IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *ENTEROBACTERIACEAE* BACTERIA ISOLATED FROM FECES OF WREATHED HORNBILL (*Rhyticeros undulatus*)

MUHAMMAD RADITYA WIDANDI

STUDY PROGRAM OF VETERINARY MEDICINE
SCHOOL OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES
IPB UNIVERSITY
BOGOR
2025

STATEMENT REGARDING THE UNDERGRADUATE-THESIS, SOURCES OF INFORMATION, AND COPYRIGHT TRANSFER

I hereby declare that the thesis entitled *"*Identification and Antimicrobial Susceptibility of *Enterobacteriaceae* Bacteria Isolated from Feces of Wreathed Hornbill *(Rhyticeros undulatus)"* is my own work, carried out under the guidance of my academic advisor, and has not been submitted in any form to any other university or institution. All sources of information, whether published or unpublished, that are cited from the works of other authors have been properly acknowledged within the text and listed in the References section at the end of this thesis.

I hereby transfer the copyright of this written work to IPB University (Institut Pertanian Bogor).

Bogor, July 2025

Muhammad Raditya Widandi
B0401211831

ABSTRAK

MUHAMMAD RADITYA WIDANDI. "Identifikasi dan Uji Kepekaan Antimikroba Bakteri *Enterobacteriaceae* yang Diisolasi dari Feses Julang Emas (*Rhyticeros undulatus*)". Dibimbing oleh DORDIA ANINDITA ROTINSULU dan AGUS WIJAYA.

Burung julang emas (*Rhyticeros undulatus*) adalah spesies burung dari famili Bucerotidae yang dilindungi karena populasinya yang semakin menurun. Upaya konservasi eksitu dilakukan untuk memulihkan spesies ini, namun tantangan seperti meningkatnya resistensi antimikroba menghambat pengobatan burung di konservasi eksitu. Penelitian ini bertujuan untuk mengidentifikasi bakteri *Enterobacteriaceae* dari feses burung julang emas di area konservasi eksitu dan menguji kepekaan bakteri tersebut terhadap antimikroba. Sebanyak 16 isolat bakteri *Enterobacteriaceae* berhasil diisolasi dari feses tujuh ekor burung julang emas. Bakteri yang diisolasi berasal dari enam genus yang terdiri dari 4 isolat *Klebsiella*, 4 isolat *Escherichia*, 3 isolat *Yersinia*, 2 isolat *Citrobacter*, 2 isolat *Proteus*, dan 1 isolat *Enterobacter*. Uji kepekaan antimikroba dilakukan terhadap doksisiklin, ampisilin, gentamisin, siprofloxacin, kloramfenikol, dan trimetropim-sulfametoksazol dengan menggunakan metode difusi cakram Kirby-Bauer. Terdapat tingkat resistensi yang tinggi terhadap doksisiklin yang terdiri dari 10 isolat resisten, diikuti oleh ampisilin dengan 5 isolat resisten, namun tidak ditemukan isolat yang *multridrug-resistant*. Semua isolat sensitif terhadap siprofloksasin dan trimetoprim-sulfametoksazol. Studi ini menunjukkan pentingnya penggunaan antimikroba yang tepat pada burung di konservasi eksitu.

Kata kunci: *Enterobacteriaceae*, julang emas, konservasi eksitu, resistansi antimikroba

ABSTRACT

MUHAMMAD RADITYA WIDANDI. "Identification and Antimicrobial Susceptibility of *Enterobacteriaceae* Bacteria Isolated from Feces of Wreathed Hornbill *(Rhyticeros Undulatus)"*. Supervised BY DORDIA ANINDITA ROTINSULU and AGUS WIJAYA.

The wreathed hornbill (*Rhyticeros undulatus*) is a bird species from the Bucerotidae family that is protected due to its declining population. Ex-situ conservation efforts are being carried out to help recover this species; however, challenges such as the rise of antimicrobial resistance complicate these efforts. This study aims to identify *Enterobacteriaceae* bacteria from the feces of wreathed hornbills in ex-situ conservation areas and to test their susceptibility to antimicrobials. A total of sixteen *Enterobacteriaceae* bacterial isolates were successfully obtained from the feces of seven wreathed hornbills. The isolated bacteria belonged to six genera: *Klebsiella* (4 isolates), *Escherichia* (4 isolates), *Yersinia* (3 isolates), *Citrobacter* (2 isolates), *Proteus* (2 isolates), and *Enterobacter* (1 isolate). Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method against doxycycline, ampicillin, gentamicin, ciprofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole. A high level of resistance was observed against doxycycline, with 10 isolates showing resistance, followed by ampicillin, with 5 resistant isolates. However, no multidrug-resistant isolates were found. All isolates were sensitive to ciprofloxacin and trimethoprim-sulfamethoxazole. This study highlights the importance of appropriate antimicrobial use in birds within ex-situ conservation.

Keywords: antimicrobial resistance, *Enterobacteriaceae*, wreathed hornbill, ex-situ conservation.

IDENTIFICATION AND ANTIBICOBIAL SUSCEPTIBILITY OF *ENTEROBACTERIACEAE* BACTERIA ISOLATED FROM FECES OF WREATHED HORNBILL (*Rhyticeros undulatus*)

MUHAMMAD RADITYA WIDANDI

Undergraduate-thesis
as one of the requirements to obtain the Bachelor's degree
in
School of Veterinary Medicine and Biomedical Sciences

STUDY PROGRAM OF VETERINARY MEDICINE
SCHOOL OF VETERINARY MEDICINE AND BIOMEDICAL SCIENCES
IPB UNIVERSITY
BOGOR
2025

Undergraduate-thesis Examination Committee:
1 Prof. Dr. drh. Aryani Sismin Satyaningtijas, M.Sc
2 Dr. drh. Supratikno, M.Si

Title : Identification and Antimicrobial Susceptibility of *Enterobacteriaceae* Bacteria Isolated from Feces of Wreathed Hornbills *(Rhyticeros undulatus)*

Name : Muhammad Raditya Widandi

NIM : B0401211831

Approved by

First Supervisor:
drh. Dordia Anindita Rotinsulu, M.Si., Ph.D.

Second Supervisor:
drh. Agus Wijaya, M.Sc., Ph.D.

Acknowledged by

Head of Veterinary Medicine Study Program:
Dr. drh. Wahono Esthi Prastyaningtyas, M.Si.
NIP. 19800618200604026

Digitally signed by:
Wahono Esthi Prasetyaningtyas
Date: 21 Jul 2025 11.21.10 WIB
Verify at disign.ipb.ac.id

Vice Dean for Academic and Student Affairs
School of Veterinary Medicine and Biomedical Sciences:
Prof. drh. Ni Wayan Kurniani Karja, M.P., Ph.D.
NIP. 196902071996012001

digitally signed
disign.ipb.ac.id

Date of Exam:
July 15th 2025

Date of Graduation: 22 JUL 2025

ACKNOWLEDGEMENTS

Praise and gratitude are sincerely offered to Allah Subhanahu wa Ta'ala for all His blessings, through which this scientific work has been completed. This research, conducted from September 2024 to January 2025, is titled "Identification and Antimicrobial Susceptibility of *Enterobacteriaceae* Bacteria Isolated from Feces of Wreathed Hornbills *(Rhyticeros undulatus)*.".

The author sincerely extends profound appreciation to the primary thesis supervisor, drh. Dordia Anindita Rotinsulu, M.Si, Ph.D., and to the academic and secondary thesis supervisor, drh. Agus Wijaya, M.Sc., Ph.D., for their invaluable guidance, support, and constructive feedback throughout the preparation and completion of this undergraduate thesis.

The author also gratefully acknowledges Jagat Satwa Nusantara, Taman Mini Indonesia Indah (TMII), for granting permission and providing the necessary samples for this study. Special thanks are conveyed to drh. M. Piter Kombo, drh. Caesar Rizal Kurniawan G., drh. Kenda Adhitya Nugraha and Mba Dela for their assistance during the research process.

Additionally, the author expresses sincere gratitude to Mr. Agus Somantri from the Division of Medical Microbiology for his significant technical support and contributions, which greatly facilitated the successful execution of this research.

The author wishes to convey his deepest gratitude to his beloved parents, Mr. Didik Haryanto and Mrs. Endang Sri Windarti, for their unwavering love, prayers, and moral support, which have been a cornerstone of strength throughout the course of this research. Sincere appreciation is also extended to his cherished siblings, Ms. Dysha Cindrati and Mr. Galih Widiyanto, whose encouragement and constant inspiration have greatly supported the author's academic journey.

The author is profoundly thankful to Rangga for his dedication and invaluable assistance as a research partner, whose contributions significantly enriched the research process

Lastly, the author warmly acknowledges his dearest friends (Atria, Fasya, Alfa, Gumi, Nugraha, Max, Radith, Nahlan, Ardy, Gifari, and Naabil) for their enduring friendship, encouragement, and presence, which have made this journey more meaningful and memorable.

Bogor, July 2025

Muhammad Raditya Widandi

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 Background

The wreathed hornbill (*Rhyticeros undulatus*) is an avian species of the Bucerotidae family. In Indonesia, there are 13 different species in the Bucerotidae family, including the wreathed hornbill itself (Jarulis *et al.* 2015). In Indonesia, wreathed hornbills are spread across Sumatra, Borneo, and Java Island (MacKinnon and Ramsay 2010). According to the International Union for Conservation of Nature (IUCN) in 2018, the status of the wreathed hornbill has been classified as vulnerable, which is defined as species that possess a very high risk of extinction a population decline of 30 to 50 percent over the previous 10 years or three generations. Conservation efforts are required to maintain and hopefully increase the number of this species. Ex-situ conservation is one of the efforts that can be made to conserve wildlife. Ex-situ conservation essentially means cultivating and preserving animals outside their natural habitats (Mahanayak 2024). This method is employed when species are critically endangered. Ex-situ conservation can complement in-situ efforts by providing additional protection and as a source for reintroducing species into the wild. This conservation method includes various facilities and technology such as zoos, semen banks, and more.

