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Isolation and Antibiotic Resistance Profile of *Klebsiella spp.* from the Gastrointestinal Tract of Broiler Chickens in Poultry Farms in Bali

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Abstract. The poultry sector in Bali has experienced substantial growth, accompanied by a notable increase in broiler chicken populations. Of many potential infectious diseases that may affect broiler chickens on a large scale, information on *Klebsiella spp.* Outbreaks in Bali have not been studied yet. *Klebsiella spp.* is an opportunistic pathogen that can induce severe respiratory disease in poultry, carrying a potential risk of zoonotic transmission. Furthermore, the increasing incidence of antibiotic resistance among *Klebsiella* strains poses a critical challenge to effective therapeutic interventions. This study was conducted to isolate and identify *Klebsiella spp.* from the gastrointestinal tract of broiler chickens in commercial farms across Bali, and to assess the antimicrobial resistance profiles of the isolates against multiple antibiotic agents. A cross-sectional study with a total of 25 cloacal swabs was conducted, and the swabs were subjected to microbiological analysis. Isolation was performed using selective MacConkey Agar (MCA), followed by Gram staining, biochemical identification, and antibiotic susceptibility testing through the Kirby-Bauer disk diffusion method. Two out of 25 (8%) samples were positively identified as *Klebsiella spp*. All of these positive isolates showed complete resistance to ampicillin, sulfamethoxazole, erythromycin, ciprofloxacin, and tetracycline. Additionally, the positive isolates also exhibited intermediate resistance against kanamycin, while 50% of the isolates demonstrated susceptibility to chloramphenicol. These findings confirm the presence of multidrug-resistant (MDR) strains among the isolates examined. The outcomes of this research emphasize the urgent need for a rigorous study to identify potential outbreaks caused by *Klebsiella spp.*, throughout commercial farms in Bali, and stringent monitoring and regulation of antibiotic usage within the poultry industry to mitigate the proliferation of antimicrobial resistance, thereby safeguarding animal health and preventing potential risks to public health.

Keywords: antimicrobial resistance; broiler chickens; multidrug resistance; *Klebsiella spp.*

I. INTRODUCTION

According to data from the Central Bureau of Statistics of Indonesia, the Directorate General of Livestock and Animal Health reported that the population of broiler chickens in the Province of Bali reached 68,720,589 birds in 2021, increasing to 72,373,629 birds in 2022 [1]. These figures indicate that the poultry industry in Bali has experienced positive growth in fulfilling the demand for animal protein consumption.

One of the significant challenges in the poultry sector is bacterial disease caused by *Klebsiella spp.*, an opportunistic pathogen with zoonotic potential. However, cases of direct transmission from animals to humans are rarely reported [2]. *Klebsiella spp.* is a pathogen that can lead to substantial production losses and mortality in poultry farming [3]. In birds, *Klebsiella* infection may cause symptoms such as lethargy, dehydration, dull plumage, fecal soiling around the cloaca, respiratory distress, diarrhea, growth retardation, and death, accompanied by pathological signs such as pneumonia, pericarditis, airsacculitis, and hepatomegaly [4]. Differential diagnoses include coccidiosis, enteritis, and other bacterial diseases [5].

To date, data on *Klebsiella spp* infection in broiler chickens in Bali have not been reported. However, in other regions of Indonesia, *Klebsiella spp.* has been successfully isolated and identified in poultry farms, particularly in East Java [6] [7] [8]. Several studies have employed cloacal swab sampling techniques for detecting *Klebsiella spp.* in both layer and broiler chickens [9].

The bacterial isolation and identification process involves the collection of cloacal swab samples, which are then cultured on MacConkey Agar (MCA) media, followed by Gram staining and a series of biochemical tests for further analysis [9] [10]. This procedure is classified as a culture-based method that relies on the growth of bacteria on selective media to allow identification. Identified isolates of *Klebsiella spp.* are subsequently subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method [11].

Klebsiella spp. Infections are commonly treated with β-lactam antibiotics, which act by covalently binding to penicillin-binding proteins (PBPs) and inhibiting cell wall synthesis [12]. Antimicrobial resistance (AMR) in *Klebsiella spp.* Represents a serious threat to both animal and human health. This bacterium is part of the normal microbiota of poultry and is known for its ability to develop resistance to various antibiotics [13]. Globally, antimicrobial-resistant and hypervirulent strains of *Klebsiella spp.* have emerged [14]. Antibiotics, whether naturally derived, semi-synthetic, or synthetic, are compounds capable of inhibiting or killing bacteria at low concentrations, and their use in poultry farming is subject to specific restrictions by the Regulation of the Minister of Agriculture of the Republic of Indonesia No. 14 of 2017 [15]. Therefore, data reflecting field conditions about the restricted use of antibiotics are critically needed.

