Research Article

Detection of ESBL Encoding Gene and the Virulence Factors of *Klebsiella pneumoniae* from Dairy Cattle Farms in South Sulawesi

Nurhashunatil Mar'ah[1], Safika Safika[2]*, Agustin Indrawati[2]

[1]Postgraduate Student of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia; [2]Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia.

Abstract | One of the multi-resistance mechanisms commonly found in dairy farms is ESBL (Extended Spectrum Beta Lactamase). This study aimed to investigate the antibiotic resistance and virulence factors of *Klebsiella pneumoniae* from Dairy Farm isolates in South Sulawesi, Indonesia. Twelve isolates obtained in the field were tested for phenotype resistance profile with four antibiotics of β-lactams using the Kirby-Bauer method. *The Polymerase Chain Reaction* (PCR) was used to detect the resistance genes and virulence factors of *K. pneumoniae*. Samples isolated from water sources showed a resistance profile to cefotaxime (100%), ceftazidime, ampicillin, and amoxicillin (50%). Samples isolated from udder rinses water were resistant to amoxicillin, cefotaxime, ampicillin (100%), and ceftazidime (60%). Samples obtained from milker hand swabs results were resistant to amoxicillin, cefotaxime, and ampicillin (100%). The cattle udder swab samples showed resistance to ampicillin and amoxicillin (100%), cefotaxime (75%), and ceftazidime (50%). Gene resistance detection found that water samples encoded bla_{TEM} gene (100%), Udder rinses water encoded bla_{TEM} (80%), bla_{SHV} (20%), bla_{CTX-M} (20%) and udder swab samples detected the presence of bla_{TEM} genes (40%). *K. pneumoniae* virulence factor genes: *mrkD* detected in all isolates (100%), *entb* and *wabG* were found in all water sources, milker hand, and udder swabs samples (100%) except samples from udder rinses water that encoded gene virulence factors 60% and 80%, respectively. All samples were negative for *rmpA* and *magA* genes. The study results showed *K. pneumoniae* of Dairy farms indicated the presence of ESBL resistance and virulence factors genes with different frequencies.

Keywords | β-lactam, Dairy, ESBL, *K. pneumoniae*, Virulence factors, Resistance

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***Correspondence** | Safika Safika, Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, 16680, Indonesia; **Email:** safika@apps.ipb.ac.id
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INTRODUCTION

An increase in domestic consumption of milk and its products may improve the national economy and face international trade competition (Jahroh *et al*., 2020). The growth of the middle and upper class as potential consumers is considered to have played a role in increasing the consumption of milk by the public (Darmawan, 2013). Nevertheless, until recently Indonesia's dairy industry has been dominated by smallholder farmers (Guntoro *et al*., 2016). One of the obstacles related to the quality of products produced by community-scale dairy farms is hygiene which includes increased incidence of mastitis, the lack of availability of clean water, and contamination of milk (Diwyanto and Iskandar, 1999). Environmental bacteria such as *E. coli, E. feundeii*, *E. aerugenes,* and *K. pneumoniae* can lead to a decrease in the quality of dairy products and exacerbate the infection (Zalizar *et al*., 2018).

K. pneumoniae is an opportunistic pathogenic bacteria from

the *Enterobacteriaceae* family, Gram-negative, short rod-shaped, and has a capsule (Chang *et al*., 2021). This bacteria is responsible for infection reports in hospitals which are associated with compromised immune systems, exacerbate local infections, and agents of foodborne illness (Russo and Marr, 2019; Richardson *et al*., 2022). Virulence factors are naturally encoded in bacteria and have the ability to cause clinical symptoms which help the process of bacterial attachment, invasion, colonization, and the formation of biofilms (Chilupuri *et al*., 2021). The existence of virulence factors is exacerbated by an increase in the ability of bacterial resistance. *K. pneumoniae* as a health-threatening bacteria has ability to acquire Mobile Genetic Element (MGE) which encodes antibiotic resistance genes.

Enterobacteriaceae bacteria, particularly *K. pneumoniae* and *E. coli* are environmental bacteria that encode ESBL gene. ESBL-producing bacteria can hydrolyze β-lactam rings and make antibiotic works ineffectively (Naelasari *et al*., 2018). In general, ESBL-producing bacteria are encoded by 3 main genes: *Cefotaxime-Munich* (*CTX-M*), *Sulfhydryl variable* (*SHV*), and *Temoneira Enzyme* (*TEM*). ESK(C)APE is an acronym for several names of Gram-negative and Gram-positive bacteria (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) that are capable of developing Multidrug-Resistant which recorded an increase rapidly in the last decade and can increase the potential for bacterial infection and reduces the choice of treatment therapy (Santajit and Indrawattana, 2016; WHO, 2021).