Jagat Satwa Nusantara (JSN) is a zoological park structured into three central units, each representing a significant animal class: Freshwater World for pisces class, the Komodo Museum and Reptile Park for herpetofauna, and the Bird Park for avifauna class. This zoological park functions as an ex-situ conservation institution, where diverse species of animals are cared for and bred to establish and cultivate new habitats, contributing to environmental protection and conservation efforts, and promoting scientific research (Alfalasifa and Dewi 2019). Zoos and other animal conservation facilities also allow visitors to engage with species not typically seen daily. As a place centered in conservation and education on animals, it is imperative to do further research on the identification of bacteria related to the animals to gain insights on how to increase animal welfare and help advance conservation efforts of wild animals in JSN.

Research about bacteria in an avian ex-situ conservation site is essential. Stress levels and crowding in wild birds can contribute to spreading infectious diseases, facilitating the rapid transmission of pathogens within their populations (Kobuszewska and Wysok 2024). Due to their large population densities, frequent social interactions, high mobility, and ability to thrive in human-altered environments, birds have a considerable potential to act as reservoirs for pathogens that can be transmitted to other vertebrate species. Additionally, since the birds in an ex-situ conservation facility are sometimes given medications using antimicrobials, they may develop antimicrobial-resistant bacteria. These reasons are why it is crucial to conduct research on the bacteria present in wild birds in JSN Zoological Park, such as the wreathed hornbills, to advance further conservation efforts and improve animal welfare.

1.2 Problem Statement

The emergence of antimicrobial resistance has been a problem in veterinary medicine. This problem has proven to be a severe threat to animals and humans alike. In recent years, multiple studies have shown antimicrobial resistance in wild

animals. This problem would have detrimental effects on veterinary medicine, especially those centered around wildlife and medical conservation. Research on the identification of bacteria and their antimicrobial resistance within wild animals, especially birds, is critical in advancing the state of animal welfare in conservation.

1.3 Objectives

This research aimed to identify *Enterobacteriaceae* present in the fresh fecal samples of wreathed hornbill and to assess their antimicrobial susceptibility profiles. The findings were expected to support appropriate antimicrobial treatment strategies for captive wreathed hornbills, thereby enhancing their medical care and welfare.

1.4 Benefits

This research is expected to provide information regarding *Enterobacteriaceae* bacteria present in the feces of the wreathed hornbill and the current status of their antimicrobial resistance. The data obtained in this study are anticipated to offer veterinarians valuable guidance on the most appropriate antimicrobial for treating wreathed hornbills in captivity.

2. LITERATURE REVIEW

2.1 Wreathed Hornbill

The wreathed hornbill (Figure 1) is a species of bird from the family Bucerotidae and the genus *Rhyticeros.* Its scientific classification is as follows (Krishna *et al.* 2012):

Kingdom : Animalia
Phylum : Chordata
Class : Aves
Order : Bucerotiformes
Family : Bucerotidae
Genus : *Rhyticerotidae*
Species : *Rhyticeros undulatus*

Figure 1 Wreathed hornbill (*Rhyticeros undulatus*)
(personal documentation)

In Indonesia, this species can be found on Sumatra, Borneo, and Java islands. MacKinnon and Ramsay (2010) state that wreathed hornbills are birds with yellow beaks, light yellow irises, and black bodies. The male individual has a bright yellow neck pocket, while the female has a blue neck. The wreathed hornbill is classified as vulnerable and listed in CITES Appendix II (IUCN 2018). This classification indicates that the species faces a high risk of extinction in the wild, and trade of this species must be regulated to prevent exploitative actions that could threaten its survival. In Indonesia, wreathed hornbills are protected under the regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. 92/2018.

As a highly protected species in Indonesia, wreathed hornbills require significant efforts from various sectors to ensure their survival in the wild. With their natural habitat and population in decline, veterinarians play a crucial role in safeguarding the health and welfare of wreathed hornbills in captivity.

2.2 *Enterobacteriaceae*

The *Enterobacteriaceae* family is a diverse group of bacteria commonly found in various environments. They represent around 80% of Gram-negative bacterial isolates and are responsible for multiple diseases in humans, including urinary tract infections, pneumonia, diarrhea, meningitis, sepsis, endotoxic shock, and more (Ng *et al.* 2010). Many different genera and species within this family frequently cause infections in humans. In animals, members of the family *Enterobacteriaceae* can be divided into three groups depending on their pathogenicity. They include major animal pathogens like *Escherichia coli*, opportunistic pathogens that occasionally cause infections in animals like *Proteus* spp., *Enterobacter* spp., and *Citrobacter* spp., and organisms of uncertain importance for animals, such as *Erwinia* spp. and *Leclercia adecarboxylata*, and can also cause various infections, including urinary tract infections, pneumonia, and sepsis (Jesumirhewe *et al*. 2022).

Several studies have highlighted the significance of *Enterobacteriaceae* species in avian feces. A study by Beleza *et al*. (2019) shows that *Enterobacteriaceae* are commonly found to cause an infection in birds of the Passeriformes order. However, the presence of predisposing factors is necessary to trigger diseases in birds. *Enterobacteriaceae* are important potential pathogens in avian clinical medicine, which could cause both primary and secondary infections. In their study, it also showed that from 300 fecal samples obtained from various parrot species, there are 508 different isolates of bacteria from various species, highlighting the massive amount of bacterial diversity within avian species and its significance in avian medicine (Hidasi *et al*. 2013).

2.3 Gut Microbiota in Avian

The avian digestive system hosts a very complex and diverse microbiota that plays a crucial role in maintaining the functionality of the gastrointestinal tract. Additionally, immunity, nutrition, metabolism, and many other physiological functions are closely linked to the gut microbiota. According to research on wreathed hornbill, great hornbill, and toco toucan birds by Sun *et al.* (2018), the microbiota of wreathed hornbill was dominated by unclassified *Enterobacteriaceae* (0.1%–44%), *Pseudomonas* (1%–45%), *Lactobacillus* (9%–68%), *Acinetobacter* (0.3%–28%), and *Clostridium* (0%–21%). The great hornbill also showed similar results in the ratio of bacteria being comprised of unclassified *Enterobacteriaceae* (0.5%–33%), *Pseudomonas* (0.2%–37%), unclassified *Clostridiaceae* (9%–47%), unclassified *Streptophyta* (0%–41%), *Epulopiscium* (0.7%–19%), and *Fusobacterium* (0%–24%). These results show that Firmicutes and Proteobacteria were the dominant phyla in the gut microbiotas of the two omnivorous species examined in this study. These two phyla are also observed to be the most common phyla in the gut of other birds.

Research by Waite *et al.* (2014) revealed that on the digestive tract of kakapo, a critically endangered New Zealand parrot, phylum-level content consisted predominantly of Proteobacteria and Firmicutes, with a frequent presence of *Bacteroidetes* and *Actinobacteria* found in the kakapo fecal samples. Another study also highlights the diversity of microbiota in other types of birds. Roggenbuck *et al.* (2014) mention that on carnivorous birds, especially vultures, the most common phyla of bacteria that can be found are the Firmicutes, Proteobacteria, and

the Actinobacteria. These studies show our current understanding of the microbiota of various bird species with different diets and behaviors.

2.4 Antimicrobial Resistance in Avian

Antimicrobial resistance among *Enterobacteriaceae* in avian is a significant concern. *Enterobacteriaceae* have been found to show resistance towards various antimicrobials, with bacterial isolates showing greater resistance to amoxicillin (78.7%), ampicillin (75.4%), streptomycin (45.9%), and sulfonamides (42.6%) (Beleza *et al.* 2019). Among the other three most frequently occurring bacterial species (*Serratia liquefaciens*, *Enterobacter aerogenes*, and *Enterobacter cloacae*), each has a high absolute frequency of amoxicillin-resistant strains, with an isolate of *Enterobacter aerogenes* showing a resistance to all 12 antimicrobials used in that study (Beleza *et al.* 2019).

A study by Sigirci *et al.* (2019) mentions that samples obtained from birds in pet shops across Istanbul have shown the presence of various bacteria, such as *Pseudomonas* spp., *Salmonella* spp., and *Shigella* spp., that were resistant to tetracycline. That study stated that 65% of 150 isolates were resistant to tetracycline, and 58% of them were classified as multidrug resistant. These studies emphasize the necessity of identifying antimicrobial-resistant bacteria in avian species, especially those that have recurrent close contact with humans, such as birds in ex-situ conservation sites.

.

3. METHODS

3.1 Time and Place

The research was performed from November 2024 until February 2025. Samples were collected from Taman Burung, Taman Mini Indonesia Indah, Jakarta, under the management of Jagat Satwa Nusantara. The research was conducted in the Laboratory of Bacteriology, Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University.

3.2 Tools and Materials

The tools that were used during this research included gloves, masks, lab coats, inoculating loops or needles, sterile petri dishes, sterile cotton swabs, filter paper, test tubes, test tube racks, pipettes, sterile forceps, micropipettes, vortex tube mixers, Durham tubes, incubators at 37 °C, microscopes, Bunsen burners, glass slides, cover slips, and refrigerators.

The materials that were used in this research included fecal samples from 7 individuals of wreathed hornbills (*Rhyticeros undulatus*), NaCl 0.9%, KOH 3%, H_2O_2 3%, alcohol 70%, alcohol 96%, distilled water, Blood Agar (BA), MacConkey Agar (MCA), Tryptic Soy Agar (TSA), Mueller Hinton Agar (MHA), Gram staining reagents (including crystal violet, lugol, safranin, distilled water, and acetone alcohol), indole, xylol, immersion oil, oxidase reagent, methyl red (MR) reagent, Voges-Proskauer (VP) reagent, Ehrlich reagent, Simon's citrate, sulphur indole motility (SIM), Triple Sugar Iron Agar (TSIA), urease test media, sugars for fermentation tests (including glucose, lactose, sucrose, maltose, and mannitol), antimicrobial discs, peptone water, and disinfectants.