Currently, no local data exist concerning the isolation and identification of *Klebsiella spp.* and its resistance patterns in broiler farms in Bali. Consequently, this study is crucial as it provides foundational data on the prevalence of *Klebsiella spp.* infections in the region and serves as a valuable reference for therapeutic strategies and antibiotic resistance control measures in the poultry sector, which in turn poses implications for both public and animal health. The objective of this study is to isolate and identify *Klebsiella spp.* From broiler chickens in Bali to determine their antibiotic resistance profiles using a culture-based approach.

II. METHODS

Approval of the Ethical Commission

Before conducting the study, ethical approval was obtained from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, under reference number B/44/UN14.2.9/PT.01.04/2024.

Study Period and Location

Sampling was conducted in August and September 2024. Cloacal swab samples were processed at the Bacteriology and Mycology laboratory, Faculty of Veterinary Medicine, Udayana University.

Samples

This study employed a cross-sectional design utilizing 25 cloacal swab samples collected from broiler chicken farms in the Bali region. Cloacal swab samples were obtained from clinically healthy broiler chickens at the finisher stage, which had not received vaccination against *bacteria.* Cloacal swabs from broiler chickens can be used as a sampling method for the isolation of *Klebsiella spp.* [9]. The swab samples were transported using Stuart transport medium. In this study, cloacal swab samples were collected randomly from five regencies in Bali, with details regarding the poultry houses presented in Table 1. All cloacal swab samples were subjected to the isolation and identification of *Klebsiella spp*. at the Microbiology Laboratory, Faculty of Veterinary Medicine, Udayana University.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method with antibiotic discs (Oxoid™, UK). The test involved inoculating the bacterial isolates onto the surface of Mueller-Hinton agar plates. The bacterial suspension was prepared using a turbidity standard equivalent to 0.5 McFarland. Antibiotic discs were then placed onto the agar surface. The antibiotics used in this study belonged to various classes, including β-lactams (Ampicillin 10 µg), Tetracyclines 30 µg, Fluoroquinolones (Ciprofloxacin 5 µg), Macrolides (Erythromycin 15 µg), Sulfonamides (Sulfamethoxazole-trimethoprim 25 µg), Amphenicols (Chloramphenicol 30 µg), and Aminoglycosides (Kanamycin 30 µg). The plates were incubated at 35–37°C for 16–18 hours. The diameter of the inhibition zones was measured in millimeters using a ruler. The results were interpreted by comparing the measurements to the standard interpretation chart provided by the Clinical and Laboratory Standards Institute (CLSI) to classify the bacterial isolates as susceptible (S), intermediate (I), or resistant (R) (16). Reference strains of *Escherichia coli* ATCC 25922 were used for quality control (Thermoscientific, LENEXA, USA)

Isolation and Identification of Klebsiella spp.

Samples were cultured on selective MacConkey Agar (MCA) medium and incubated for 18–24 hours at 37°C. Gram staining was performed to identify *Klebsiella pneumoniae* colonies. Presumptive *Klebsiella spp i*solates were subjected to biochemical tests, including bacterial culture on Triple Sugar Iron Agar, carbohydrate fermentation tests, and the Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) tests [6] [10].

Analysis

The collected data were analyzed descriptively.

III. RESULTS AND DISCUSSION

Klebsiella spp. is an opportunistic pathogen that contributes to production losses and mortality within the poultry industry (3). Members of the genus *Klebsiella* (*K.*) are classified under the family *Enterobacteriaceae*. To date, the genus comprises eight species: *K. pneumoniae* (which includes three subspecies), *K. oxytoca*, *K. planticola*, *K. ornithinolytica*, *K. granulomatis*, *K. mobilis*, *K. terrigena*, and *K. variicola* [17].

