_rev	TGGTAGCCATATCCCTTTGG			
Wzx-K5_ for	TGGTAGTGATGCTCGCGA	Serotype K5	280	Turton *et al.* (2008)
Wzx-K5_ rev	CCTGAACCCACCCCAATC			
*mrk*D_for	AAGCTATCGCTGTACTTCCGGCA	fimbriae type 3	340	Compain *et al.* (2014)
*mrk*D_rev	GGCGTTGGCGCTCAGATAGG			
*rmp*A_for	CATAAGAGTATTGGTTGACAG	Regulator mucoid phenotype A	461	Compain *et al.* (2014)
*rmp*A_rev	CTTGCATGAGCCATCTTTCA			
entB_for	GTCAACTGGGCCTTTGAGCCGTC	Enterobactin	400	Compain *et al.* (2014)
entB_rev	TATGGGCGTAAACGCCGGTGAT			
ybtS_for	GACGGAAACAGCACGGTAAA	Yersiniobactin	242	Compain *et al.* (2014)
ybtS_rev	GAGCATAATAAGGCGAAAGA			

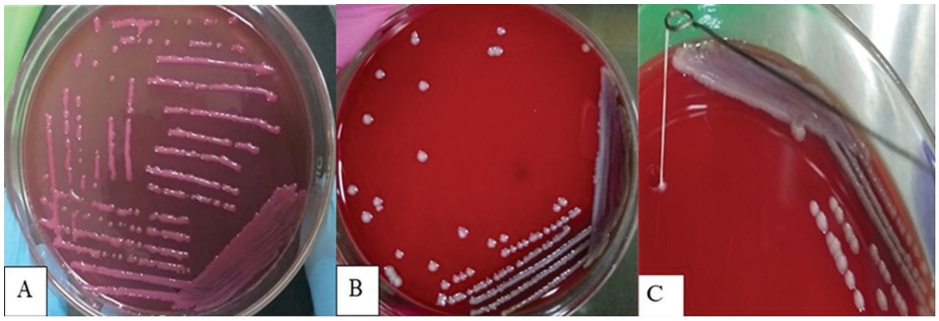

Fig. 1. Phenotypic characteristics of isolates grown on agar medium. Pink mucoid colonies on MacConkey agar (A), whitish-gray colonies on blood agar (B), and formation of viscous strings measuring >5 mm (C).

of the *K. pneumoniae* isolates were string test-positive. Hyperviscosity of *K. pneumoniae* was observed with string formation (>5 mm) on a single colony (Fig. 1c). Serotype characterization using PCR showed that 22 (40.7%) isolates carried the *Mag*A gene and were associated with serotype K1, 16 (29.6%) carried the k2A gene and were associated with serotype K2, and 16 (29.6%) lacked the *Mag*A, k2A, and K5 genes and were grouped as non-K1/K2/K5 (Fig. 3). Based on the string test and molecular serotyping, isolates K1 and K2 showed hypermucoid characteristics, and only three isolates of Non-K1/K2/K5 possessed similar characteristics.

Detection of virulence-associated genes in K. pneumoniae isolates

Virulence factors, such as *rmp*A, *mrk*D, *ent,* and *ytbs*, were analyzed using a multiplex PCR assay. Table 2 shows the products of the PCR assay. The results showed that the *rmp*A gene detected in K1 and

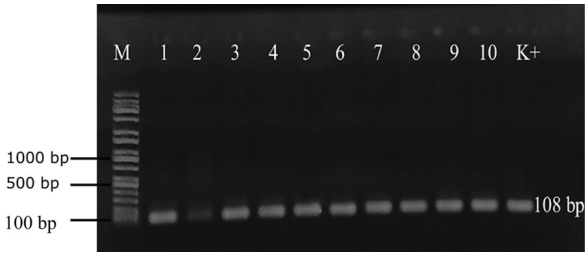

Fig. 2. PCR amplification of the *rpoB* gene for *Klebsiella pneumoniae* in gel electrophoresis. M: marker, Lane 1-10: *K. pneumoniae* isolates from pneumonic Aceh cattle.

K2 isolates was 63.6% (*n* =22) and 62.5% (*n* =16), respectively. The *mrk*D and *ent* genes were detected in all isolates, including Non-K1/K2/K5, while *ytbs* was detected in 72.7% (*n* =22) and 75% (*n* =16) of K1 and K2 isolates, respectively.

Table 2. Prevalence of capsular types and virulence factors of *K. pneumoniae* isolated from pneumonic Aceh cattle.