3.3 Methodology

3.3.1 Sample Collection

Fecal samples were collected from 7 wreathed hornbill individuals in Taman Mini Indonesia Indah, Jakarta. The fecal droppings from each bird were collected by placing a large clean sheet of plastic underneath the cage of the hornbills. The plastic was placed there for approximately 30 minutes to an hour. After that, the droppings were collected using sterile forceps or other sterile tools and put inside a clear zip lock bag. The bags were properly labelled before being placed inside a cooler box to maintain the viability of the samples, and were brought to the Laboratory of Bacteriology, SVMBS, IPB University.

3.3.2 Bacteria Isolation from Fecal Sample

The fecal sample was diluted using a 0.9% NaCl solution at a 1:9 ratio, then homogenized using a vortex. The resulting bacterial suspension was taken with an inoculating loop and inoculated onto BA and MCA. The inoculation method used the three-quadrant or plate T method with the streak plate technique. The petri dish was then incubated at 37 °C for 24-48 hours. Bacterial inoculation was performed under sterile conditions, with the inoculating loop sterilized before streaking, done near a Bunsen burner, and the petri dish opened as little as possible. After incubation, the separated single colonies were cultured on TSA, then incubated at 37 °C for 24 hours.

3.3.3 Bacteria Identification from Isolates

Bacterial identification was performed by observing colonies macroscopically, including their morphology, color, edges, and elevation on selective media. A pure culture was taken from the TSA plate and placed on a microscope slide, followed by Gram staining for microscopic identification. The 3% KOH test was used to distinguish between Gram-positive and Gram-negative bacteria, where a positive result for Gram-negative bacteria is indicated by the formation of a slimy texture. This test was performed by smearing the bacterial colony onto a slide and adding a few drops of 3% KOH reagent. The oxidase test was done by placing a few drops of oxidase reagent on filter paper. In contrast, the catalase test involves smearing the bacterial colony on a slide and adding a few drops of catalase reagent (Green and Goldman 2021).

The urease, TSIA, and Simmon's Citrate tests were conducted by inoculating the bacterial culture onto the respective media. Color changes in the media were observed after 24–48 hours. The SIM test was also performed, and media changes were observed after 24 hours, followed by the addition of Ehrlich reagent to the surface of the media. The Methyl Red test was carried out by inoculating the bacterial culture and incubating it for 24 hours, after which 3–5 drops of methyl red reagent were added. The Voges-Proskauer test was performed by inoculating the bacterial culture, incubating it for 48 hours, and then adding five drops of alpha-naphthol and 10 drops of 40% KOH to the medium. The carbohydrate fermentation tests included glucose, sucrose, maltose, mannitol, and lactose. These tests determined the bacteria's ability to ferment these sugars. A color change from red to yellow in the media indicated a positive result (Green and Goldman 2021).

3.3.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method (CLSI 2023). First, colonies from the culture were picked up with a loop and placed into a test tube containing 0.9% NaCl, then mixed thoroughly with a vortex. The bacterial suspension was then standardized using the 0.5 McFarland suspension. After that, 100 µL of the suspension was pipetted onto MHA and spread evenly using a sterile cotton swab. Once the surface had settled, antimicrobial disks (ampicillin 10 µg, doxycycline 30 µg, gentamicin 10 µg, ciprofloxacin five µg, trimethoprim-sulphamethoxazole 1.25/23.75 µg, chloramphenicol 30 µg) were carefully placed on the MHA plates. Each test was repeated twice to ensure reproducibility. The plates were then incubated at 37 °C for 24 hours. After incubation, the inhibition zones around each antimicrobial disc were measured with a caliper or ruler. Each test was performed in duplicate. These measurements were compared against the standard antimicrobial sensitivity guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) to determine the resistance levels of bacteria (CLSI 2023)(Table 1).

Table 1 Breakpoints of antimicrobial susceptibility testing using the disk diffusion method

Antimicrobial	Class	Dose (µg)	Diameter of Inhibitory Zone (mm)*		
			Sensitive	Intermediate	Resistant
Ampicillin	Penicillin	10	≥ 17	14–16	≤ 13
Doxycycline	Tetracycline	30	≥ 14	11–13	≤ 10
Trimethoprim-sulfamethoxazole	Sulfonamide	1.25/ 23.75	≥ 16	11–15	≤ 10
Chloramphenicol	Amphenicol	30	≥ 18	13–17	≤ 12
Ciprofloxacin	Fluoroquinolone	5	≥ 26	22–25	≤ 21
Gentamicin	Aminoglycoside	10	≥ 18	15–17	≤ 14

*Reference: CLSI (2023)

3.4 Data Analysis

The obtained data were analyzed descriptively and quantitatively. The data were presented in tables and images containing results from isolation, identification, characterization, and inhibition zones from the antimicrobial susceptibility test of *Enterobacteriaceae* bacteria isolates from the fecal sample of wreathed hornbills.

4. RESULT AND DISCUSSION

4.1 Bacterial Identification

Fecal samples from seven wreathed hornbills were cultured on MacConkey agar (MCA) (Figure 2) and blood agar (BA) (Figure 3) to isolate *Enterobacteriaceae*. A total of 16 isolates were obtained, with 14 from MCA: 13 showed pink coloration and one was pale yellow (Table 2). Lactose fermenters appear pink on MCA, while non-lactose fermenters are colorless or pale (Mazumder *et al.* 2022). MCA is a selective and differential medium that supports the growth of Gram-negative bacteria while inhibiting Gram-positive bacteria (Jung and Hoilat 2024).

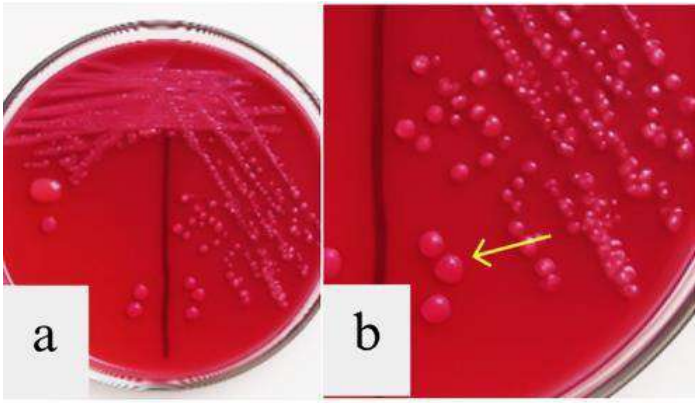

Figure 2 Macroscopical examination of bacterial isolates on MacConkey Agar (a); closer view of isolated colony (b)

Table 2 Macroscopic and microscopic examination of isolates on MacConkey agar

Bird Code	Isolate Code	Macroscopic		Microscopic	
		Color	Lactose Fermentation	Shape	Gram-staining
JE02	JE02M1	Pink	+	Coccobacilli	Gram-negative
JE05	JE05M1	Pink	+	Coccobacilli	Gram-negative
	JE05M2	Pink	+	Coccobacilli	Gram-negative
	JE05M3	Pink	+	Coccobacilli	Gram-negative
	JE05M4	Pink	+	Coccobacilli	Gram-negative
JE04	JE04M1	Pink	+	Coccobacilli	Gram-negative
JE08	JE08M1	Pale Pink	+	Coccobacilli	Gram-negative
	JE08M3	Pink	+	Coccobacilli	Gram-negative
JE09	JE09M1	Pink Edges	+	Coccobacilli	Gram-negative
JE12	JE12M1	Pink	+	Cocci	Gram-negative
	JE12M2	Pale Yellow	+	Coccobacilli	Gram-negative
	JE12M3	Pink	+	Bacilli	Gram-negative
JE32	JE32M1	Pink	+	Coccobacilli	Gram-negative
	JE32M2	Pink	+	Coccobacilli	Gram-negative

From the BA (Figure 3), two isolates were isolated, in which one isolates produced β-hemolysis while the other produced γ-hemolysis (Table 3). Blood agar is used as a medium because it is a differential medium that can distinguish bacteria based on their hemolytic character (Turista and Puspitasari 2019). A β-hemolytic reaction implies the complete lysis of red blood cells, which causes a clear zone on the agar surrounding the colony. Meanwhile, the α-hemolytic response is when the hemoglobin in the red blood cells is reduced to methemoglobin, causing a greenish discoloration surrounding the colonies. Lastly, γ-hemolysis had no hemolysis (Mogrovejo *et al.* 2020).

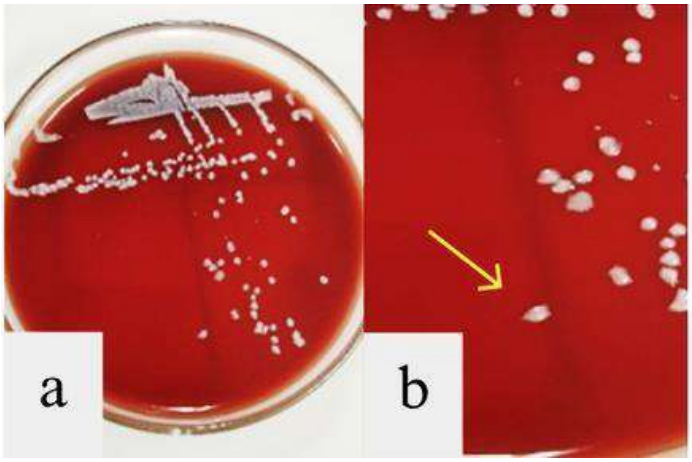

Figure 3 Macroscopical examination of bacterial isolates on blood agar (a); closer view of isolated colony (b)

Table 3 Macroscopic and microscopic examination of isolates on blood agar

Bird Code	Isolate Code	Macroscopic		Microscopic	
		Colony color	Hemolysis	Shape	Gram-staining
JE05	JE05B1	White	β-Hemolysis	Coccobacilli	Gram-negative
JE08	JE08B1	White	γ-Hemolysis	Cocci	Gram-negative

In addition to the macroscopical examination, Gram-staining was also done to observe the microscopical structure and shape of the bacterium. Based on Gram staining, all 16 isolates were identified as Gram-negative bacteria, which are characterized by the pink color of the isolates under a microscope, as seen in Figure 4. Gram-positive bacteria have thick peptidoglycan walls without an outer membrane, while Gram-negative bacteria have thinner peptidoglycan layers and lipid-rich outer membranes. In Gram staining, crystal violet stains all cells purple, and iodine forms large dye complexes. Alcohol removes the outer membrane in Gram-negative bacteria, making them lose the purple dye, while Gram-positive bacteria retain the dye. Safranin was added, and then the colorless Gram-negative cells were stained pink. Thus, Gram-positive bacteria appear purple and Gram-negative bacteria appear pink or red under a microscope (Paray *et al.* 2023). From the obtained results, most colonies were circular in shape, shiny, and small in size. The colonies were either pale yellow or pink. KOH 3% test was also done on the

isolated bacteria to further confirm the Gram identity of each isolate, with every isolate showing a positive result, which is indicative of Gram-negative bacteria. The KOH string test was used to distinguish bacterial characteristics based on the formation of mucus when bacterial colonies are reacted with KOH 3% reagent. Gram-negative bacterial colonies will show a positive reaction with the formation of mucus, while Gram-positive bacterial colonies will not show any reaction (Rinihapsari and Julianasya 2021).