This study aimed to determine the prevalence of *Klebsiella* in broiler farms across Bali. A total of 25 cloacal swab samples were collected from five regencies and subjected to bacterial isolation and identification (Table 1). The isolates were identified and characterized using MacConkey Agar (MCA) medium to observe colony morphology and microscopic features. Further biochemical tests were conducted to assess characteristics such as cellular shape, arrangement, colony appearance, temperature preferences, and growth behaviour. Additional analyses included the indole production test using Sulphide Indole Motility (SIM) medium, the Methyl Red (MR) and Voges-Proskauer (VP) medium, citrate utilization test using Simmons Citrate Agar (SCA), urease agar, catalase tests using 3% H_2O_2, the Triple Sugar Iron Agar (TSIA), and growth assessment at 37°C. The results were documented and summarized in Table 2.

Initial isolation of the samples was performed by inoculating them into MCA. The MCA medium is a selective and differential medium that supports the growth of Gram-negative bacterial species based on their ability to metabolize lactose [18]. In this study, the bacterial colonies observed were consistent with previous findings [19], which described *Klebsiella* colonies on MCA as large (3–4 mm), mucoid, lactose-fermenting, irregular in shape, and comprising plump rods with a prominent capsule (Figure 1).

The subsequent identification step involved Gram staining, which microscopically revealed pink-stained, rod-shaped bacteria, indicating that they were likely Gram-negative bacilli (Figure 2a). The catalase test revealed the production of oxygen bubbles, indicating the presence of the catalase enzyme in the bacterial isolate. This enzyme catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen (Figure 2b). *Klebsiella* species are known to be short, Gram-negative rods, and preliminary catalase testing of the isolates showed positive results [20].

Further identification was performed through a series of biochemical tests. In this study, biochemical analysis using TSIA revealed that isolates A6 and A7 were capable of fermenting lactose, sucrose, and glucose, as indicated by the presence of acid in both the slant and butt, gas production, and the absence of hydrogen sulfide (H_2S) formation (Figure 3a). The SIM medium indicated that the bacteria were non-motile, indole-negative, and did not produce H_2S (Figure 3b). Additional biochemical assays showed negative results for the MR test and positive results for the VP test (Figures 3c and 3d). The citrate utilization test showed positive results, as evidenced by a color change from green to blue (Figure 3e). The glucose fermentation test showed a positive outcome, indicated by a color change from blue to yellow, accompanied by gas production in the Durham tube (Figure 3f). Similarly, the urease test was positive, as evidenced by a color change to red or pink (Figure 3g).

Klebsiella species are non-motile, usually capsulated, and facultatively anaerobic bacteria [18]. On TSIA medium, they characteristically exhibit an acid slant, acid butt, gas production, and no H_2S production [21]. Biochemical test outcomes may vary depending on the species being tested. *K. ornithinolytica*, *K. oxytoca*, *K. planticola*, and *K. pneumoniae* exhibit varying VP test results. The MR test is positive in *K. ornithinolytica* and *K. planticola*, variable in *K. oxytoca*, and negative in *K. pneumoniae*. Citrate utilization and indole tests are positive for *K. ornithinolytica* and *K. oxytoca*, variable in *K. planticola* and *K. pneumoniae*. Glucose fermentation is positive across all species [6] [19]. Based on these findings, the isolates in this study were identified as *K. pneumoniae*. Of the 25 cloacal swab samples analyzed, two isolates (8%) were confirmed to be positive for *K. pneumoniae*. This result aligns with a previous study conducted in East Java using the Polymerase Chain Reaction (PCR) method, where 11 out of 141 samples (7.8%) tested positive for *Klebsiella* [7]. Older chickens were found to have a higher prevalence risk [3].

One of the significant challenges in poultry production is bacterial infection, which is often accompanied by increasing antibiotic resistance [22]. The growing incidence of multidrug-resistant (MDR) bacteria presents a substantial threat to both medical and veterinary professionals, substantially limiting therapeutic options. All identified *Klebsiella* isolates were tested for

antimicrobial susceptibility using the disk diffusion method, and the results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) guidelines [23] (Table 3). Antibiotic susceptibility testing utilized Escherichia coli ATCC 25922 as a control strain, and the results for all antibiotics fell within the expected reference range. Among the two samples tested, 100% of the bacteria were resistant to five antibiotics: Ampicillin, Sulfamethoxazole, Erythromycin, Ciprofloxacin, and Tetracycline. Additionally, both isolates showed intermediate resistance to Kanamycin, and for Chloramphenicol, one isolate (50%) was sensitive while the other (50%) was resistant. These findings are consistent with previous studies reporting *K. pneumoniae* resistance in poultry to Ampicillin (100%), Erythromycin (100%), Tetracycline (95%), Chloramphenicol (25%) [10], and Ciprofloxacin (90.9%) [7], as well as intermediate resistance to Kanamycin [5]. One of the leading contributors to AMR infections is the improper and excessive use of antimicrobials in both human medicine and animal husbandry [24].