Livestock acts as a potential reservoir in the environment from humans and animals (Ibebkwe*et al*., 2023). The research conducted by Podschun *et al* (2001) shows that the strains in the field are as virulent as clinical isolates. The existence of antibiotics resistance genes and virulence factors that are encoded naturally by *K. pneumoniae* have ability to develop resistance and makes these bacteria more difficult to treat. Research over the decade was extensively done with *K. pneumoniae* as ESBL-producing bacteria isolated from human and clinical isolates. However, research related to antibiotic resistance in the environment and dairy farms especially in β-lactam antibiotics as an important factor, is still lacking. This study aims to identify *K. pneumoniae* bacteria that show resistance to several β-lactam antibiotics and virulence factors that support bacteria to attack the host potentially. This study reports the *K. pneumoniae* ESBL resistance profile and virulence factors gene of *K. pneumoniae* isolated from dairy farms in South Sulawesi, Indonesia.

MATERIALS AND METHODS

Study area

This research was located at a total of five dairy farms that are located close to each other in Enrekang Regency South Sulawesi Province. These 5 farms are still traditional community-scale farms with a total population of no more than 10 dairy cows in each farm. The research was conducted time from December 2022 to August 2023.

Sample collection

The samples from this research were collected in the morning to minimize bacterial contamination before milking with a description of the total sample as follows: udder swabs from cattle (n=11) from 5 farms, samples from the environment in the form of water sources (n=5) and udder rinse water (n=11) and skin swab from dairy farmers (n=5). All of the water samples were taken using a bottle with a volume of 250 ml while the swab sample was taken using a transport swab (Amies). Swab samples on the milker's hand are taken from the entire surface of the hand and between the fingers and for each udder swab sample is taken from 4 quarters of the udder. The samples were sealed using wrap and taken using the *coolbox* at 4°C for further bacteria culture and analysis at the Research Laboratory, Division of Medical Microbiology, School of Veterinary Medicine and Biomedicine Sciences, IPB University.

Biochemical analysis and molecular confirmation of *K. pneumoniae* bacteria

Samples were isolated with *Macconkey Agar (MAC)* (Oxoid, UK) as differential selective media and for 24 hours were incubated at 37°C. Macroscopically, *K. pneumoniae* bacteria colonies are characterized by their ability to ferment glucose and mucoid (Safika *et al*., 2022). The bacteria were then cultivated using *Tryptic Soy Agar* (TSA) media (Oxoid, UK). Cultured bacteria from TSA media continued for iMVIC test (*simmon citrate, methyl red, indole,* and *voges-proskauer*). Confirmation of suspected bacteria (*K. pneumoniae*) was done molecularly by finding the presence of *rpoB* gene. Forward primer: AACCAGTTCCGCGTTGGCCTGG and Reverse primer: CCTGAACAACACGCTCGGA (Almeida *et al*., 2023).

Antibiotic sensitivity test (AST)

The Kirby-Bauer method was used as phenotype confirmation of antibiotic sensitivity test in this study. The suspension was homogenized and the result of the turbidity was adjusted to *McFarland* standard solution (0.5 or 1, 5 x 10^8 CFU/ml). The suspension was spread into Mueller Hinton Agar (MHA) using glass spreader and placing paper discs containing antibiotics with certain concentrations (Oxoid, UK) to incubated at 37°C for 18 hours. Ampicillin (10µg/disk), amoxicillin (10µg/disk), cefotaxime (30µg/disk), and ceftazidime (10µg/disk). After incubation, the clear zone that formed was then measured by vernier calipers and adjusted according to the CLSI Standard (2021).

Table 1: Genes used in research.