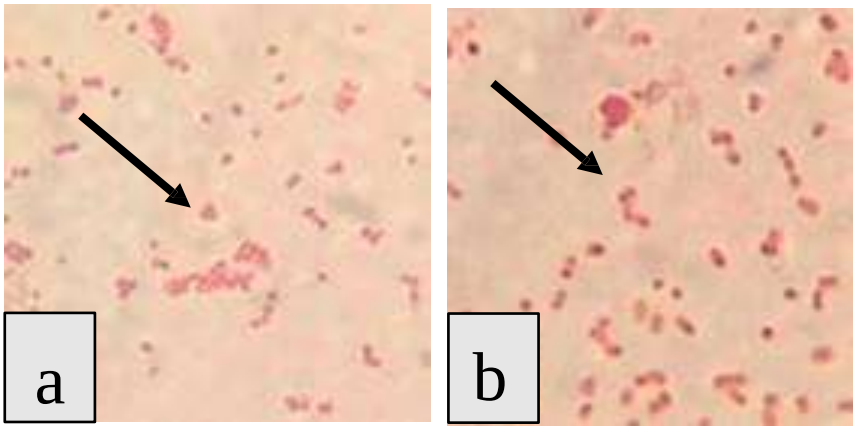

Figure 4 Microscopical examination of bacteria colored using Gram-staining. Magnification: 10x100. Cocci-shaped bacteria of JE12M1 isolate (a), Coccoid-shaped bacteria of JE32M2 isolate (b).

Further identification refers to the biochemical test results (Cowan and Steel 2003). According to Arbefeville *et al*. (2024), the difference in bacterial biochemical characteristics, such as protein and fat metabolism, carbohydrate metabolism, and enzyme production, shows enough information to help classify bacteria into different groups based on their reactions. Based on biochemical test results (Table 4), 16 bacterial isolates were identified consisting of four isolates of *Klebsiella* genus (25%), four isolates of *Escherichia* genus (25%), three isolates of *Yersinia* genus (18.75%), two isolates of *Citrobacter* genus (12.5%), two isolates of *Proteus* genus (12.5%), and one isolate of *Enterobacter cloacae* (6.25%).

Table 4 Biochemical test results of isolates from wreathed hornbill fecal samples

Bird code	Isolate	O	I	M	C	MR	VP	U	TSIA	G	Mn	Mal	S	L	Genus/ Species
JE02	JE02M1	-	-	-	+	-	+	+	A/A	+	+	+	+	+	*Klebsiella pneumoniae*
JE05	JE05M1	-	-	-	+	-	+	+	A/A	+	+	+	+	+	*Klebsiella pneumoniae*
	JE05M2	-	+	+	d	+	-	+	K/A/H_2S	+	+	+	+	+	*Proteus* sp.
	JE05M3	-	+	+	-	+	-	-	A/A	+	+	+	d	+	*Escherichia coli*
	JE05M4	-	-	+	+	d	d	d	A/A	+	+	+	+	d	*Enterobacter cloacae*
	JE05B1	-	d	+	+	+	-	+	K/A/H_2S	+	+	+	+	+	*Citrobacter freundii*
JE04	JE04M1	-	+	-	-	+	-	-	A/A	+	+	+	+	+	*Escherichia coli*
JE08	JE08M1	-	+	+	+	-	-	+	A/A	+	+	+	+	+	*Citrobacter* sp.
	JE08M3	-	+	-	-	+	-	-	A/A/gas	+	+	+	+	+	*Escherichia coli*
	JE08B1	-	-	-	-	+	+	+	A/A	+	+	d	+	-	*Yersinia enterocolitica*
JE09	JE09M1	-	-	-	-	+	-	+	A/A	+	+	d	+	+	*Yersinia* sp.
JE12	JE12M1	-	+	-	-	+	+	-	A/A	+	+	+	+	+	*Escherichia coli*
	JE12M2	-	+	-	-	d	-	-	A/A	+	+	d	+	+	*Yersinia* sp.
	JE12M3	-	-	+	-	+	-	+	A/A/H_2S	+	-	-	-	+	*Proteus* sp.
JE32	JE32M1	-	+	-	-	-	+	+	A/A	+	+	+	+	+	*Klebsiella oxytoca*
	JE32M2	-	-	-	+	-	+	+	A/A	+	+	+	+	+	*Klebsiella pneumoniae*

Description: O = Oxidase Test; I = Indole Test; ; M = Motility; C = Simmon's Citrate Test; MR = Methyl Red Test; VP = Voges-Proskauer Test; U = Urease Test; TSIA = Triple Sugar Iron Agar Test; TSIA = Triple Sugar Iron Agar; G = Glucose; Mn = Mannitol; Mal = Maltose; G = Glucose; S = Sucrose; L = Lactose; + = positive reaction, - = negative reaction; d = dubius; A/A = Acid slant/Acid butt/no gas/; A/A/gas = Acid slant/Acid butt/gas production; A/A/ H_2S = Acid slant/Acid butt/ H_2S production; K/A/H_2S = Alkaline slant/Acidic butt/no gas/H_2S production.

From seven fecal samples of wreathed hornbill birds, four *Klebsiella* spp. isolates were identified in three birds (42.8%). These four *Klebsiella* spp. isolates were identified as three isolates of *Klebsiella pneumoniae* (JE02M1, JE05M1, JE32M2) and one isolate as *Klebsiella oxytoca* (JE32M1). *Klebsiella pneumoniae* is a Gram-negative, non-motile, bacilliform, late lactose-fermenting bacterium (Nakhaee *et al.* 2022). It is an encapsulated opportunistic pathogen member of the *Enterobacteriaceae* family. *Klebsiella oxytoca* is also a Gram-negative pathogenic bacterium of environmental origin, which can cause infection in healthcare settings. This species mainly resides in the intestinal system as a commensal bacterium. However, it can also spread to the bloodstream and cause infection, especially in individuals with an immunosuppressed condition. This species of bacterium is also commonly associated with septicemia, pneumonia, and urinary tract infections in humans and animals (Moradigaravand *et al.* 2017; Chang *et al.* 2018). The *Klebsiella* genus is classified into a wide variety of species, namely *K. indica, K. terrigena, K. spallanzanii, K. huaxiensis, K. oxytoca, K. grimontii, K. pasteurii,* and *K. michiganensis* (Dong *et al.* 2022). *Klebsiella pneumoniae* and *Klebsiella oxytoca* were isolated from stool samples and oropharyngeal swabs of several species of parrots, gulls, and passerines that are clinically healthy (Davies *et al*. 2015; Chang *et al.* 2018). However, *K. pneumoniae* frequently acts as a respiratory pathogen among immunosuppressed birds. Systemic infections are more common in birds, but local infections involving the upper respiratory tract, skin, oral cavity, and crop may occur, especially in psittacine birds (Davies *et al.* 2015). This bacterium can cause kidney failure, lung infections, and encephalitis in birds (Davies *et al.* 2015). Additionally, *Klebsiella* infections are frequently associated with respiratory and urinary tract infections, sepsis, and mastitis in other animals. These bacteria can also develop resistance towards multiple antimicrobials and can cause nosocomial infections in animals (Nakhaee *et al.* 2022).

The *Escherichia* genus was present in 3 of 7 wreathed hornbills (42.8%). Out of the 16 isolates, four isolates (JE04M1, JE05M3, JE08M3, and JE12M1) (25%) were identified as *Escherichia coli*. *Escherichia coli* is a Gram-negative bacterium that exists as part of the normal gut microbiota in humans and animals, but can act as an opportunistic pathogen under certain conditions. When found outside the intestinal tract, these bacteria can cause urinary tract infections, pneumonia, bacteremia, and peritonitis, among others. Its virulence factors enable *E. coli* to evade the host's immune system and develop resistance to commonly used antimicrobials (Nasrollahian *et al.* 2024). *Escherichia coli* are commensal bacteria in the gastrointestinal tract, the pharynx, and trachea of birds, animals, and humans (Guabiraba and Schoulder 2015). However, some *E. coli* strains are known to cause serious diseases such as cystitis and colibacillosis in birds, and pyelonephritis, sepsis, and gastroenteritis in humans due to their various virulence factors (Stromberg *et al.* 2017). These strains are known as extraintestinal pathogenic *E. coli*, which cause diseases outside the gastrointestinal tract, and are epidemiologically and phylogenetically distinct from intestinal pathogenic *E. coli* (Fancher *et al.* 2021).