The *K. pneumoniae* isolates identified in this study are classified as MDR bacteria, as evidenced by their resistance to multiple classes of antibiotics. The presence of MDR bacteria poses a serious threat to both public and animal health, as it significantly reduces available treatment options [7]. The mechanisms of antimicrobial resistance include enzymatic inactivation of antimicrobial compounds, alterations in membrane permeability, and modifications of antimicrobial target sites [17].

This study has several limitations that should be acknowledged. First, the sample size was relatively small, comprising only 25 cloacal swab samples collected from broiler farms in five regencies across the island of Bali. This limited number of samples may not adequately represent the broader broiler population in the region, potentially affecting the generalizability of the findings. Second, molecular confirmation methods, such as Polymerase Chain Reaction (PCR), were not employed to validate the identification of *Klebsiella pneumoniae*. The identification relied solely on phenotypic and biochemical characteristics, which, although informative, lack the specificity and sensitivity offered by molecular techniques. Therefore, future research is recommended to incorporate larger sample sizes and employ molecular diagnostic methods to enhance the validity and accuracy of the results.

TABLE 1
SWAB CLOACAL SAMPLE DATA FROM POULTRY FARMS IN BALI PROVINCE

Sample Code	Regency	Age Stage	Clinical Symptoms*)	Number of Samples (n)
A1-A5	Tabanan	Finisher	None	5
A6-A10	Gianyar	Finisher	None	5
A11-A15	Badung	Finisher	None	5
A16-A20	Klungkung	Finisher	None	5
A21-A25	Bangli	Finisher	None	5
		Total Sample		25

*) Clinical symptoms in chickens indicating infection with *Klebsiella pneumoniae.*

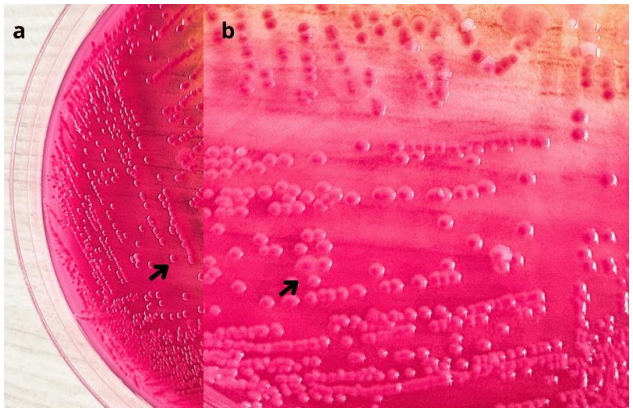

Figure 1. Growth of *Klebsiella* bacteria on MacConkey Agar (MCA), with *Klebsiella* colonies showing lactose fermentation (pink colonies) (a), and mucoid colonies (b).

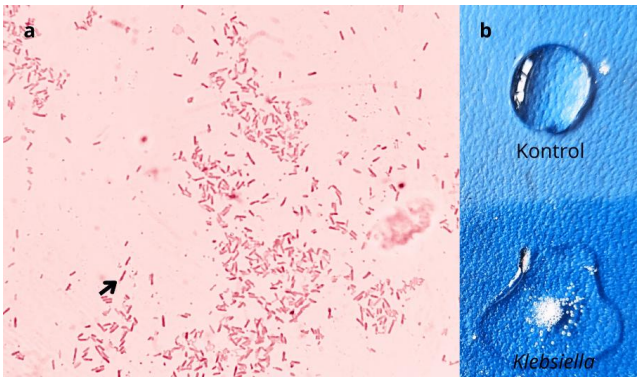

Figure 2. Gram staining results of *Klebsiella pneumoniae* showing Gram-negative, rod-shaped bacteria (a). The primary biochemical test indicates a positive catalase reaction.