Serotype	Total isolate (%)	Phenotype (%)		Virulence factor detection (%)			
		Mucoid	Hypermucoid	*rmp*A	*mrk*D	*ent*	*ytbs*
K1	22 (40.7%)	0	100	63.6	100	100	72.7
K2	16 (29.6%)	0	100	62.5	100	100	75
Non-K1/K2/K5	16 (29.6%)	81.3	18,7	0	100	100	0

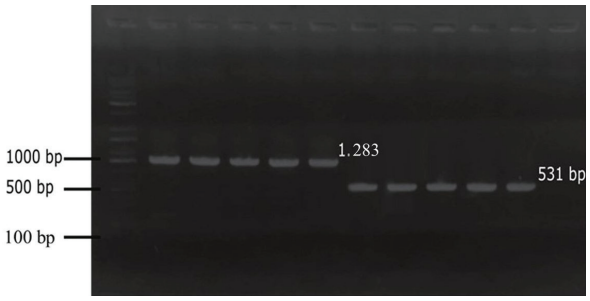

Fig. 3. Molecular typing of *K. Pneumoniae* capsular antigens. M: Marker. Lane 1–5: K1 Serotype (*Mag*A), Lane 6–10: K2 serotype (k2A).

Discussion

Klebsiella pneumoniae is an important opportunistic pathogen that causes a variety of infectious diseases in humans, animals, and plants, especially in immunocompromised hosts (Huang *et al.,* 2016; Liu *et al.,* 2023). The emergence of hypervirulent strains increases the occurrence of infection in healthy and immunocompetent hosts (Shon *et al.,* 2013; Zhuo *et al.,* 2025). The virulence factors of the pathogen and the decrease in mucosal immunity can increase the colonization rates of *K. pneumoniae* in the lungs (Teo *et al.,* 2024). In the present study, *K. pneumoniae* was isolated from the infected lungs of Aceh cattle exhibiting hyperemia, congestion, consolidation, and atelectasis in the interstitial region. *Klebsiella pneumoniae* as the causative agent in the pneumonic lungs of Aceh cattle has been confirmed through molecular and immunohistochemistry studies in bovine pneumonic lung tissues (Darniati *et al.,* 2021a).

The presence of the *rpo*B gene was confirmed in all 54 *K. pneumoniae* isolates. The *rpo*B gene encodes an RNA polymerase subunit that plays an important role in transcription and contains a conserved region that is effective for identifying bacteria and as a locus for phylogenetic analysis (Chander *et al.,* 2011). Amplification of the *rpo*B gene has been demonstrated to be more sensitive and discriminatory for detecting *K. pneumoniae* species than the 16S rRNA gene and can be used to identify organism levels at the species and subspecies (He *et al.,* 2016).

Although *K. pneumoniae* was a part of the normal flora in the upper respiratory system, the pathogen has been reported capable of infecting the bovine lower respiratory system (Darniati *et al.,* 2021b). However, the virulence factors, genetic, and phenotypic variations among *K. pneumoniae* isolated from pneumonic lungs of cattle in Indonesia have not been widely reported (Darsana *et al.,* 2015).

In this study, *K. pneumoniae* was isolated from 62.3% (38/61) individuals. Positive samples were obtained from cattle showing symptoms of depression (cachexia) with or without respiratory symptoms observed before the animal was slaughtered (antemortem observations). *Klebsiella pneumoniae* isolates showed mucoid, non-hemolytic, and whitish-gray colonies on blood agar and formed pink mucoid colonies (lactose fermenter) on MacConkey Agar media. The results of the microviscosity test with the string test showed that 41 (75.9%) bacterial isolates had hypermucoid characters, including K1 and K2 serotypes, and 3 isolates of non-K1/K2/K5. Capsule plays a dominant role in *K. pneumoniae* virulence and pathogenicity. Hypercapsule production provides a more robust defense against the host's immune system compared with non-encapsulated *K. pneumoniae* (Dai and Hu, 2022).

In general, *K. pneumoniae* is classified into two pathotypes, namely hypervirulent strains (hvKp) and classic strains (cKp) based on their clinical and phenotypic characteristics (Pomakova *et al.,* 2012). The classical *K. pneumoniae* exhibits high resistance to a wide range of antibiotics and causes severe pneumonia, especially in patients with chronic alcoholism, chronic obstructive pulmonary disease, and diabetes (Paczosa and Mecsas, 2016). Hypervirulent *K. pneumoniae* was first described in Taiwan in the mid-1980s and 1990s for causing pyogenic liver abscesses (Liu *et al.,* 1986; Wang *et al.,* 1998). This new variant of *Klebsiella* can cause serious infections in healthy individuals and infect various organs, such as the lungs, pleura, urinary tract, abdominal cavity, blood, skin, eyes, and central nervous system (Chen *et al.,* 2010; Pomakova *et al.,* 2012; Shon *et al.,* 2013; Prokesch *et al.,* 2016; Martin and Bachman, 2018).