Three *Yersinia* spp. isolates (JE08B1, JE09M1, and JE12M2) out of sixteen isolates (18.75%) were identified in three individuals. The genus *Yersinia* is a group of Gram-negative, rod-shaped bacteria that consists of 11 species, with three species that are particularly important due to their role in causing human diseases (Delibato

et al. 2018). These include *Yersinia pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*. Two species of this genus, namely, *Y. enterocolitica and Y. pseudotuberculosis*, can cause yersiniosis, which is an acute gastroenteritis in humans and agricultural animals, especially swine (Seabaugh and Anderson 2024). Yersiniosis in birds most commonly causes sudden death, with the clinical symptoms being lethargy, diarrhea, and anorexia. Bright green feces have also been reported in blue-fronted amazons (*Amazona aestiva*) and yellow-headed amazons (*A. oratrix*), which were likely related to anorexia or hepatic changes observed during necropsy (Galosi *et al.* 2015). A study reveals that out of 468 fecal samples from 57 different species of migratory birds, 12.8% of collected samples were found to have *Yersinia* spp. Most isolated *Yersinia* strains belong to nonpathogenic species. However, pathogenic strains of *Y. pseudotuberculosis* and *Y. enterocolitica* have also been isolated from two song thrushes (*Turdus philomelos*) and one redwing bird (*T. iliacus*) (Niskanen *et al.* 2003).

Bacteria from the genus *Citrobacter* were found in two wreathed hornbills. Two (JE05B1 and JE08M1) out of 16 isolates (12.5%) were identified as *Citrobacter* sp., with one isolate (JE05B1) being further classified as *Citrobacter freundii. Citrobacter* spp. are Gram-negative, non-spore-forming, rod-shaped bacteria that are facultative anaerobes belonging to the *Enterobacteriaceae* family (Jabeen *et al.* 2023). They are commonly isolated from various environments, including soil, sewage, sludge, water, food, and the intestinal tracts of both animals and humans. *Citrobacter* spp. are also considered opportunistic nosocomial pathogens that are often associated with urinary tract infections (UTIs), bloodstream infections, intra-abdominal sepsis, brain abscesses, pneumonia, and other neonatal infections such as meningitis, neonatal sepsis, joint infections, or common bacteremia (Jabeen *et al.* 2023). *Citrobacter* spp. have been identified in various exotic birds in the past. A sun conure (*Aratinga solstitialis)* was admitted to a clinic due to symptoms of diarrhea and lameness. Biochemical tests revealed the presence of *Citrobacter diversus* in the fecal sample (Ajayan *et al*. 2024). In a separate case, a pair of Australian king parrots (Alisterus scapularis) underwent necropsy, revealing *Citrobacter freundii* in the liver and spleen of both birds. No parasitic or other bacterial infections were detected aside from *C. freundii*, indicating that its presence was not a secondary infection. This suggests that under certain conditions, an opportunistic pathogen like *C. freundii* can breach the intestinal mucosa and lead to severe bacteremia (Churria *et al*. 2014).

Two isolates (12.5%) (JE05M2, JE12M3) were identified as *Proteus* sp. and were found in two fecal samples of wreathed hornbills. *Proteus* spp. are Gram-negative, facultatively anaerobic, heterotrophic, and proteolytic rod-shaped bacteria that belong to the family *Enterobacteriaceae*. The other members of the Protea family are *Proteus*, *Providencia*, and *Morganella. P. mirabilis, P. penneri, P. vulgaris, P. myxofaciens, and P. hauseri* make up the genus *Proteus* (Anju *et al.* 2023). These bacteria are commonly found in various environments, including soil, water, and the gastrointestinal tracts of humans and animals. *Proteus* spp. is also an opportunistic pathogen frequently associated with urinary tract infections, wound infections, and nosocomial infections, particularly in immunocompromised individuals. Among them, *P. mirabilis* is the most clinically significant species, responsible for most *Proteus*-related infections (Drzewiecka 2016). The *Proteus* genus has also been reported in wild birds. A study reported

that lovebirds (*Agapornis* sp.) infected with *Proteus* sp. exhibited signs of upper respiratory tract infection, including seropurulent nasal shedding. In contrast, Magellanic penguins (*Spheniscus magellanicus*) showed clinical signs of deep pododermatitis in the footpad region (Olinda *et al*. 2012). Humidity, poor hygiene, trauma, improper diet, and inadequate housing predispose birds to bacterial infection (Olinda *et al.* 2012). This shows *Proteus* characteristics as an opportunistic pathogen typically considered non-pathogenic, which can still lead to infections and diseases, particularly when the host defense mechanisms are compromised.

One isolate (JE5M4) was identified as *Enterobacter cloacae. Enterobacter* is a genus of Gram-negative, rod-shaped, facultatively anaerobic bacteria of the *Enterobacteriaceae* family. It is a non-spore-forming, flagella-containing, urease-positive, and lactose-fermenting bacterium. In humans, *Enterobacter* infections are associated with an extensive range of clinical manifestations such as bacteremia, lower respiratory tract infections, surgical site infections, and urinary tract infections. *Enterobacter* infections can have similar clinical presentations to other facultative anaerobic gram-negative rod bacterial infections, so they can often be indistinguishable (Ramirez and Giron 2023). In birds, *Enterobacter* spp. are also regarded as opportunistic pathogens that can multiply and cause intestinal or extra-intestinal infections under certain conditions. In a study of 167 cloacal swabs collected from psittacine species, 20% of the bacteria isolated were *Enterobacter cloacae.* The *Enterobacter* genus is considered necessary in birds due to their association with systemic infections in the respiratory and intestinal systems. However, species *like E. sakazakii*, *E. cloacae*, and *E. aerogenes* can be isolated from clinically healthy birds (Lopes *et al.* 2015).

4.2 Antimicrobial Resistance of Bacteria

All isolated bacteria (n=16) from the feces of wreathed hornbills in this study were tested for their antimicrobial susceptibility using the Kirby-Bauer disc diffusion method and interpreted according to CLSI (2023) by measuring the diameter of the inhibition zone surrounding the antimicrobial discs as seen in Figure 5. Results showed various resistance levels towards six different antimicrobials (Table 5, Table 6): chloramphenicol, ampicillin, ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, and doxycycline.

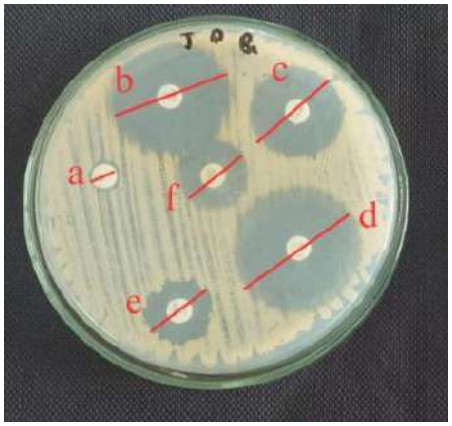

Figure 5 Kirby-Bauer disc diffusion test result: inhibition zones of chloramphenicol (a); sulfamethoxazole/trimethoprim (b); gentamicin (c); ciprofloxacin (d); ampicillin (e); doxycycline (f).

Table 5 Antimicrobial susceptibility of bacteria isolated from the feces of wreathed hornbills

Bird Code	Isolate Code	Species	Antimicrobial Susceptibility Test*					
			AMP	DO	CN	CIP	SxT	C
JE02	JE02M1	*Klebsiella pneumoniae*	S	R	S	S	S	S
JE05	JE05M1	*Klebsiella pneumoniae*	R	R	I	S	S	I
	JE05M2	*Proteus* sp.	S	S	I	S	S	S
	JE05M3	*Escherichia coli*	S	R	S	S	S	S
	JE05M4	*Enterobacter cloacae*	S	R	S	S	S	I
	JE05B1	*Citrobacter freundii*	S	R	S	S	S	S
JE04	JE04M1	*Escherichia coli*	R	R	S	S	S	S
JE08	JE08M1	*Citrobacter* sp.	S	S	S	S	S	S
	JE08M3	*Escherichia coli*	S	S	S	S	S	S
	JE08B1	*Yersinia enterocolitica*	I	S	S	S	S	R
JE09	JE09M1	*Yersinia* sp.	R	S	S	S	S	S
JE12	JE12M1	*Escherichia coli*	R	R	S	S	S	S
	JE12M2	*Yersinia* sp.	S	R	S	S	S	S
	JE12M3	*Proteus* sp.	R	R	S	S	S	S
JE32	JE32M1	*Klebsiella oxytoca*	I	R	S	S	S	S
	JE32M2	*Klebsiella pneumoniae*	I	S	S	S	S	S

Description: AMP = Ampicillin 10 µg; DO = Doxycycline 30 µg; CN = Gentamicin 10 µg; CIP = Ciprofloxacin 5 µg; SXT = Trimethoprim-Sulfamethoxazole 1.25 µg /23.75 µg; C = Chloramphenicol 30 µg; S = susceptible; I = Intermediate; R = Resistant.

Table 6 Percentage of susceptible-resistant bacteria to antimicrobials tested

Anti-microbials	Antimicrobial Class	Number of Isolates (Percentage %) (n=16)		
		Susceptible	Intermediate	Resistant
DO	Tetracycline	6 (37.5%)	0 (0%)	10 (62.5%)
AMP	Penicillin	8 (50%)	3 (18.75%)	5 (31.25%)
C	Phenicols	13 (81.25%)	2 (12.5%)	1 (6.25%)
CN	Aminoglycosides	14 (87.5%)	2 (12.5%)	0 (0%)
SXT	Folate Pathway Antagonists	16 (100%)	0(0%)	0 (0%)
CIP	Fluoroquinolones	16 (100%)	0 (0%)	0 (0%)

Description: AMP = Ampicillin 10 µg; DO = Doxycycline 30 µg; CN = Gentamicin 10 µg; CIP = Ciprofloxacin 5 µg; SXT = Trimethoprim-Sulfamethoxazole 1.25 µg /23.75 µg; C = Chloramphenicol 30 µg.

