Research Article

Antibiotic Resistance Coding Genes in *Klebsiella pneumoniae* from Clinical Cats in Bogor Indonesia

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Abstract | Infectious agents like as viruses, bacteria, fungi, and parasites can cause feline respiratory conditions. Antibiotics can be used to treat diseases caused on by bacterial organisms. This research aims to determine antibiotic resistance and the gene coding for antibiotic resistance in *Klebsiella pneumoniae* isolated from clinical cats in Bogor, Indonesia. This study's total sample comprised of 58 clinical cat laryngeal swabs. Samples were isolated and biochemically and molecularly identified. The Kirby-Bauer disk diffusion method was used to test positive isolates, followed by PCR method to discover the resistance coding genes. The results showed that ampicillin showed 76.0% resistance to *Klebsiella pneumoniae*, followed by oxytetracycline (72.0%) and tetracycline (68.0%), enrofloxacin (52.0%), and gentamicin (44.0%). All *Klebsiella pneumoniae* isolates carried the bla_{TEM} gene, 57.20 % contained the *tet*A gene, 30.40 % contained the bla_{SHV} gene, 33.3 % contained the *aac*3-IV gene, and 28.50 % contained the *qnr*S gene. In this investigation, all *Klebsiella pneumoniae* were isolates were resistant to antibiotics and were categorized as multi drug resistant (MDR), demonstrating variable proportions of antibiotic resistance coding genes. Antibiotic resistance in Klebsiella pneumoniae has been detected in the majority of veterinary clinics in Bogor, although at varying degrees in each group. Resistance gene activity against each antibiotic can measure both the phenotype and genotype of Klebsiella pneumoniae resistance development.

Keywords | Antibiotic resistance, Cats, Feline respiratory, *Klebsiella pneumoniae*, Multi drug resistant

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INTRODUCTION

Respiratory tract infections are a common disease in cats. Infectious agents like as viruses, bacteria, fungi, and parasites can cause feline respiratory conditions. Infection with the bacterium Klebsiella pneumoniae can lead to respiratory system diseases. *Klebsiella* is a gram-negative bacterium that measures 2.0–3.0, 0.6 m in diameter, is a member of the intestinal and respiratory tracts natural flora, contains capsules, and lives facultatively as an anaerobe, resulting in a highly mucoid colony culture. Numerous lung infections, including pneumonia, sepsis, and urinary tract infections, are caused by *K. pneumoniae* (Brooks et al., 2004). *Klebsiella pneumoniae* continues to be a leading cause of pneumonia with a high mortality rate in many countries (Brisse et al., 2009), pyometra (Stone et al., 1988), urinary tract infections (Ling et al., 2001), upper respiratory tract infections (Adler et al., 2007), and septicemia (Roberts et al., 2000).

The effectiveness of antibiotics against bacterial infections. Antibacterial chemicals produced by microorganisms

are antibiotics. The use of antibiotics in the treatment of bacterial infections should be based on standards of clinical effectiveness, low toxicity, and the least possible impact on the use of multi-resistant bacteria, balancing the need for effective therapy with the minimization of the development and spread of antimicrobial resistance in animals and humans (Weese et al., 2015). Inappropriate antibiotic choice, indications, doses, administration techniques, frequency, and duration all contribute to inefficient infection treatment (Saleem et al., 2019). Additionally, irresponsible antibiotic use can result in antibiotic resistance, multidrug-resistant (MDR) infections, superinfections, and other negative side effects. The incorrect use of antibiotics, according to Huddleston (2014), causes mutations and genetic anomalies in the DNA of bacterial cells.

Klebsiella spp. are a severe global concern. Antibiotic resistance to *K. pneumoniae* isolates isolated from pets has been documented in recent years in a number of European countries, including Germany (Stolle et al., 2013; Ewers et al., 2014), Italy (Donati et al., 2014), France (Haenni et al., 2012; Poirel et al., 2013), Spain (Hidalgo et al., 2013), and Switzerland (Wohlwend et al., 2015). *K. pneumoniae* isolates from dogs and cats were resistant to ampicillin, cephalexin, ceftazidime, enrofloxacin, gentamicin, and tetracycline, according to Brisse and Duijkeren (2005).