Gene Target	Nucleotide sequence (5'-3')	Amplicon (bp)	Annealing (°C)	Reference
rpoB	F:AACCAGTTCCGCGTTGGCCTGG R: CCTGAACAACACGCTCGGA	1090	54	Almeida *et al.* (2023)
blaSHV	F: CCTGTTAGCCACCCTGCC R: CCGCAGATAAATCACCAC	768	52	Momtaz *et al.* (2012)
blaTEM	F: ATCAGCAATAAACCAGC R: CCCCGAAGAACGTTTTC	516	54	Colom *et al.* (2003)
blaCTX-M	F: ATGATGAAAAAATCGTTATGC R: CAGCATCTCCCAGCCTAAT	551	57	Lyimo *et al.* (2016)
magA	F: GGTGCTCTTTACATCATTGC R: GCAATGGCCATTTGCGTTAG	1.283	59	El-Fertas Aissani *et al.* (2016)
rmpA	F: ACTGGGCTACCTCTGCTTCA R: CTTGCATGAGCCATCTTTCA	535	50	
mrkD	F: CCACCAACTATTCCCTCGAA R: ATGGAACCCACATCGACATT	240	54	
entB	F: ATTTCCTCAACTTCTGGGGC R: AGCATCGGTGGCGGTGGTCA	371	57	
wabG	F: CGGACTGGCAGATCCATATC R: ACCATCGGCCATTTGATAGA	683	54	Brisse *et al.* (2009)

DNA EXTRACTION

Extraction of bacterial DNA was done by using the boiling method (Junior *et al.*, 2016) with several modifications. Colonies bacteria that incubated were cultured to the Tryptic Soy Broth (TSB) media and continued to be centrifuged with 25,000 rpm for 30 minutes. Pellets from the centrifuged process were added to nuclease-free water with a total volume of 100 µl and placed in the water bath for 6 minutes; at 95-100°C and vortexed. The suspension was centrifuged at 12,000 rpm for 7 minutes. Supernatant was taken as master stock DNA.

DETECTION OF VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE GENES OF *K. PNEUMONIAE*

Detection of resistance β-lactams genes in this study using PCR focused on detecting the bla_{TEM}, $bla_{CTX-M,}$ and bla_{SHV} as the main genes of ESBL. The reaction volume was made up of 12 µl consisting of 6 µl MyTaqTM HS RedMix (Bioline), 1 µl for each primer consisting of Forward and Reverse (Genetika Science), 2 µl dH2O (DNase, RNAse free) (Invitrogen™, USA), and made up to 12 µl using 2 µl as DNA template. The Reaction of PCR was done in a Thermal Cycler T100TM (Bio-Rad, California, USA) and visualized on 1% agarose. The electrophoresis was performed for 35 minutes at 60 volts using 5 µl samples and 1 µl FloroSafe DNA Stain (1st base). All of the primers used in this study are listed in Table 1.

Amplification resistance gene using protocol as follows: $bla_{TEM:}$ 95°C for 1 minute, followed by 30 cycles that consisting of 95°C for 15 seconds, 54°C for 15 seconds, 72°C for 1 minute and final elongation 72°C for 10 minutes; $bla_{CTX-M:}$ 95°C for 5 minutes, followed by 30 cycles, consisting of 95°C for 30 seconds, 57°C for 1 minute, 72°C for 1 minute, and final elongation 72°C for 5 minutes; bla_{SHV}: 95°C for 5 minutes, followed by 30 cycles that consisting of 95°C for 30 seconds, 52°C for 1 minute seconds, 72°C for 45 seconds, and final elongation 72°C for 5 minutes.

Amplification virulence factors under the condition as follows: *rmpA, magA, mrkD,* and *entB*: denaturations start at 94°C for 4 minutes, followed by 30 cycles that consisting of 94°C for 30 seconds, annealings a range of 54-59°C, 72°C for 1 minute, and final elongation at 72°C for 10 minutes; *wabG* gene detection condition: denaturation at 94°C for 5 minutes followed by 35 cycles that consisting of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 1 minute and final elongation for 1 minute.

DATA ANALYSIS

The study result of phenotype confirmation by *kirby-bauer* method, virulence factors, and resistance genes of *K. pneumoniae* are shown with tables and analyzed descriptively.

RESULT AND DISCUSSION

BIOCHEMICAL AND MOLECULAR CONFIRMATION RESULT

Twelve out of a total of thirty-two isolates obtained at the Dairy farm, Enrekang Regency, South Sulawesi were positive for *K. pneumoniae* bacteria. The colonies of *klebsiella* sp are shown in Figure 1. The results of biochemical confirmation are shown in Table 2. This accordance with researches by Effendi *et al.* (2018),

Table 2: Biochemical test interpretation of suspect *Klebsiella* sp.