Based on the antimicrobial susceptibility test results in Tables 5 and 6, ten isolates (62.5%) were resistant to doxycycline, five isolates were resistant to ampicillin (31.1%), and one isolate (6.25%) was resistant to chloramphenicol. Intermediate susceptibility was also seen with three isolates (18.75%) to ampicillin, two isolates (12.75%) to gentamicin, and two (12.75%) isolates to chloramphenicol. The remaining isolates were susceptible to the tested antimicrobials. Specifically, all sixteen isolates (100%) were susceptible to ciprofloxacin and trimethoprim/sulfamethoxazole. Fourteen isolates (87.5%) showed susceptibility to gentamicin, thirteen isolates (81.75%) were susceptible to chloramphenicol, eight isolates (50%) were susceptible to ampicillin, and six isolates (37.5%) were susceptible to doxycycline. These results showed that doxycycline has the highest resistance level among the tested antimicrobials, while ciprofloxacin and trimethoprim/sulfamethoxazole have the lowest resistance level. No isolate is considered multidrug resistant, or resistant to three or more classes of antimicrobials (Catalano *et al.* 2022).

Ten isolates (62.5%) were resistant to doxycycline. These isolates were JE02M1 (*K. pneumoniae*), JE05M1 (*K. pneumoniae*), JE05M3 (*E. coli*), JE05M4 (*E. cloacae*), JE05B1 (*C. freundii*), JE04M1 (*Escherichia* sp.), JE12M1 (*E. coli*), JE12M2 (*Yersinia* sp.), JE12M3 (*Proteus* sp.), and JE32M1 (*Klebsiella* sp.). Doxycycline is a part of the tetracycline antimicrobial that kills and prevents the growth of Gram-positive and Gram-negative bacteria. It works by binding the bacterial ribosome and preventing amino acids from attaching themselves to the ribosome of the bacteria, which would then disrupt proper protein formation and, in turn, lead to bacterial death (Chopra and Roberts 2001). A lot of bacteria from the *Enterobacteriaceae* family are resistant to doxycycline. A study reported that 86% *of Klebsiella* isolates, 90% of *Citrobacter* isolates, 92% of *Proteus* isolates, and 55% of *E. coli* bacteria isolated from the fecal sample and rectal swab of 151 dogs and 182 cats were resistant to doxycycline (Aleshina *et al*. 2024). Due to the overuse of antimicrobials in veterinary medicine, some *Yersinia* sp. isolated from pigs were resistant to doxycycline; however, the number varies across countries,

ranging from 31% of samples in Croatia to 62.5% in Malaysia (Angelovska *et al.* 2023).

Five isolates out of 16 (31.2%) were resistant to ampicillin, namely JE05M2 (*Proteus sp*), JE08M1 (*Citrobacter* sp.), JE09M1 (*Yersinia* sp.), JE12M1 (*Escherichia coli*), and JE12M3 (*Proteus* sp.). Additionally, three isolates (16.6%), which are JE08B1 (*Yersinia* sp.), JE32M1 (*Klebsiella* sp.) and JE32M2 (*Klebsiella pneumoniae*) showed intermediate susceptibility. Ampicillin is a type of beta-lactam antimicrobial that kills bacteria by stopping them from building cell walls by binding to penicillin-binding proteins, which prevents the bacteria from creating a strong protective wall (Bereda 2022). However, some bacteria produce an enzyme called penicillinase that breaks the beta-lactam ring, thus creating resistance (Penwell *et al.* 2015). The obtained results align with a study by Bedenić *et al*. 2025, that shows bacteria from the genus *Proteus* that are isolated from a hospital environment have been known to produce TEM-52, a type of beta-lactamase enzyme, which causes resistance to ampicillin. *Citrobacter freundii* isolated from the hospital environment contains a carbapenemase-producing gene, which causes the bacteria to be able to resist beta-lactam antimicrobials (Yao *et al.* 2021). In that study, all carbapenemase-producing *Citrobacter* isolates were resistant to ampicillin. *Yersinia* spp. isolated from wild boars exhibited resistance to ampicillin, with 98% of *Y. enterocolitica* and 13% of *Y. pseudotuberculosis* affected (Hulankova 2022). Similarly, *Escherichia coli* isolated from a human patient also demonstrated resistance to ampicillin (Li *et al*. 2019). *Klebsiella pneumoniae* is naturally resistant to ampicillin because it possesses the SHV-1 penicillinase enzyme encoded in its chromosome (Wyres and Holt 2018). Meanwhile, resistance to other antimicrobials may sometimes arise through mutations within its DNA. Most antimicrobial resistance in *K. pneumoniae* occurs by acquiring resistance genes from other bacteria via horizontal gene transfer by sharing large conjugative plasmids.

Isolate JE08B1 (*Yersinia* sp.) was resistant to chloramphenicol, while two other isolates (12.5%), comprising JE05M1 (*K. pneumoniae*) and JE05M4 (*E. cloacae*), had intermediate susceptibility. Chloramphenicol is a synthetically manufactured broad-spectrum antimicrobial that inhibits protein synthesis (Bale *et al.* 2023). It belongs to its antimicrobial class and its derivatives such as florfenicol, thiamphenicol, and azidamphenicol. Thiamphenicol and azidamphenicol are used alongside chloramphenicol in human medicine. Florfenicol, on the other hand, is often used in veterinary medicine. Factors that influence chloramphenicol resistance are chloramphenicol acetyltransferases, which inactivate chloramphenicol through acetylation, and efflux pumps that actively remove the drug from bacterial cells upon entry (Bale *et al.* 2023). This study aligns with previous studies where *Klebsiella pneumoniae* isolated from duck cloacal swabs was resistant to chloramphenicol (Thesia *et al.* 2025). *Yersinia* sp. isolates collected from swine fecal samples also showed resistance to chloramphenicol (Angelovska *et al.* 2023). A study reported that 28% of *Enterobacter* spp. *Resisted* chloramphenicol (Sood 2016).

Two isolates (12.5%), namely JE05M2 (*Proteus* sp.) and JE05M3 (*Escherichia coli*), displayed intermediate susceptibility to gentamicin, while the rest of the isolates were susceptible. Gentamicin is a part of the aminoglycoside antimicrobial class and has bactericidal activity against aerobic Gram-

negative bacteria. Gentamicin binds to the 16S rRNA of the 30S ribosomal subunit, interfering with mRNA translation and causing the production of incomplete or non-functional proteins (Beganovic *et al.* 2018). It is suggested that inserting these defective proteins into the cell wall may weaken its structure. The most common mechanism of aminoglycoside resistance is modifying the antimicrobial with special enzymes called aminoglycoside-modifying enzymes (AMEs). These enzymes change parts of the aminoglycoside molecule, which prevents it from binding effectively to the bacterial ribosome (Tsodikova and Labby 2015).

All sixteen isolates (100%) were susceptible to ciprofloxacin. Ciprofloxacin is an antimicrobial agent in the fluoroquinolone class used to treat bacterial infections such as urinary tract infections and pneumonia. It works by inhibiting bacterial DNA topoisomerase and DNA gyrase, enzymes that play a role in DNA replication. Mutations in the DNA gyrase, plasmid transfer, and efflux pumps can make bacteria more resistant to fluoroquinolones like ciprofloxacin (Shariati *et al.* 2022).

All 16 isolates (100%) were susceptible to trimethoprim-sulfamethoxazole. Sulfamethoxazole is a sulfonamide antimicrobial that interferes with folate production in bacteria. It competes with p-aminobenzoic acid (PABA) and blocks the enzyme dihydropteroate synthase, which stops the formation of dihydrofolate. Trimethoprim blocks a different enzyme called dihydrofolate reductase, preventing the production of the active form of folate, tetrahydrofolate (Eyler and Shvets 2019). When combined, sulfamethoxazole and trimethoprim create a more substantial, synergistic effect by blocking two steps in the folate pathway. Since tetrahydrofolate is needed to make DNA and proteins, this combination can kill bacteria (bactericidal), whereas each drug alone only stops bacterial growth (bacteriostatic). Resistance towards sulfamethoxazole-trimethoprim antimicrobial is due to a mutation in the bacteria that causes the bacteria to acquire genes that allow folate synthesis to continue even in the presence of sulfonamides. Bacteria can also mutate to bypass the need for folate-dependent DNA synthesis, making them resistant to trimethoprim (Cattoir 2022).

This study proved that doxycycline was the least effective antimicrobial, with 62.5% of tested bacteria showing resistance, followed by ampicillin, where 31.25% of bacteria tested showed resistance, and 18.8% showed intermediate susceptibility. On the other hand, ciprofloxacin and trimethoprim-sulfamethoxazole were the most effective antimicrobials, as all isolates were susceptible to these antimicrobials. However, the usage of fluoroquinolone antimicrobial class, such as ciprofloxacin must be strictly supervised. The World Health Organization (WHO) in 2018 classified fluoroquinolones, including ciprofloxacin, as *Highest Priority Critically Important Antimicrobials* (HPCIAs). This designation is based on three prioritization factors: their use in treating serious infections in high-risk populations with limited therapeutic alternatives, their frequent use in human medicine, and documented evidence of resistance transmission from non-human sources. In Indonesia, fluoroquinolones are likewise classified as prescription-only drugs under Regulation of the Minister of Agriculture No. 14/PK.350/5/2017, requiring strict veterinary oversight and cautious administration. In contrast, trimethoprim-sulfamethoxazole, a folate pathway antagonist, is not classified as a hard drug under Indonesian law and is not ranked as highly as ciprofloxacin on the WHO list of critically important antimicrobials. Considering this, both drugs could potentially

be used in the antimicrobial treatment of wreathed hornbills in Taman Mini Indonesia Indah. However, it is essential for veterinarians to conduct appropriate monitoring to minimize the risk of developing antimicrobial resistance. These findings emphasize the importance of carefully selecting antimicrobial treatments for wreathed hornbills to ensure optimal effectiveness in addressing bacterial infections. Additionally, it is crucial to administer antimicrobial therapy judiciously to prevent the emergence and spread of resistant bacteria in the ex-situ conservation area.