K. pneumoniae isolates from Indonesian broiler chickens in West Jawa were resistant to erythromycin (100 percent), oxytetracycline (97.5%), ampicillin (97.5%), tetracyclines (95%), nalidixic acid (87.5%), enrofloxacin (82.5%), ciprofloxacin (75%) gentamicin (45%) and chloramphenicol (25%) (Safika et al., 2022). Cat-isolated *Escherichia coli* is resistant to 66% ampicillin, 60% amoxicillin, 54% oxytetracycline, 24% doxycycline, 38% enrofloxacin, and 28% ciprofloxacin (Yamin et al., 2020).

Antibiotics are typically used to treat bacterial infections in a veterinary clinic, but they are also frequently used to prevent secondary infections in cases of viral illnesses and after surgery. Thus, the improper use of antibiotics can result in bacterial resistance to antibiotics. Understanding bacterial resistance to antibiotics is crucial, as resistant bacteria can cause serious sickness in animals and complicate their treatment. A veterinary facility in Bogor has isolated *K. pneumoniae* bacteria from cats, necessitating an inquiry into the pathogen's resistance to numerous medications.

MATERIALS AND PROCEDURES

Samples isolation

Before the sample was taken, a questionnaire was administered at the animal clinic. The questionnaire consists of three sections: the first section contains the identity of the clinic, the second section contains clinical activities, including data on inpatient cats used in the study, and the third section contains information on the antibiotics used in the clinic. The selection of the antibiotics utilized in the study will be based on the antibiotic data collected from the five medications used most frequently in each clinic.

This investigation employed cat laryngeal swabs collected at a veterinary clinic in Bogor, Indonesia. A total of 58 samples were obtained from 10 clinics. When the cat was hospitalized, specimens were collected. Before administering antibiotics, swabs were taken. The samples were preserved in 0.1% peptone water buffer at 4 degrees Celsius. When samples arrive at the laboratory, they are placed in the refrigerator until isolation (Safika et al., 2022).

The samples were grown on Mac Conkey (MC) Agar (Oxoid, UK) for 18-24 hours at 37° C. One loop of bacteria swab was fixed on microscope slides with sterile physiological NaCl for Gram staining, after which crystal violet solution was applied for 3 minutes, lugol for 1 minute, 95% acetone alcohol for 30 seconds, and safranin for 2 minutes. The morphology of bacteria was observed under a microscope at 10 x 100 magnification. Gram positive bacteria will produce a violet color, whereas Gram negative bacteria will produce a red color (Brooks et al., 2004). *Klebsiella* bacteria ferment lactose on MCA media and have the mucoid morphology and red bacilli characteristic of Gram-negative bacteria.

Identification by molecular and biochemical methods

Based on a macroscopic examination and ferment lactose, bacteria suspected of being colonies of the genus *Klebsiella* were next identified using biochemical assays. The biochemical tests performed were the Triple Sugar Iron Agar (TSIA) test (Oxoid, UK), the Urease test (Oxoid, UK), the IMVIC test (Oxoid, UK) consisting of the Sulfide Indole Motility (SIM) test, the Methyl Red-Voges Proskauer (MR-VP) test, and the Simmon's Citrate test, and the carbohydrate fermentation test consisting of the (Oxoid, UK). *Klebsiella pneumoniae* is positive for the Methyl Red test, Simmon's Citrate test, Urease test, gas and sugar fermentation tests in the Triple Sugar Iron Agar (TSIA), and the fermentation tests for glucose, lactose, sucrose, maltose, dulcitol, and mannitol, but negative for the Indole, Voges Proskauer, and H2S tests (TSIA) (Safika et al., 2022).