Capsular characterization of *K. pneumoniae* isolates from the respiratory tract of Aceh cattle showed that bacteria with hypermucoid characteristics had *Mag*A or k2a genes. The *Mag*A gene was detected in 22 (40.7%)

isolates, and k2A was identified in 16 (29.6%%) isolates. The wzx-K5 gene specific to the K5 serotype was not detected in the respiratory tract of Aceh cattle. *Klebsiella pneumoniae* serotype K1 was detected by PCR with the target gene *Mag*A located in the operon specific for serotype K1 and yielded a fragment measuring 1.283 bp, matching the size obtained by Turton *et al.* (2008). The K2 serotype was detected by targeting the k2A gene and yielded a fragment size of 543 bp, matching the size obtained by Yu *et al.* (2008). Non-K1/K2/K5 isolates generally had lower viscosity than K1 and K2 isolates, although 3 isolates showed a string length of ± 5 mm in this study. Although capsular serotypes K1 and K2 are often associated with hvKp, cKp can also have a hypermucoid character (Shon *et al.,* 2013). Evolutionary mechanisms contribute to the emergence of identical K-types, even in the absence of phylogenetic relationships between the serotypes (Brisse *et al.,* 2009).

In this study, the K1 serotype was more predominant than the K2 and non-K1/K2/K5. Although serotype K1 is considered the most virulent strain of *K. pneumoniae*, capsular serotypes K1 and K2 are associated with hvKp, and major capsular serotypes are involved in liver abscess and metastatic septic complications (De Francesco *et al.,* 2023). In the current study, the prevalence of capsular serotypes K1 and K2 was approximately 70.4%. This is higher than the study by Mario *et al.* (2023), which identified a prevalence of hvKp at 24.2% in Giza Governorate, Egypt (Mario *et al.,* 2023). Previous studies have reported that K1 and K2 strains have higher virulence than other serotypes (Wang *et al.,* 2021). The presence of thick polysaccharide capsules at the cell surface of K1 and K2 serotypes directly or indirectly contributes to anti-phagocytic activity (Lee *et al.,* 2014; Opal, 2014; Al Ismail *et al.,* 2025). Capsules could protect *K. pneumoniae* by inhibiting host immune cell phagocytosis, preventing early immune response activation, and hampering lysis by complement and antimicrobial peptides. Capsule confers resistance to opsonophagocytosis in *K. pneumoniae* and contributes to biofilm formation (Dai and Hu, 2022). Macrophage lectin receptors initiate phagocytosis through the recognition of mannose or rhamnose sugar patterns on the microbial capsule (Xu *et al.,* 2024). On the other hand, K1 and K2 capsule types lack these repeated sugars, hence facilitating the evasion from lectinophagocytosis and macrophage killing (Athamna *et al.,* 1991; Kabha *et al.,* 1995, Pan *et al.,* 2015). Moreover, K1 isolates generally express the *fba* gene in high glucose concentrations, causing inhibition of neutrophil-mediated phagocytosis and intracellular killing (Lee *et al.,* 2020). Sialic acid (Sia) is a component of CPS in the hvKp phenotype that is directly or indirectly responsible for anti-phagocytic activity (Lee *et al.,* 2014; Opal, 2014).

Virulence factors of *K. pneumoniae* play a critical role in determining its prevalence and pathogenicity within the host. Several virulence factors, including capsules, LPS, siderophores, and fimbriae, have been identified in *K. pneumoniae*. These factors play an important role in the existence of pathogens in the environment and host (Paczosa and Mecsas, 2016). In this study, the virulence factor of capsular serotype K1, K2, and non-K1/K2/K5 characterization was performed to detect the presence of regulator of mucoid phenotype A (*rmp*A), type 3 fimbrial adhesin gene (*mrk*D), enterobactin (*ent*), and yersiniabactin (*ytbs*). Molecularly, there are two genes in *K. pneumoniae* that are associated with invasive infections, namely mucoviscosity-associated gene A (*Mag*A) and mucoid regulator phenotype A (*rmp*A) (Lin *et al.,* 2020). The *rmp*A gene is a plasmid-carried gene and plays a role in capsular polysaccharide synthesis and enhances the hypermucoviscus phenotype (Compain *et al.,* 2014). In this study, the *rmp*A gene was detected in 62.5% and 63.6% of the K2 and K1 serotypes, respectively.