5. CONCLUSION AND SUGGESTION

5.1 Conclusion

The study identified and characterized a diverse range of *Enterobacteriaceae* from 6 genera from the fecal samples of wreathed hornbills, namely *Citrobacter*, *Escherichia, Enterobacter, Klebsiella, Yersinia*, and *Proteus. Escherichia* spp. and *Klebsiella* spp. were the most commonly isolated bacteria in this research. Most of the isolates were resistant to doxycycline, followed by ampicillin. However, no multidrug-resistant isolates were detected. Furthermore, all isolates were susceptible to trimethoprim-sulfamethoxazole and ciprofloxacin, making it the most effective antimicrobial tested in this research. These findings highlight the importance of microbiological monitoring in captive wreathed hornbills and the importance of selective antimicrobial treatments to reduce the risk of antimicrobial resistance within the conservation site.

5.2 Suggestion

Further testing to confirm the identity of the bacteria can be done, such as using a more complete biochemical testing, PCR, and 16S rRNA sequencing. Further research on antimicrobial resistance using different antimicrobials can help expand antimicrobial resistance data in wreathed hornbills towards different classes of antimicrobials. Additional research in other species of hornbills should be done to develop further data regarding bacterial identification and antimicrobial resistance in birds in ex-situ conservation.

REFERENCES

Aleshina YM, Yeleussizova A, Mendybayeva A, Shevchenko P, Rychshanova R. 2024. Prevalence and antimicrobial resistance of *Enterobacteriaceae* in the north of Kazakhstan. *Open Veterinary Journal*. 14(2):604-616.

Alfalasifa N, Dewi B. 2019. Konservasi satwa liar secara ex-situ di taman satwa lembah hijau bandar lampung. *Jurnal Sylva Lestari*. 7(1):71-81.

Ajayan H, Safwan P, Abraham T. 2024. Isolation, identification and antibiogram of citrobacter diversus from sun conure. *International Journal of Veterinary Sciences and Animal Husbandry*. 9(4): 566-567.

Angelovska M, Zaharieva M, Dimitrova L, Dimova T, Gotova I, Urshev Z, Ilieva Y, Kaleva D, Kim C, Naydenska S. 2023. Prevalence, genetic homogeneity, and antimicrobial resistance of pathogenic *yersinia enterocolitica* strains isolated from slaughtered pigs in Bulgaria. *Antimicrobials*. 12(4):716.

Anju M, Kuruvilla, Thomas S. 2023. Characterization of genus *Proteus* isolated from various clinical specimens and detection of extended-spectrum β-lactamase production. *Chrismed Journal of Health and Research*. 10(1): 11-15.

Arbefeville S, Timbrook T, Garner C. 2024. Evolving strategies in microbe identification—a comprehensive review of biochemical, MALDI-TOF MS and molecular testing methods. *Journal of Antimicrobial Chemotherapy*. 79(1):2–8.

Bale B, Elebesunu E, Manikavasagar P. 2023. Antimicrobial resistance in ocular bacterial infections: an integrative review of ophthalmic chloramphenicol. *Tropical Medicine and Health*. 51(15):1-10.

Bedenić B, Pospišil M, Nađ M, Bandić Pavlović D. 2025. Evolution of β-Lactam Antimicrobial Resistance in *Proteus* Species: From Extended-Spectrum and Plasmid-Mediated AmpC β-Lactamases to Carbapenemases. *Microorganisms*. 13(3):508.

Beganovic M, Luther M, Rice L, Arias C, Rybak M, LaPlante K. 2018. A review of combination antimicrobial therapy for enterococcus faecalis bloodstream infections and infective endocarditis. *Clinical and Infection Disease*. 67(2):303-309.

Beleza A, Maiciel W, Cerreira A, Bezerra W, Carmo C, Havt A, Gaio F, Teixeira R. 2019. Detection of *Enterobacteriaceae*, antimicrobial susceptibility, and virulence genes of Escherichia coli in canaries (*Serinus canaria*) in northeastern Brazil. *Pesquisa Veterinária Brasileira*. 39(3):201-208.

Bereda G. 2022. Clinical pharmacology of ampicillin. *Journal of Pharmaceutical Research and Reports*. 3(3):1-3.

[CLSI] Clinical and Laboratory Standards Institute. 2023. *Performance Standards for Antimicrobial Susceptibility Testing 33rd Edition*. West Valley (US): Clinical and Laboratory Standards Institute. [Accessed 2024 Sep 20].

Catalano A, Iacopetta D, Ceramella J, Scumaci D, Giuzio F, Saturnino C, Aquaro S, Rosano C, Sinicropi MS. 2022. Multidrug Resistance (MDR): a widespread phenomenon in pharmacological therapies. *Molecules*. 27(3):616.

Cattoir V. 2022. *Mechanisms of Streptococcus pyogenes Antimicrobial Resistance. Streptococcus pyogenes: Basic Biology to Clinical Manifestations*. Oklahoma City (OK): University of Oklahoma Health.

Cowan ST, Steel KJ. 2003. *Cowan and Steel's Manual for The Identification of Medical Bacteria*. London: Cambridge University Press.

Chang H, Dai F, Duan G, Zhao S, Lu H, Xiang X. 2018. Pathogenicity Analysis of *Klebsiella oxytoca* Isolated from *Larus ridibundus* Migratory Birds. *Pakistani Veterinary Journal*. 38(4): 449-451.

Chopra I, Roberts M. 2001. Tetracycline antimicrobials: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*. 65(2):232-260.

Churria C, Arias N, Origlia J, Netri C, Marcantoni H, Loyola M, Petrucelli M. 2014. Citrobacter freundii infection in two captive Australian king parrots (*Alisterus scapularis). Journal of Zoo and Aquarium Research.* 2(2):52-53.

Davies Y, Cunha M, Oliveira M, Philadelpho N, Romero D, Milanelo L, Guimarães M, Ferreira A, Moreno A, Sá L, *et al*. 2015. Virulence and antimicrobial resistance of *Klebsiella pneumoniae* isolated from passerine and psittacine birds. *Avian Pathology*. 45(2): 194–201.

Delibato E, Luzzi I, Pucci E, Proroga Y, Capuano F, De Medici D. 2018. Fresh Produce and Microbial Contamination: Persistence During The Shelf Life and Efficacy of Domestic Washing Methods. *Annali dell'Istituto Superiore di Sanità*. 54(4):358-363.

Dong N, Yang X, Chan W, Zhang R, Chen S. 2022. Klebsiella species: taxonomy, hypervirulence and multidrug resistance. e*BioMedicine.* 79:103998.

Drzewiecka D. 2016. Significance and roles of *Proteus* spp. bacteria in natural environments. *Microbial Ecology*. 72(4): 741–758.

Eyler RF, Shvets K. 2019. Clinical Pharmacology of Antimicrobials. *Clinical Journal of the American Society of Nephrology*. 14(7):1080-1090.

Fancher CA, Thames HT, Colvin MG, Smith M, Easterling A, Nuthalapati N, Zhang L, Kiess A, Dinh T, Theradiyil A. 2021. Prevalence and molecular characteristics of avian pathogenic Escherichia coli in "no antimicrobials ever" broiler farms. *Microbiol Spectrum*. 9(3):e0083421.

Galosi L, Farneti S, Rossi G, Cork S, Ferraro S, Magi G, Petrini S, Valiani A, Cuteri V, Attili A. 2015. *Yersinia pseudotuberculosis*, serogroup o:1a, infection in two Amazon Parrots (*Amazona aestiva* and *Amazona oratrix*) with hepatic hemosiderosis. *Journal of Zoo and Wildlife Medicine*. 46(3): 588-91.

Green L, Goldman E. 2021. *Practical Handbook of Microbiology 4th Edition.* CRC Press.

Guabiraba R, Schouler C. 2015. Avian Colibacillosis: Still Many Black Holes. *FEMS Microbiology Letters*. 362(15).

Hidasi H, Neto J, Moraes D, Coelho G, Jayme V, Andrade M. 2013. Enterobacterial detection and Escherichia coli antimicrobial resistance in parrots seized from the illegal wildlife trade. *Journal of Zoo and Wildlife Medicine*. 44(1):1-7.

Hulankova R. 2022. Higher resistance of *Yersinia enterocolitica* in comparison to *Yersinia pseudotuberculosis* to antimicrobials and cinnamon, oregano, and thyme essential oils. *Pathogens.* 11(12):1456.

[IUCN] The IUCN Red List of Threatened Species Wreathed Hornbill *Rhyticeros undulatus.* 2018. BirdLife International.

Jabeen I, Islam S, Hassan A, Tasnim Z, Shuvo S. 2023. A brief insight into *Citrobacter* species – a growing threat to public health. *Frontiers in Antimicrobials*. 2:1276982.

Jarulis, Budiman, Solihin D, Mardiastuti. 2015. Hornbills (Bucerotidae) in elephant conservation centre (ECC) forest in Seblat, Bengkulu. Journal of Global Forest and Environmental Science. 4(1): 108-117

Jesumirhewe C, Springer B, Allerberger F, Ruppitsch W. 2022. Genetic characterization of antimicrobial-resistant *Enterobacteriaceae* isolates from bovine animals and the environment in Nigeria. *Frontiers in Microbiology*. 13.

Jung B, Hoilat GJ. 2024. MacConkey Medium. Treasure Island (FL): StatPearls Publishing.

Krishna M, Kuladip S, Awadhesh K. 2012. Rapid Assessment of Wreathed Hornbill *Aceros undulatus* (Aves: Bucerotidae) Populations and Conservation Issues in Fragmented Lowland Tropical Forests of Arunachal Pradesh, India. *Journal of Threatened Taxa*. 4. 3342-3348.

Kobuszewska A, Wysok B. 2024. Pathogenic Bacteria in Free-Living Birds, and Its Public Health Significance. *Animals*. 14(6):968.

Li M, Liu Q, Teng Y, Ou L, Xi Y, Chen S, Duan G. 2019. The Resistance Mechanism of *Escherichia coli* Induced by Ampicillin in Laboratory. *Infection and Drug Resistance*. 12:2853-2863.

Lopes E, Maciel W, Albuquerque Á, Machado D, Bezerra W, Vasconcelos R, Lima B, Gonçalves G, Teixeira R. 2015. Prevalence and antimicrobial resistance profile of enterobacteria isolated from psittaciformes of illegal wildlife trade. *Acta Scientiae Veterinariae.* 43: 1313.