Klebsiella pneumoniae is identified by determining the *rpoB* gene. Primers forward 5'- AAC CAG TTC CGC GTT GGC CTG G-3 'and reverse 5'- CCT GAA CAA CAC GCT CGG A-3 'with an amplicon length of 1090 bp used to identify the *rpoB* gene using a version of the method reported by Alves et al. (2006). *Klebsiella pneumoniae* ATCC

700603 served as a control positive. DNA amplification of *Klebsiella pneumoniae* with a final volume of 25 µl containing 4 µl of DNA template, 2 µl of forward primer (20 M), 2 µl of reverse primer (20 M), 12 µl of MytaqTM HS Red Mix (Bioline, UK), and ddH2O to a final volume of 25 µl. The PCR procedure was conducted using the Thermal Cycler GeneAmp® PCR System 9700 (Applied BiosystemsTM, United States). Predenaturation at 94°C for 4 minutes then 30 cycles (denaturation at 94°C for 30 seconds, annealing at 54°C for 1 minute, and extension at 78°C for 4 minutes) and a final extension at 72°C for 1 minute. PCR results were seen using electrophoresis on a 1% agarose gel in Tris-Acetate-EDTA (TAE) buffer 1X (Thermo Fisher Scientific USA).

Test for antibiotic susceptibility using the kirby-bauer disk diffusion technique

The antibiotic sensitivity test utilizes the Kirby-Bauer Disk Diffusion technique with Mueller-Hinton agar in accordance with Guidelines the Clinical and Laboratory Standards Institute's (2021). The bacterial colonies were diluted with physiological NaCl until according to the Mcfarland standard, 0.5 or 1.5, 10^8 CFU/ml, and then cultured at 35°C for 18 to 24 hours. The inhibition zone is assessed using Clinical Laboratory Standards Institute Guidelines (2021). 10g of ampicillin (AMP), 30g of tetracycline (TET), 30g of oxytetracycline (OT), 10 g of gentamicin (CN), and 5g of enrofloxacin (EN) were employed as antibiotics (Oxoid, UK) This antibiotic sensitivity test was conducted three times simultaneously.

Detection of genes for antibiotic resistance

The resistance genes *qnr*S (enrofloxacin), *tet*A (tetracycline, and oxytetracycline), *aac*3-IV (Gentamicin), and bla_{SHV}, bla_{TEM}, bla_{CTXM} were utilized to identify *Klebsiella pneumoniae* isolates exhibiting phenotypic intermediates and resistance (Ampicillin). Antibiotic resistance genes were identified using PCR using primers listed in Table 1.

RESULTS AND DISCUSSION

Isolation and identification

According to questionnaire responses, the most commonly used antibiotics at ten clinics include the tetracycline group the tetracycline group (29%), quinolones (26%), β-lactam (19%), aminoglycosides (16%), and sulfonamides (16%) (data not shown). Tetracycline is the most used tetracycline group in clinics (50%), followed by oxytetracycline (33%) and doxycycline (17%). The most commonly administered β-lactam antibiotics prescribed are ampicillin (34%), amoxicillin (30%), cephalosporins (11%) and other antibiotics (25%). Enrofloxacin was the most commonly prescribed quinolone antibiotic (56%), followed by ciprofloxacin (30%) and other antibiotics (14%). In contrast, the most commonly prescribed aminoglycoside antibiotic was gentamicin (60%), followed by neomycin (10%) and other antibiotics (30%). Based on the findings of the questionnaire, this test utilized tetracycline, oxytetracycline, ampicillin, enrofloxacin, and gentamicin.

A total of 58 cats' laryngeal swabs were obtained from ten veterinary clinics in Bogor. The amount of obtained samples differs due to the changing number of cat patients visited by each clinic. In addition to cats hospitalized for gastrointestinal disorders (18 samples= 31.04%) and pyometra (6 samples= 10.34%), respiratory problems accounted for 58.62% (n= 34/58) of the samples collected.

Cat laryngeal swabs were isolated and identified as *Klebsiella* in 49 (84.48%) of 58 samples grown on MC Agar. 63% (31/49) of the samples displayed the biochemical features of *Klebsiella pneumoniae*, as indicated

Table 1: Lists primers used detection of antibiotic resistance genes.