Test result	Methyl red	Indole	Simmon's citrate	Voges proskauer	TSIA
Suspect klebsiella sp.	-	-	+	+	A/A, (+) Gas, (-) H2S

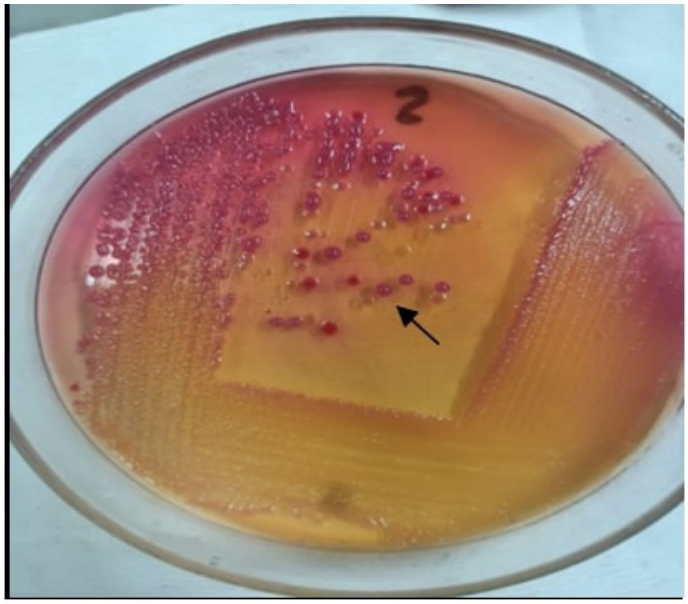

Figure 1: *K. pneumoniae* bacteria shown mucoid, pink and convex colonies in MAC media. Arrow: single colonies of *K. pneumoniae*.

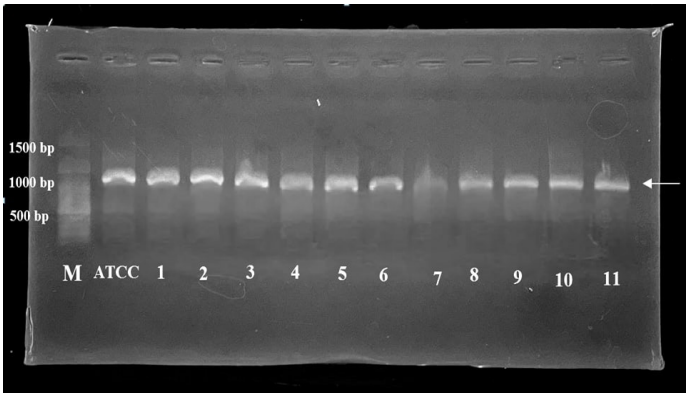

Figure 2: Electrophoresis results on molecular confirmation of *K. pneumoniae* (1090 bp). M: Marker; ATCC: positive control; 1-11: positive isolates of *K. pneumoniae*.

Dita *et al.* (2019); Salaudin *et al.* (2019), Permatasari *et al.* (2020). Bacteria that were suspected as *K. pneumoniae* biochemically continue to be confirmed by detecting the *rpoB* gene which is known to encode RNA polymerase β-subunit (Lin *et al.*, 2023). Relatively, detection using *rpoB* is a more appropriate way than using the 16rRNA gene. Some researchers suggest that *K. pneumoniae* detection is effective by using the *rpoB* gene (Urbaniak *et al.*, 2018; Michodigni *et al.*, 2021). The amplicon of *rpoB* gene was interpreted in Figure 2. From this results, it was found that the dominant isolate of *K. pneumoniae* was from water sources on farms. This was based on *K. pneumoniae* bacteria which are ubiquitous in aquatic environments such drinking water (Aromolaran and Amodu, 2021; Hasan and Aburesha, 2021), Lakes (Bartley *et al.*, 2019), wastewater (Rawy *et al.*, 2020), Rivers (Hasan and Aburesha, 2021; Henriot *et al.*, 2019) and Seawater (Podschun *et al.*, 2001).

Table 3: β-lactam antibiotic resistance profile of *K. pneumoniae* isolated in dairy farm.