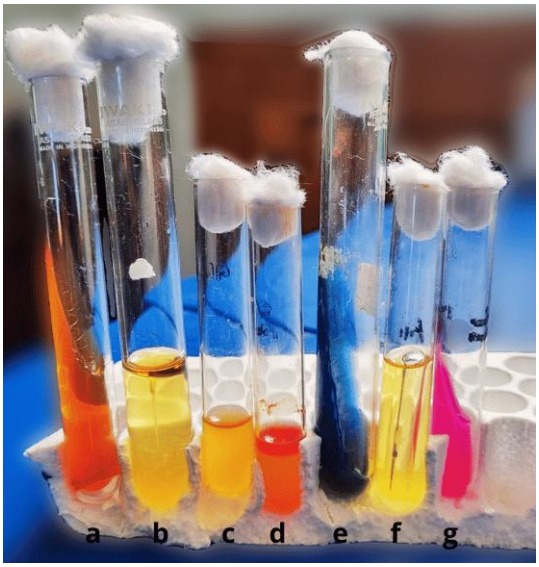

Figure 3. Results of biochemical tests using Triple Sugar Iron Agar (a), Sulfide Indole Motility (b), Methyl Red (c), Voges-Proskauer (d), Simmons Citrate Agar (e), Glucose Fermentation Test (f), and Urease Agar (g).

TABLE 2
GROWTH SAMPLE ON MEDIA AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES

Sample Code	MCA*)	Gram Stain	Catalase	TSIA	Indole	MR	VP	SCA	Motility	H_2S	Urease	Glucose	Identified Bacteria
A1	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A2	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A3	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A4	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A5	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp.*
A6	LF	-ve, bacil	+	A/A/+	-	-	+	+	non motile	-	+	+	*Klebsiella pneumoniae*
A7	LF	-ve, bacil	+	A/A/+	-	-	+	+	non motile	-	+	+	*Klebsiella pneumoniae*
A8	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A9	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp.*
A10	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp.*
A11	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A12	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A13	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A14	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp*
A15	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp*

A16	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp*
A17	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A18	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A19	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A20	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A21	NLF	-ve, bacil	+	Alk/A/+	-	+	-	+	motile	+	-	+	*Salmonella sp.*
A22	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A23	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A24	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*
A25	LF	-ve, bacil	+	A/A/+	+	+	-	-	motile	-	-	+	*Escherichia coli*

*) LF: Lactose Fermented; NLF: Non-Lactose Fermented
**) A: Acid Slant/butt; Alk: Alkali Slant/butt, Gas +/-

TABLE 3
INHIBITION ZONE DIAMETER MEASUREMENTS

No	Antibiotic	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (mm)*)			Positive Samples of *Klebsiella pneumoniae* (mm) *)								
						A6			A7			ATCC *Escherichia coli* 25922		
			S	I	R	S	I	R	S	I	R	Disk diffusion QC ranges*)	Diameter	Criteria*)
1.	Ampicillin	10 µg	≥17	14-16	≤13	-	-	6	-	-	12	15-22	20	S
2.	Sulphamethoxazole	25 µg	≥16	11-15	≤10	-	-	6	-	-	7	23-29	26	S
3.	Kanamycin	30 µg	≥18	14-17	≤13	-	16	-	-	16	-	17-25	22	S
4.	Erythromycin	15 µg	≥23	14-22	≤13	-	-	7	-	-	6	14-22	18	S
5.	Ciprofloxacin	5 µg	≥26	22-25	≤21	-	-	10	-	-	8	29-38	24	S
6.	Tetracycline	30 µg	≥15	12-14	≤11	-	-	6	-	-	8	18-25	18	S
7.	Chloramphenicol	30 µg	≥18	13-17	≤12	-	-	12	22	-	-	21-27	26	S

*) According to the Clinical and Laboratory Standards Institute (CLSI, 2020), bacterial susceptibility is classified into three categories: Sensitive (S), Intermediate (I), and Resistant (R) based on zone diameter breakpoints.

CONCLUSION

This study successfully isolated and identified *K. pneumoniae* from the gastrointestinal tract of broiler chickens at several farms in Bali, with a prevalence rate of 8% (2/25). The identified isolates exhibited morphological, microscopic, and biochemical characteristics consistent with those of the *K. pneumoniae* species. Antibiotic susceptibility testing revealed that both isolates demonstrated resistance to five antibiotics (ampicillin, sulfamethoxazole, erythromycin, ciprofloxacin, and tetracycline), intermediate susceptibility to kanamycin, and partial sensitivity to chloramphenicol. These findings indicate that the *K. pneumoniae* isolates identified in this study are classified as multidrug-resistant (MDR), posing a significant threat to both animal and public health, and underscoring the critical importance of monitoring antibiotic usage in the livestock sector.

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