Antibiotic (Gene resistant)	Primer sequences	Amplicon (bp)	Annealing (°C)	References
Ampicillin (bla_{SHV})	(F) 5'-TCG CCT GTG TAT TAT CTC CC-3'	768	54	Colom *et al.* (2003)
	(R) 5'-CGC AGA TAA ATC ACC ACA ATG-3'			
Ampicillin (bla_{TEM})	(F) 5'-ATC AGC AAT AAA CCA GC-3'	516	54	
	(R) 5'-CCC CGA AGA ACG TTT TC-3'			
Ampicillin (bla_{CTXM})	(F) 5'-ATG ATG ACT CAG AGC ATT CG-3'	866	54	
	(R) 5'-TGG GTT ACG ATT TTC GCC GC-3'			
Enrofloxacin (*qnr*S)	(F) 5'- ACG ACA TTC GTC AAC TGC AA-3'	417	55	Robicsek *et al.* (2006)
	(R) 5'- TAA ATT GGC ACC CTG TAG GC-3'			
Gentamicin (*aac*3-IV)	(F) 5'-CTT CAG GAT GGC AAG TTG GT-3'	286	55	
	(R) 5'-TCA TCT CGT TCT CCG CTC AT-3'			
Tetracyclines and oxytetracyclines (*tet*A)	(F) 5'-GTA ATT CTG AGC ACT GTC GC-3'	965	62	Chuah *et al.* (2018)
	(R) 5'-CTG CCT GGA CAA CAT TGC TT-3'			

by biochemical tests performed to confirm the presence of the bacteria. *Klebsiella pneumoniae* is positive for the Methyl Red test, Simmon's Citrate test, Urease test, gas and sugar fermentation tests in the Triple Sugar Iron Agar (TSIA), and the fermentation tests for glucose, lactose, sucrose, maltose, dulse, and mannitol, but negative for the Indole, Voges Proskauer, and H2S tests (TSIA).

Further analysis was conducted to confirm the presumptive *K. pneumoniae* isolates by using the presence of specific *rpoB* gene in 25 of 31 isolates based on biochemical test findings with an amplicon length of 1090 base pairs. The *rpo*B gene, which codes for the β-subunit of RNA polymerase, has emerged as a candidate gene for phylogenetic studies and identification (Mollet et al., 1997; Borukhov and Nudler, 2003; Adékambi, 2009). The consequences of the *rpo*B gene amplification are depicted in Figure 1.

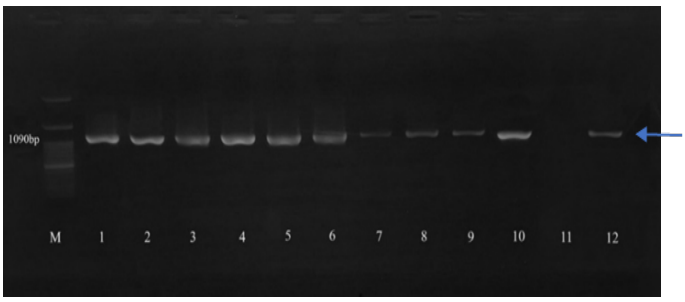

Figure 1: Amplification of the *rpo*B gene (1090 bp) in *Klebsiella pneumoniae* isolates. M: 100 bp DNA marker; 1 control positive ATCC 700603; 2-10 and 12 are positive for *Klebsiella pneumoniae*, and 11 are negative for *Klebsiella pneumoniae*.

Antibiotic susceptibility test of *Klebsiella pneumoniae*

The inhibitory zones formed by antibiotic sensitivity testing on Mueller-Hinton agar (MHA) (Figure 2). The test results for antibiotic resistance were correlated with the CLSI 2021 standards (Table 2). The results suggested that *K. pneumoniae* had a high level of resistance to five antibiotics. Isolates from this investigation were resistant to at least three classes of antibiotics.