No	Samples code	β-lactam antibiotics			
		AMX	AMP	CTX	CAZ
Water sources					
1	4.2	S	I	R	S
2	10.2	R	R	R	R
n = 2		1	1	2	1
		50%	50%	100%	50%
Udder rinse water					
1	2.3	R	R	R	R
2	6.1	R	R	R	S
3	14.2	R	R	R	I
4	15.1	R	R	R	R
5	16.3	R	R	R	R
n = 5		5	5	5	3
		100%	100%	100%	60%
Milker hand swab					
1	J.2	R	R	R	S
n = 1		100%	100%	100%	0
Udder swabs					
1	B.3	R	R	R	S
2	E.2	R	R	I	S
3	H.1	R	R	R	R
4	O.3	R	R	R	R
n = 4		100%	100%	75%	50%

S: Susceptible; I: Intermediete; R: Resistant

Antibiotic resistance

Twelve isolates were confirmed as *K. pneumoniae* by PCR and then tested to show the profile resistance with the four β-lactam antibiotics given. Phenotypic confirmation of ESBL-producing bacteria by the *Kirby-Bauer* Disc method showed a fairly high resistance status. The clear zone of inhibition that formed was adjusted per the CLSI Standard (2021) to determine the antibiotic sensitivity. The phenotype confirmation of β-lactam resistance results are shown in Table 3. Samples from water sources showed that *K. pneumoniae* were resistant to ceftazidime, ampicillin, amoxicillin, (50%), and cefotaxime (100%). The udder rinses water signified resistance condition to cefotaxime, ampicillin, amoxicillin (100%), and ceftazidime

(60%). Milker hand swab samples indicated resistance to cefotaxime, ampicillin, and amoxicillin (100%). The cattle udder swab samples represented resistance to amoxicillin, ampicillin (100%), cefotaxime (75%), and ceftazidime (50%). In general, the resistance of β-lactam antibiotics in isolates shows a percentage above 50%. Reports of β-lactam resistant bacteria have been a concern this decade. The presence of high-resistance bacteria was shown in research by Jelic *et al.* (2019) found *K. pneumoniae* isolated from water samples were resistant to β-lactam, specifically to penicillin and all cephalosporin generations. Geographically, the 5 farms that are used as sampling sites are located in adjacent areas that may allow the spread of resistance genes from one source to another place. Water is one of the reservoirs that have the ability to spread resistance genes from the livestock to the environment which live ubiquitously in aquatic environments (Aromolaran and Amodu, 2021).

In general, the process of β-lactam binding to the specific site results in blocking the activity of transpeptidase enzyme. Veterinary medicine reports widely used β-lactam antibiotics because of their high specificity, lower toxicity, and generally good bactericidal effect (Seiffert *et al.*, 2013). The use of antibiotic therapy for dairy cows on farms was dominated by β-lactams belonging to the cephalosporin group (USDA, 2017; Dong *et al.*, 2020; FDA, 2022) for clinical mastitis and respiratory infections caused by *Klebsiella* spp. use the ampicillin, tetracycline, and oxytetracycline (FDA, 2022). In addition, A report by Schrag *et al.* (2020) showed the use of cephalosporin and penicillin in dairy farm have a higher frequency than other antibiotics for mastitis treatment.

Enterobacteriaceae bacteria has a natural ability to hydrolyze β-lactam ring from several antibiotics by producing Extended Spectrum Beta Lactamase (ESBL) enzyme (CLSI, 2021). This enzyme is coded chromosomally and plasmid. In general, resistance in *K. pneumoniae* involves inactivation and enzymatic modification by bacteria, changes in antibiotic targets, porin mutations, increased efflux pump expression, and formation of biofilm (Mulani *et al.*, 2019). Some cases of *K. pneumoniae* as ESBL bacteria have been found in hospitals (Martin *et al.*, 2018), zoos (Seguel *et al.*, 2017), animal products (Klaper *et al.*, 2021), pets (Ochoa *et al.*, 2022), and dairy farms (Soekoyo *et al.*, 2020; Gelalcha and Dego, 2022). The natural ability of *K. pneumoniae* to raise resistance combined with the ubiquitous habitat such as soil, vegetation, and water makes these bacteria have a higher potential for public health risks.