Mackinnon, Ramsay J. 2010. *Burung-burung di Sumatera, Jawa, Bali dan Kalimantan: termasuk Sabah, Sarawak, dan Brunei Darussalam.* Rahardjaningrah W, Editor. Jakarta. Puslitbang Biologi LIPI.

Mahanayak B. 2024. Ex-situ and in-situ conservation of wildlife. *World Journal of Biology, Pharmacy and Health Sciences.* 18(03): 277–282.

Mazumder R, Hussain A, Phelan J, Campino S, Haider S, Mahmud A, Ahmed D, Asadulghani M, Clark T, Mondal D. 2022. Non-lactose fermenting Escherichia coli: following in the footsteps of lactose fermenting *E.coli* high-risk clones. *Frontiers in Microbiology.*

Mogrovejo D, Perini L, Gostinčar C, Sepčić K, Turk M, Ambrožič-Avguštin J, Brill F, Gunde-Cimerman N. 2020. Prevalence of antimicrobial resistance and hemolytic phenotypes in culturable arctic bacteria. *Frontiers in Microbiology*. 3(11):570.

Moradigaravand D, Martin V, Peacock SJ, Parkhill J. 2017. Population structure of multidrug resistant *Klebsiella oxytoca* within hospitals across the UK and Ireland identifies sharing of virulence and resistance genes with K. pneumoniae. *Genome Biology and Evolution*. 9(3):574-587.

Nakhaee P, Moghadam Z, Shokrpoor S, Razmyar J. 2022. Klebsiella pneumoniae infection in canaries (Serinus canaria Domestica): a case report. *Iranian Journal of Veterinary Research.* 23(3): 280-284.

Nasrollahian S, Graham J, Halaji M. 2024. A review of the mechanisms that confer antimicrobial resistance in pathotypes of *E.coli. Frontiers in Cellular and Infection Microbiology.* 14:1387497.

Ng LS, Tan TY, Yeow SC. 2010. A cost-effective method for the presumptive identification of *Enterobacteriaceae* for diagnostic microbiology laboratories. *Pathology*. 42(3):280-3.

Niskanen T, Waldenström J, Fredriksson-Ahomaa M, Olsen B, Korkeala H. 2003.virF-Positive Yersinia pseudotuberculosis and Yersinia enterocolitica found in migratory birds in Sweden. *Applied and Environmental Microbiology*. 69(8): 4670-4675.

Olinda R, Feijo F, Bezerra F. 2012. Diagnosis of Proteus spp. in wild birds raised under captivity in Rio Grande do Norte, Brazil. *Arquivos do Instituto Biológico.* 79(2): 301-303.

Paray A, Singh M, Mir M, Kaur A. 2023. Gram Staining: A Brief Review. *International Journal of Research and Review.* 10(9): 336-341.

Penwell W, Shapiro A, Giacobbe R, Gu R, Gao N, Thresher J, McLaughlin R, Huband M, DeJonge B, Ehmann D, Miller A. 2015. Molecular Mechanisms of Sulbactam Antibacterial Activity and Resistance Determinants in *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*. 59(3):1680-9.

[Permen] Peraturan Menteri Lingkungan Hidup dan Kehutanan Republik Indonesia. Nomor P.92/Menlhk/Setjen/Kum.1/8/2018 Tentang Jenis Tumbuhan dan Satwa Yang Dilindungi. 2018

[Permen] Peraturan Menteri Pertanian Republik Indonesia Nomor 14/Permentan/PK.350/2017 Tentang Klasifikasi Obat Hewan. 2017

Ramirez D, Giron M. 2023. *Enterobacter Infections*. Treasure Island (FL): StatPearls Publishing.

Rinihapsari E, Julianasya S. 2021. Penggunaan KOH String Test Sebagai Alternatif Identifikasi Awal Bakteri Gram Negatif. *Jurnal Ilmu Kedokteran Dan Kesehatan Indonesia.* 1(1): 100–110.

Roggenbuck M, Schnell B, Blom N. 2014. The Microbiome of New World vultures. *Nature Communications* 5. 1(1): 1-7.

Seabaugh J, Anderson D. 2024. Pathogenicity and virulence of Yersinia. *Virulence.* 15(1): 2316439.

Shariati A, Arshadi M, Khosrojerdi M, Abedinzadeh M, Ganjalishahi M, Maleki A, Heidary M, Khoshnood S. 2022. The Resistance Mechanisms of Bacteria Against Ciprofloxacin and New Approaches for Enhancing The Efficacy of This Antimicrobial. *Frontiers in Public Health.* 10:1025633.

Sigirci B, Celik B, Kahraman B, Bagcigil F, Seyyal A. 2019. Tetracycline Resistance of *Enterobacteriaceae* Isolated From Feces of Synanthropic Birds. *Journal of Exotic Pet Medicine.* 28: 13-18.

Sood S. 2016. Chloramphenicol - A Potent Armament Against Multi-Drug Resistant (MDR) Gram Negative Bacilli. *Journal of Clinical and Diagnostic Research*. 10(2): 1-3.

Stromberg Z, Johnson J, Fairbrother J, Kilbourne J, Van Goor A, Curtiss R, Mellata M. 2017. Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. *PLoS One*. 12(7):e0180599.

Sun CH, Liu HY, Zhang Y, Lu CH. 2019. Comparative analysis of the gut microbiota of the hornbill and toucan in captivity. *Microbiology open*. 8(7):e00786.

Thesia N, Khairullah A, Effendi M, Tyasningsih W, Puspitasari Y, Ahadini S, Moses I, Yanestria S, Riwu K, Wasito W, Baihaqi Z, Ahmad R. 2025. Detection of multidrug-resistant *Klebsiella pneumoniae* isolated from duck cloacal swabs in Jombang, Indonesia. *Open Vet J*. 15(1):300-306.

Tsodikova S, Labby K. 2016. Mechanisms of Resistance to Aminoglycoside Antimicrobials: Overview and Perspectives. *Medchemcomm*. 7(1): 11–27.

Turista D, Puspitasari E. 2019. The growth of Staphylococcus aureus in the blood agar plate media of sheep blood and human blood groups A, B, AB, and O. *Jurnal Teknologi Laboratorium*. 8(1): 01 – 07.

Waite DW, Eason DK, Taylor MW. Influence of hand rearing and bird age on the fecal microbiota of the critically endangered kakapo. 2014. *Applied and Environmental Microbiology*. 80(15):4650-8.

[WHO] World Health Organization. 2018. *Critically important antimicrobials for human medicine WHO 6th revision.* Geneva (CH): World Health Organization. [Accessed 2025 June 23].

Wyres K, Holt K. 2018. Klebsiella pneumoniae is a key trafficker of drug resistance genes from the environment to clinically significant bacteria. *Current Opinion in Microbiology*. 45:131 139.

Yao Y, Falgenhauer L, Falgenhauer J, Hauri AM, Heinmüller P, Domann E, Chakraborty T, Imirzalioglu C. 2021. Carbapenem-resistant *citrobacter* spp. as an emerging concern in the hospital-setting: results from a genome-based regional surveillance study. *Frontiers in Cellular and Infection Microbiology*. 11:744.

APPENDIX

Appendix 1 Kirby-Bauer Disc Diffusion Test Diameter Measurement

Sample	Isolate Code	Species	Diameter of Inhibitory Zone (mm)					
			AMP (10 µg)	DO (30 µg)	CN (10 µg)	CIP (5 µg)	SxT (1.25/ 23.75 µg)	C (30 µg)
JE02	JE02M1	*Klebsiella pneumoniae*	23	0	27	43	28	18
JE05	JE05M1	*Klebsiella pneumoniae*	24	0	22.5	35	27.5	17.5
	JE05M2	*Proteus* sp	24	18	16	28	23*	23
	JE05M3	*Escherichia coli*	19.5	10	17	35	27	24
	JE05M4	*Enterobacter cloacae*	23	0	20	31	20	16
	JE05B1	*Citrobacter freundii*	25.5	0	25.5	36	29	21
JE04	JE04M1	*Escherichia coli*	22.5	0	25	35.5	27.5	19.5
JE08	JE08M1	*Citrobacter* sp	12	21.5	25	34	30	27.5
	JE08M3	*Eschericia coli*	20	21	22	36.5	28.5	28.5
	JE08B1	*Yersinia enterocolitica*	16.5	18	24.5	31.5	31.5	8
JE09	JE09M1	*Yersinia* sp	9.5	20.5	20.5	33.5	26.5	22.5
JE12	JE12M1	*Escherichia coli*	0	0	21.5	26	27.5	24
	JE12M2	*Yersinia* sp	23	0	27.5	40	29	24
	JE12M3	*Proteus* sp	0	13	21.5		29.5	27.5
JE32	JE32M1	*Klebsiella oxytoca*	13.5	0	26	37.5	30.5	22.5
	JE32M2	*Klebsiella pneumoniae*	15.5	24	24.5	37.5	28	21

AUTOBIOGRAPHY

The author was born in Jakarta on January 10, 2004, as the third child of Mr. Didik Haryanto and Mrs. Endang Sri Windarti. The author completed high school at SMAN 65 Jakarta Barat and graduated in 2021. The author enrolled in IPB University as an undergraduate student at the School of Veterinary Medicine and Biomedical Sciences, IPB University, in the same year through the international undergraduate program.

The author was actively engaged in various organizational roles during the undergraduate program. He served as a staff member in the Information and Communication Division of the Indonesian chapter of the International Veterinary Students Association (IVSA) from 2021 to 2022. Furthermore, he was a member of the Student Executive Board (BEM) of the School of Veterinary Medicine and Biomedical Sciences (SKHB IPB), where he held positions as a staff member in the Media and Branding Bureau during the 2022–2023 period and in the Student Mental Health Advocacy unit for the 2023–2024 period. Additionally, he served as the Head of the Wild Ornith Cluster within the Wild Animals Professional Association (Himpro Satwa Liar) for the 2023–2024 term.