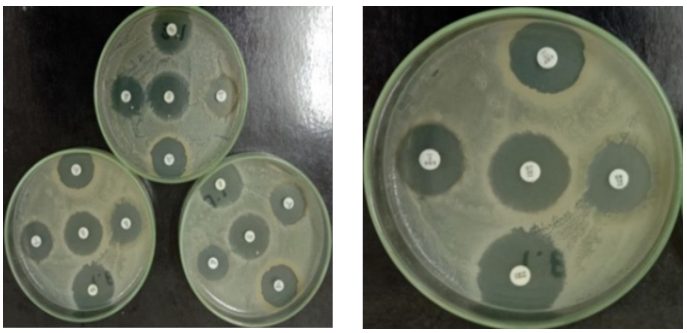

Figure 2: Inhibition zone of an antibiotic disc on Muller Hinton Agar.

Table 2: Antibiotic resistance pattern of *K. pneumoniae* (n= 25).

Sample	Antibiotic				
	Ampicillin	Enrofloxacin	Gentamicin	Tetracycline	Oxytetracycline
1	R	R	R	R	R
2	R	R	R	R	R
3	R	R	R	R	R
4	R	R	R	R	R
5	R	R	R	R	R
6	R	R	R	R	R
7	R	R	R	R	R
8	R	R	R	R	R
9	R	S	R	R	R
10	R	R	R	I	R
11	R	S	R	I	R
12	R	R	S	R	R
13	S	R	I	I	R
14	I	S	S	R	R
15	R	S	S	R	R
16	R	R	S	I	S
17	R	S	S	R	R
18	I	S	S	R	R
19	R	S	S	R	S
20	S	S	S	R	R
21	R	S	S	R	S
22	R	S	S	S	S
23	I	S	S	S	S
24	I	S	S	S	S
25	R	S	S	S	S

Note: S: Susceptible; I: Intermediate; R: Resistant.

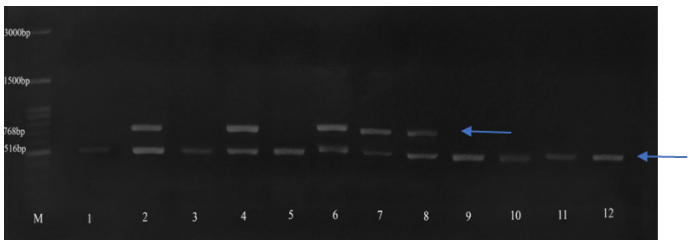

Figure 3: Amplification of the blaTEM (516 bp), blaSHV (768 bp) and blaCTXM (866 bp) genes. M: 100 bp DNA marker, 1 - 12: *Klebsiella pneumoniae* isolate that is resistant and intermediate to ampicillin antibiotics.

Detection of genes for antibiotic resistance

Detection of antibiotic resistance genes based on the target genes bla_{SHV}, bla_{TEM}, and bla_{CTXM} (ampicillin), *qnr*S (enrofloxacin), *aac*3-IV (gentamicin), and *tet*A (gentamicin) (tetracyclines and oxytetracyclines). The results of resistance genes of *Klebsiella pneumoniae* isolates were bla_{TEM} (100.00%), bla_{SHV} (30.40%), bla_{CTXM} gene (0.00%), *qnr*S (28.50%), *aac*3-IV (33.30%), and *tet*A (57.20%) (Figures 3, 6, Table 3).

Table 3: Results of amplification of genes encoding resistance in *Klebsiella pneumoniae* isolates.

Antibiotic-coding genes	β-lactam (n=23)			Quinolone (n=14)	Aminoglycoside (n=12)	Tetracycline (n=21)
	bla_{TEM}	bla_{SHV}	bla_{CTXM}	*qnr*S	*aac*3-IV	*tet*A
Percentage (%)	100.00	30.40	0.00	28.50	33.30	57.20

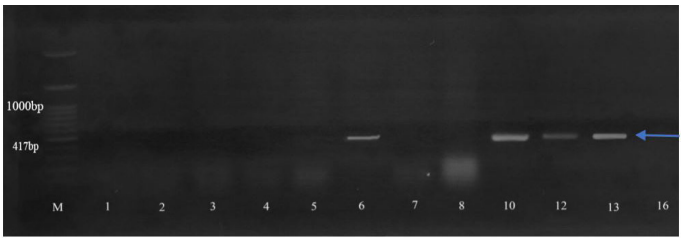

Figure 4: Amplification of the QnrS gene (417 bp). Isolates 6, 10, 12, and 13 were positive for QnrS gene. M: 100 bp DNA marker.