Antibiotic resistance gene

Confirmation of antibiotic resistance gene using bla_{TEM}, bla_{SHV}, and bla_{CTX-M}. This study found that the frequency of gene presence from water sources, udder rinses water, and udder swabs were as follows: bla_{TEM} (100%; 80%, 50%), bla_{SHV} (0; 40%; 25%), bla_{CTX-M} (0; 20%; 0) and the milker hand swab samples did not show any resistance gene from this study (Figures 3, 4, 5, Table 4). The bla_{TEM} gene has the highest frequency compared to all the genes detected in the isolates. The bla_{TEM} gene is encoded by a plasmid, responsible for resistance in the new generation of antibiotics, and was the main cause of pathogenesis of clinical isolates such as Urinary Tract Infection and resistance to antibiotics from *K. pneumoniae* (Sarshar *et al.*, 2021). Previously, studies reported high frequencies in food-producing and clinical animal samples (90% and 100%, respectively) (Effendi *et al.*, 2018; Arafa *et al.*, 2022).

Figure 3: Detection of amplicon *blaTEM* gene (516 bp). M: 100 bp DNA Marker. Isolates 1-5,9,10,12 were positives for *blaTEM* gene.

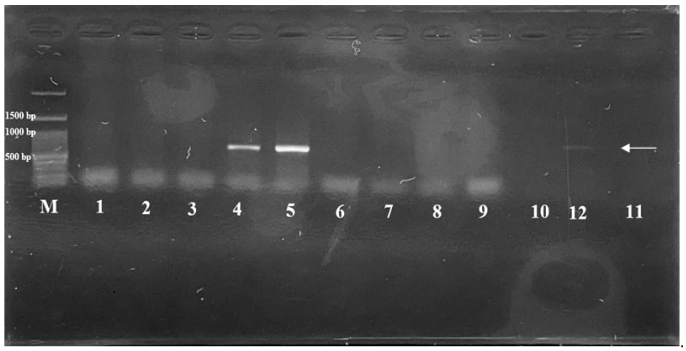

Figure 4: Detection of amplicon *blaSHV* gene (768 bp). M: 100 bp DNA Marker. Isolates 4,5, and 12 were positives for *blaSHV* genes.

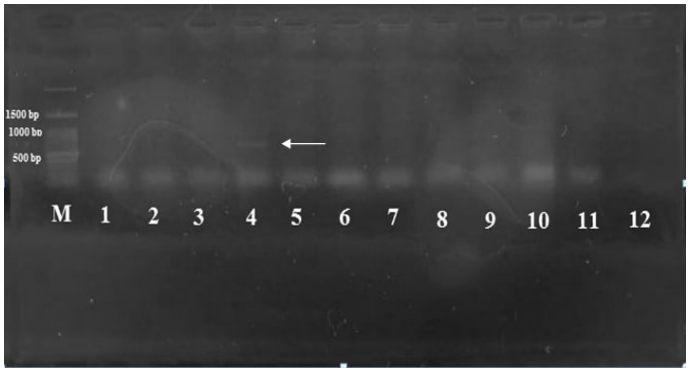

Figure 5: Detection of amplicon *blaCTX-M* gene (551 bp). M: 100 bp DNA Marker. Isolate 4 were positive for *blaCTX-M* gene.

Table 4: Profile of *rpoB*, genes encoding ESBL and virulence factors of *K. Pneumoniae.*

No	Samples code	*rpoB*	ESBL Coding gene			Virulence factors of *K. pneumoniae*				
			blaTEM	*blaSHV*	*blaCTX-M*	*rmpA*	*magA*	*mrkD*	*entB*	*wabG*
Water sources										
1	**4.2**	+	+	-	-	-	-	+	+	+
2	10.2	+	+	-	-	-	-	+	+	+
n = 2		2/2 100%	2/2 100%	0 0	0 0	0 0	0 0	2/2 100%	2/2 100%	2/2 100%
Udder rinses water										
1	2.3	+	+	-	-	-	-	+	-	-
2	6.1	+	+	+	-	-	-	+	-	+
3	14.2	+	-	-	-	-	-	+	+	+
4	15.1	+	+	-	-	-	-	+	+	+
5	16.3	+	+	+	+	-	-	+	+	+
n = 5		5/5 100%	4/5 80%	2/5 40%	1/5 20%	0 0	0 0	5/5 100%	3/5 60%	4/5 80%
Milker hand swab										
1	J.2	+	-	-	-	-	-	+	+	+
n = 1 Udder swabs		1/1 100%	0 0	0 0	0 0	0 0	0 0	1/1 100%	1/1 100%	1/1 100%
1	B.3	+	-	-	-	-	-	+	+	+
2	E.2	+	+	-	-	-	-	+	+	+
3	H.1	+	+	+	-	-	-	+	+	+
4	O.3	+	-	-	-	-	-	+	+	+
n = 4		4/4 100%	2/4 50%	1/4 25%	0 0	0 0	0 0	4/4 100%	4/4 100%	4/4 100%

(+): Positive result for the gene which being tested; (-): Negative result.