The pathogenicity of *K. pneumoniae* is determined by the ability of the pathogen to secrete multiple types of siderophores to scavenge ferric iron from the environment. Hypervirulent *K. pneumoniae* strains express four siderophores: enterobactin, yersiniabactin, salmochelin, and aerobactin (Miethke and Marahiel, 2007; Bachman *et al.,* 2012). Enterobactin (*ent*) has the highest affinity for iron of any molecule and is carried by almost all cKp and hvKp strains (Lawlor *et al.,* 2007). In this study, enterobactin was detected in all isolates of *K. pneumoniae*. In addition, yersinibactin was detected in approximately 72.7% and 75% of K1 and K2 serotypes, respectively. Yersiniabactin is an important virulence factor in cKp and promotes respiratory tract infections (Lawlor *et al.,* 2007; Miethke and Marahiel, 2007). Yersiniabactin was detected in approximately 18% of the classical isolates and 90% of the hypervirulent isolates (Bachman *et al.,* 2011). Lipocalin-2 cannot neutralize yersiniabactin in the lungs, thus increasing the risk of infection in the lungs. However, yersiniabactin is unable to access the iron required for *K. pneumoniae* growth in the presence of the host protein transferrin in the blood. Thus, isolates that only produce yersiniabactin will not be capable of disseminating to the blood (Bachman *et al.,* 2011).

Fimbrial adhesins enable *K. pneumoniae* to adhere to host cells, facilitating a critical step in the initiation of host invasion. Type 1 and type 3 fimbriae mediate adhesion factors in *K. pneumoniae*. Type 1 and 3 fimbriae are detected in almost all *K. pneumoniae* strains to mediate cell attachment and promote biofilm formation. Biofilms are useful for evading host defense mechanisms, antimicrobials, and disinfectants (Johnson, 2011). The *mrk*ABCD gene cluster encodes type 3 fimbriae (Tarkkanen *et al.* 1998). The *mrk*A subunit is the largest structure of this gene, with the adhesion factor *mrk*D at the end of *mrk*A. Type 3 fimbriae mediate Klebsiella adhesions to tracheal cells,

tongue cells, and lungs via *mrk*D (Hornick *et al.,* 1992; Sonbol *et al.,* 2013). *Mrk*D gene can be detected in all K1, K2, and non-K1/K2/K5 isolates.

Although K1 and K2 are considered the most virulent serotypes, clinical and epidemiological studies have shown that some non-K1/K2/K5 serotypes can also cause various infections in humans and exhibit high mortality rates (Qu *et al.,* 2015; Yao *et al.,* 2015; Yu *et al.,* 2015). Therefore, the presence of these serotypes can still pose a risk to animal and human health. According to Paczosa and Mecsas (2016), cKp infection generally occurs in hosts who are immunosuppressed or have immune response disorders. Decreased immune response in the host can be caused by heat stress, injury, fatigue, dehydration, and nutritional deficiencies in the process of weaning and livestock transportation (McGill and Sacco, 2020). Extensive smallholder farming conditions, hot environment/climate, and limited nutrition have the potential to cause animals to experience prolonged stress or depression, which triggers a decrease in immune response, making them more easily infected with various diseases, including *K. pneumoniae*. This statement supports the results of studies showing a fairly high prevalence of *K. pneumoniae* in the respiratory tract of extensively reared Aceh cattle. In conclusion, *K. pneumoniae* is among the pathogens potentially causing lower respiratory infection in Aceh cattle. The proportion of Hypermucoviscous strain isolates among *K. pneumoniae*-positive cultures is higher than that of low-mucoid strains, indicating a high prevalence of hvKP. Identified hvKp underscores the need for closer surveillance of livestock populations. Effective surveillance requires comprehensive data on the genetic diversity, antimicrobial resistance mechanisms, and transmission dynamics of these isolates. Molecular studies comparing isolates from animals and humans in the same region are essential for assessing zoonotic potential. In addition to controlling the spread of isolated *K. pneumoniae*, it is strongly recommended to improve biosecurity at the farm level, implement targeted vaccination strategies, and enforce responsible antibiotic use policies.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

DN, SS; Methodology, validation, and analysis. DN, EH; Data curation and writing-original draft preparation. DN, SS, EH; Executed the analysis, interpreted the data, and revised the manuscript.

Data availability

All data supporting the findings of this study are available within the manuscript.

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