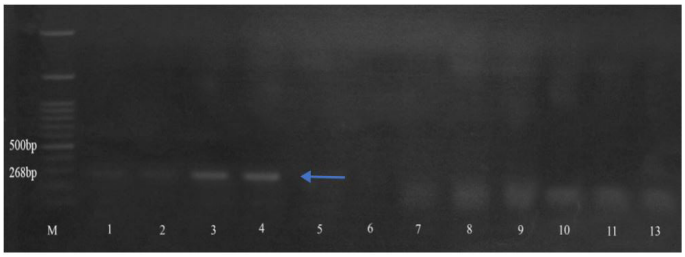

Figure 5: Amplification of the aac3-IV (286 bp) gene. Isolates 1-4 13 were positive for aac3-IV gene. M: 100 bp DNA marker.

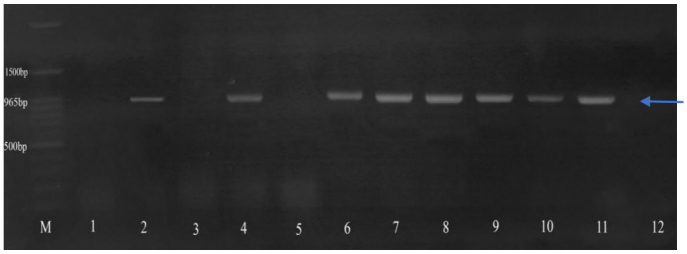

Figure 6: Amplification of the tetA gene (965 bp). Isolates 2, 4, 6-11 were positive for the tetA gene. M: 100 bp DNA marker.

Klebsiella were discovered in 49 (84%) of 58 samples from ten veterinary clinics in Bogor with pink and mucoid colony formations on MC Agar media, according to the findings of this investigation. 63% (31/49) of bacterial confirmation biochemical studies yielded a positive result. 25 of the 31 positive isolates had the target gene *rpo*B, indicating that they were *Klebsiella pneumoniae*. The *rpo*B gene encodes the essential transcription component of the RNA polymerase enzyme. The *rpo*B gene has alternating conserved and variable sections. The *rpo*B has emerged as one of the few promising options for bacterial phylogenetic analysis and identification. Contributions of *rpo*B gene sequencing to bacterial identification and taxonomy to serve as a guide for clinical microbiologists to implement *rpo*B gene sequencing in routine practice. Due to the reported *rpo*B gene-sequence divergence. Sequence analysis enabled not only the identification of species within a phylogenetic framework, but also the differentiation of bacterial strains at the intraspecies level. Even at the subspecies level, the *rpo*B gene analysis revealed valid information (Borukhov and Nudler, 2003; Adékambi et al., 2009). Since the *rpo*B gene may be used to identify the species and subspecies of *Klebsiella* isolates, it is commonly employed in phylogenetic analyses of the *Klebsiella* genus (He et al., 2016).

Klebsiella spp. are opportunistic bacteria that are isolable from a variety of clinical animal samples. *Klebsiella pneumoniae* can cause upper respiratory tract infections (Adler et al., 2007) and can be diagnosed using laryngeal swabs. Laryngeal swabs are an easy way to confirm a bacterial upper respiratory infection (Calderaro et al., 2022). *K pneumoniae* can be isolated from nasal swabs, as reported by Cheng et al. (2018). Typically, *Klebsiella pneumoniae* affects the upper respiratory tract, although it can also infect the lower lobes. Typical indications of unilateral consolidation include crepitus, bronchial breathing, and enhanced vocal resonance (Ashurst and Dawson, 2022). These microbes can cause acute, subacute, and chronic pneumonia, which increases the animal mortality rate. When an infected animal's immune system is impaired, the sickness becomes more dangerous. When *Klebsiella pneumoniae* enters an animal's circulatory system, it results in septicemia, which is exceedingly detrimental to its health (Lenchenko et al., 2020). *Klebsiella pneumoniae* has been linked to otitis (Foster et al., 2020), urogenital infections (Loncaric et al., 2020), pneumonia and acute upper respiratory tract infections (Brisse and Duijkeren, 2005).