Another gene of resistance that was also found in this study is bla_{SHV} gene. Three isolates that encoded the bla_{SHV} gene and were expressed in udder rinsed water and udder swabs (40% and 25%, respectively). In addition to the water, the udder of dairy cattle can allow the transmission of bacteria that carry resistant genes from the environment to humans (Hoque *et al*., 2020). The cattle shed floor is one of the common contamination from coliform bacteria in the environment (Hamel *et al*., 2021). The bla_{SHV} gene is responsible for resistance to the antibiotics ceftazidime, ampicillin, and penicillin (Russo and Marr, 2019).

Previous studies show that the *CTX-M* gene was found in clinical isolates of pneumonia patients (Kakuta *et al*., 2020), livestock, and slaughterhouses in Asia (Kock *et al*., 2018) and is responsible for the resistance of the cephalosporin antibiotic group (Hasibuan *et al*., 2018). Over time, *K. pneumoniae* that produces bla_{CTX-M} increased by 1.7% during 2005-2009 to 26.4% during 2010-2012. This is inconsistent with the results of a study that found that there is just one isolate (20%) encoded the bla_{CTX-M} gene from all of the samples. The frequency of this gene is lower than the other two ESBL coding genes in *K. pneumoniae*. Other supporting research is from Wang *et al*. (2013) which discovered only 2 isolates (1.7%) of 121 ESBL bacteria encode the bla_{CTX-M} gene found in clinical samples of *K. pneumoniae.*

Research conducted by Imasari *et al*. (2017) found there was a transmission of transfer of ESBL bacteria in the dairy farming environment and cattle breeders by 79.1% through direct or indirect contact. A fairly high percentage indicate the potential for cross-transfer from the environment to livestock. *K. pneumoniae* is capable of obtaining the Mobile Genetic Elements (MGE) as plasmids that contain antibiotic resistance genes. The undetected genes in this study indicates that several possibilities affect the expression gene such as other resistance genes that were not tested in the study, mutations in the genes, and low in vitro expression (Urmi *et al*., 2020).

Virulence factors gene

Five genes of virulence factor were tested in this study: *rmpA, magA, mrkD, entB,* and *wabG*. The study result shows the prevalence of *K. pneumoniae* isolates encoding the *mrkD* gene was 100% in all types of samples. The gene

of *wabG* and *entB* gene detection for water sources, milker hand swabs, and udder swabs had a percentage of 100% each while the same gene in udder rinses water samples showed percentages of 60% and 80%, respectively. All the isolates show negative results of *rmpA* and *magA* detection (Figures 6, 7, 8, Table 4).

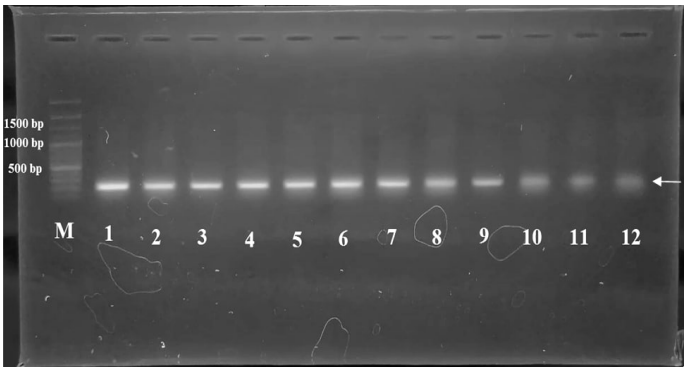

Figure 6: Detection of amplicon *mrkD* gene (240 bp). M: 100 bp DNA Marker. Isolates 1-12 were positives for *mrkD* gene.