The efficacy of the treatment is dependent upon the precision of the administered dose, the duration of administration, and the selection of the most appropriate medication. Due to continued use, antibiotic resistance may develop. Human and animal antibiotic resistance may increase the chance of antibiotic failure. Antibiotic resistance in animals is a global concern. It is vital to be aware of the prevalence of bacterial resistance to antibiotics, as resistant germs have been demonstrated to cause serious illness in dogs and complicate treatment. *Klebsiella* spp. were resistant to ampicillin, cephalexin, and ceftazidime in Germany (Stolle et al., 2013; Ewers et al., 2014), Italy (Donati et al., 2014), France (Poirel et al., 2013), Spain (Hidalgo et al., 2013), and Switzerland (Wohlwend et al., 2015), Hong et al. (2019) reported that *Klebsiella pneumoniae* isolated from dogs and cats included genes for cephalosporin resistance.

Many have reported antibiotic resistance in Indonesia, primarily in poultry (Indrawati et al., 2021; Hardiati et al., 2021; Safika et al., 2022), cattle (Juwita et al., 2022), and pigs (Kallau et al., 2018). There are relatively little data on antibiotic resistance in veterinary hospitals. Cat-isolated *Escherichia coli* is resistant to 66% ampicillin, 60% amoxicillin, 54% oxytetracycline, 24% doxycycline, 38% enrofloxacin, and 28% ciprofloxacin (Yamin et al., 2020), whereas resistance to *Klebsiella pneumoniae* in cats occurred in the β-lactam group, followed by the tetracycline group, then the quinolone group and aminoglycoside group (Ramadhan et al., 2021).

The results of the Kirby-Bauer Disk Diffusion antibiotic resistance test were correlated with the CLSI 2021 guidelines. The findings of an examination of *Klebsiella pneumoniae* resistance to five drugs indicated a significant level of resistance. The highest level of resistance was to Ampicillin (76%) and the lowest level was to Gentamicin (44%). This finding is consistent with the findings of Brisse and Duijkeren (2005), who found that *Klebsiella pneumoniae* isolates from dogs and cats were resistant to Ampicillin, enrofloxacin, and tetracyclines. *Klebsiella pneumoniae* isolates from cats, dogs, and people were resistant to Ampicillin, tetracycline, and enrofloxacin, according to Marques et al. (2019).

The resistance coding genes found in resistant and intermediate *Klebsiella pneumoniae* isolates were bla_{TEM} (100%), *tet*A (57.2%), *aac*3-IV (33.3%), bla_{SHV} (30.4%), and *qnr*S (28.5%); no isolates had the bla_{CTXM} gene (0%) The β-lactamase enzyme is responsible for β-lactam group resistance (Munita and Caesar, 2016). Gram-negative bacteria's resistance to the β-lactam class of antibiotics is mostly attributable to extended-spectrum β-lactamase (ESBL) (Rawat and Nair, 2010). The gene encoding β-lactamase enzyme was initially located on the bacterial chromosome; however, it was later discovered on plasmids in specimens recovered following antibiotic exposure. This gene's mobility is determined by the interaction it has with transposons and integrons. In the periplasmic area, the gene encoding the β-lactamase enzyme will hydrolyze the Ampicillin β-lactam ring. The broken connection renders the antibiotic ineffective and so prevents an antibiotic response (Munita and Caesar, 2016).