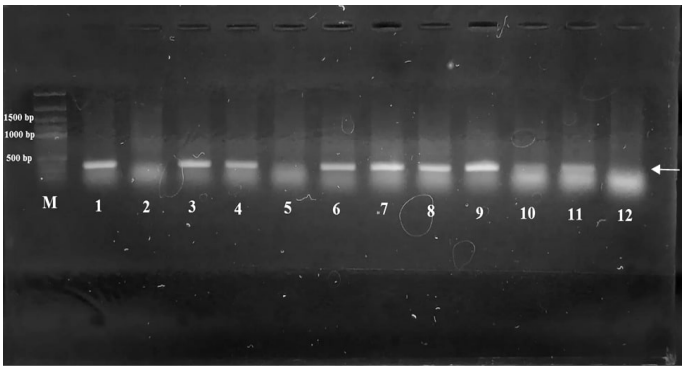

Figure 7: Detection of amplicon *entB* gene (371 bp). M: 100 bp DNA Marker. Isolates 1,3,4,6-11 were positives for *entB* gene.

Figure 8: Detection of amplicon *wabG* gene (683 bp). M: 100 bp DNA Marker. Isolates 1, 3-12 were positives for *wabG* gene.

The pathogenicity of *K. pneumoniae* has a major role in increasing infection in the host. The *mrkD* gene encodes cell surface factor and can mediate the attachment of biotic and abiotic surfaces and adequate biofilm formation which can inhibit the efficiency of antibiotic therapy (Martin and Bachman, 2018). Several studies indicate a higher prevalence of *mrkD* gene in field and clinical isolates and it has been widely studied (Liu *et al*., 2019; Bakhtiari *et al*., 2021; Mohammed *et al*., 2023). The *wabG* gene is responsible for LPS formation and plays a role in clinical symptoms such as sepsis and immune modulation during infection (Tutelyan *et al*., 2022). The enterobactin (*entB*) gene acts as the main system of iron uptake in *K. pneumoniae* and maintains the life of bacteria in the host (Effah *et al*., 2020). Through this study, it did not detect the presence of the *magA* and *rmpA* genes. The *magA* gene is encoded chromosomally and prevents the bacteria from being recognized by the immune system (Hager and Khattab, 2022) whereas *rmpA* is encoded by plasmid, and acts as a regulator of extracapsular polysaccharide (Mohammed and Flayyih, 2018). This is presumably because the *rmpA* and *magA* genes are commonly found in clinical isolates that lead to liver abscesses, invasive infections and as markers of hvKP (Mohammed and Flayyih, 2018; Hager and Khattab, 2022).

Antimicrobial resistance (AMR) due to excessive and incorrect use of human, environmental, and animal has become a serious global health threat (Velazquez-Meza *et al*., 2022) The presence of *K. pneumoniae* bacteria that have ESBL coding genes from field samples is a potential concern for farmers (Enferad and Mahdavi, 2020) and can increase the contribution of bacteria to affect host tissues (Remya *et al*., 2020). Resistance that is related to the plasmids occurs in the environment which can also carry virulence factor determinants through horizontal transmission (Michaelis and Grohmann, 2023). Commensal and pathogenic *K. pneumoniae* show a diversity of geographical/climatic conditions, use of antibiotics, and interactions between bacterial species cause bacteria to acquire virulence factor genes and have the potential to become pathogens. The presence of virulence factors and resistance genes and their relations have been conducted by some researchers (Ostria-Hernandez *et al*., 2018; Wang *et al*., 2020; Ahmadi *et al*., 2021).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, *K. pneumoniae* isolated in South Sulawesi dairy cattle farms indicated the presence of the ESBL and virulence factors gene. All of the samples of water sources, udder rinses water, and udder swabs have the potential for gene transfer between species of ubiquitous bacteria in the same environment. The existence of virulence factors present in all samples is a concern because of its affect on effectiveness of antibiotics as well as its capabilty to increase the virulence of bacteria.

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NOVELTY STATEMENT

The study is the first time an ESBL resistance test of *K. pneumoniae* isolated from dairy farms in Enrekang, South Sulawesi.
The first study conducted to detect ESBL resistance genes and virulence factors genes of *K. pneumoniae* isolated from dairy farms in Enrekang, South Sulawesi

AUTHOR'S CONTRIBUTION

NM contributed to collecting samples, doing research, data analysis, and preparing the manuscript. SS and AI contributed to revising the manuscript and supervised the research.

Ethical approval

Ethical approval on this study was obtained from Health Research Ethics Commission of Hasanuddin University Hospital Number: 105/UN4.6.4.5.31/PP36/2021.

Conflict of interest

The authors have declared no conflict of interest.

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