Through interfering with the target enzyme or by antibiotic entrance into bacterial cells, resistance to quinolones can arise in one of two ways. It is possible to suppress one or both enzymes that bind quinolone antibiotics (DNA gyrase and topoisomerase IV). Antibiotics are unable to enter bacterial cells due to mutations in the genes encoding the efflux pump in the bacterial membrane. The *qnrS* gene is a plasmid-derived gene that encodes the protective *qnr* protein against quinolone family medications (Hooper and George, 2015).

The aminoglycoside enzyme changes the antibiotic, resulting in aminoglycoside-resistant bacteria. Frequently found in the periplasmic region are aminoglycoside N-acetyltransferases, O-nucleotidyltransferases, and O-phosphotransferases. In particular, *aac, ant*, and *aph* genes represent each enzyme (Ramirez and Tolmasky, 2010; Munita and Caesar, 2016). This enzyme neutralizes the antibiotic by phosphorylating or adenylylating hydroxyl groups and acetylating free amino acids. Changes in the permeability of the outer membrane of bacteria can also contribute to the development of resistance, as they disrupt the process through which aminoglycoside antibiotics enter the cell. In addition, resistance may develop because of alterations to the aminoglycoside receptors on the 30s subunit ribosomes (Munita and Caesar, 2016; Abu-Saleh et al., 2020).

The efflux pump is responsible for bacterial resistance to tetracycline-class drugs. The theta gene codes for the efflux pump, which is responsible for removing tetracyclines from bacterial cells (Tuckman et al., 2000). According to Hoek et al. (2011), there are additional genes capable of producing bacterial resistance to the tetracycline group, in addition to the *tet*A gene. Over 20 *tet* genes have been found to date (Poole, 2005). Additional *tet* genes that may encode the tetracycline efflux pump include *tet*B, *tet*C, *tet*D, and *tet*E (Nguyen et al., 2014). The efflux pump can cause resistant bacterial isolates that lack the theta gene to acquire another tetracycline-coding gene.

In addition, *Klebsiella pneumoniae* contains metallo β-lactamase (MBL) enzymes with a broad substrate spectrum that may hydrolyze penicillins, cephalosporins, and carbapenems (Jeong et al., 2014). Contains the newly discovered bla_{IMP}, bla_{VIM}, and bla_{NDM} groups. First found in *K. pneumoniae* in 2008, bla_{NDM}-1 (New Delhi metallo β-lactamase) is a significant problem for infection therapy as this bacterium is frequently resistant to a broad spectrum of antimicrobial medicines (Urmi et al., 2020). bla_{NDM}-1 can deactivate most β-lactams, barring aztreonam (Shoja et al., 2017).

Using the genotypic method, Indonesian farms typically identify the antibiotic resistance genes blaTEM, gyrA, tetA, tetB, aac (3)-IV ermB, and mecA (Hardiati et al., 2021; Indrawati et al., 2021; Safika et al., 2022; Juwita et al., 2022). This research was limited by the fact that the resistance gene amplification results were not sequenced, making it unable to compare base sequences with GenBank data to establish whether there is variance.

CONCLUSIONS AND RECOMMENDATION

In the majority of veterinary clinics in Bogor, *Klebsiella pneumoniae* was found to be resistant to antibiotics, but to varying degrees in each group. Resistance gene activity against each antibiotic can be used to assess both the phenotype and genotype of *Klebsiella pneumoniae* resistance.

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NOVELTY STATEMENT

This research has novelties:

1. The first time a resistance test was carried out on sick cats at a clinic in the city of Bogor.
2. For the first time, resistance gene detection was carried out in sick cats at a clinic in the city of Bogor.

AUTHOR'S CONTRIBUTION

Conceptualization, S and NLPI. M; methodology, S and NLPI. M; validation, S and NLPI. M; formal analysis, J.R.; investigation, J.R; data curation, S, NLPI. M and J.R; writing-original draft preparation, S, NLPI. M and J.R; writing-review and editing, S; visualization, S, NLPI. M and J.R; funding acquisition, S. All authors have read and agreed to the published version of the manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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