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Identification of gram-negative enteric bacteria and antimicrobial resistance patterns in captive psittacine birds from Indonesia

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ABSTRACT: Psittacines are vulnerable to bacterial diseases, including gram-negative infections. A key challenge in treating these birds is antibiotic resistance, where antibiotics become ineffective against the bacteria. This study aimed to identify gram-negative bacteria present in 11 fecal samples of psittacine from Taman Mini Indonesia Indah (TMII) in East Jakarta, as well as to determine the presence of antibiotic resistance in these bacteria. In this study, bacteria were isolated using MacConkey Agar media, followed by Gram staining and biochemical tests for bacterial identification. Antibiotic resistance testing was conducted using the Kirby-Bauer method against seven antibiotics. The results of bacterial identification showed the presence of *Klebsiella* sp., *Proteus mirabilis*, and *Escherichia coli* in the fecal samples of Psittacine from TMII. Resistance to the antibiotics nalidixic acid, doxycycline, tetracycline, gentamicin, erythromycin, cefotaxime, and trimethoprim-sulfamethoxazole was observed, with trimethoprim-sulfamethoxazole demonstrating the lowest resistance rate among fecal Psittacine bacteria.

Keywords:
antibiotic resistance, biochemistry test, gram-negative bacteria, psittacine

■ INTRODUCTION

Psittacines are popular avian species kept as companion animals because of their cognitive abilities and social behavior. Despite their popularity, population declines in several psittacine species over the past few decades have raised concerns about their conservation and health management (Warsito & Bismark 2010). Among the health challenges, gram-negative bacterial infections remain significant in clinical and captive settings. A major obstacle in managing these infections is the increasing antimicrobial resistance, which threatens disease control and causes therapeutic failure, increased morbidity, mortality, and environmental contamination. Resistant bacteria can circulate between populations and cross species barriers, including humans (Poirel *et al*. 2018). This study aimed to identify gram-negative bacteria from psittacine feces and characterize their antimicrobial resistance profiles.

■ MATERIALS AND METHODS

Fecal samples were collected from eleven Psittacine birds, comprising Yellow-crested Cockatoo (*Cacatua sulphurea*), Salmon-crested Cockatoo (*Cacatua mouluccensis*), Blue and gold macaw (*Ara ararauna*), Green wings macaw (*Ara chloropterus*), Electus parrots (*Eclectus roratus*) and Palm Cockatoo (*P. aterrimus*). Samples from Taman Mini Indonesia Indah in Jakarta were used to grow bacteria on MacConkey Agar. The bacteria were identified using Gram staining and various tests, such as catalase and oxidase tests. Their resistance to seven antibiotics was assessed using the Kirby-Bauer disk method. The results were interpreted according to the CLSI 2023 guidelines.

■ RESULTS AND DISCUSSION

In this study, 15 isolates from 11 fecal samples were identified as gram-negative bacteria. The isolates included fermenters, appearing pink or red, and non-fermenters forming cream-colored colonies due to the absence of lactose fermentation (Abd El-Mongy *et al*. 2017). Microscopic examination showed all isolates stained red with bacilli and cocco-bacilli morphology. Biochemical tests identified *Klebsiella* sp.,

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Table 1. Bacterial identification results from Psittacine isolates

Isolate Code	Species	Identified Bacteria
1.1, 2.1, 3.1, 4.1, 4.2, 5.1, 6.1, 8.1, 10.2, 11.1	Blue and Gold Macaw, Green-winged Macaw, Eclectus Parrot, Yellow-crested Cockatoo, Palm Cockatoo	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*
7.1	Salmon-crested Cockatoo	*Klebsiella pneumoniae* subsp. *ozaenae*
9.1	Yellow-crested Cockatoo	*Klebsiella pneumoniae* subsp. *aerogenes*
9.2	Yellow-crested Cockatoo	*Klebsiella pneumoniae* subsp. *pneumoniae*
10.1	Macaw	*Escherichia coli*
3.2	Yellow-crested Cockatoo	*Proteus mirabilis*

Table 2. Results of antibiotic resistance test of fifteen isolates

Isolates Category	Antibiotics						
	NA 30 µg	DO 30 µg	TE 30 µg	CN 10 µg	E 15 µg	CTX 30 µg	SXT 25 µg
1.1.	R	S	S	R	I	R	S
2.1.	R	R	R	I	I	R	S
3.1.	S	S	S	I	I	R	I
3.2.	I	S	S	I	I	R	S
4.1.	R	R	R	R	S	R	R
4.2.	R	I	R	R	S	R	S
5.1.	R	S	S	S	S	R	I
6.1.	S	I	S	R	R	I	S
7.1.	R	S	S	S	I	R	S
8.1.	R	R	R	R	I	R	S
9.1.	S	S	S	I	R	I	S
9.2.	S	S	S	S	R	R	I
10.1.	I	R	R	I	R	S	I
10.2.	R	R	R	R	R	R	R
11.1.	R	S	R	R	S	R	S
Total Isolate							
S	4	8	8	3	4	1	9
I	2	2	-	5	6	2	4
R	9	5	7	7	5	12	2

Note: NA = Nalidixic acid; DO = Doxycycline; TE =Tetracycline; CN = Gentamycin; E = Erythromycin; CTX = Cefotaxime; SXT = Trimetropim-Sulfamethoxazole; S = Sensitive; I = Intermediate; R = Resistant

Proteus sp., and *Escherichia coli* in psittacine isolates, as interpreted by Cowan and Steel (2003) with reference to Enterobacteriaceae differentiation (Table 1).

Antimicrobial testing of 15 bacterial isolates against seven antibiotics showed distinct resistance patterns (Table 2). Resistance to nalidixic acid arises from target mutations, reduced drug accumulation, and bacterial plasmids (Jacoby 2005). Doxycycline and tetracycline resistance involves efflux pumps, ribosomal modification, and enzymatic inactivation (Markley & Wencewicz 2018). Gentamicin resistance occurs via aminoglycoside-modifying enzymes (Ahmadian *et al*. 2021). Erythromycin resistance mechanisms include reduced permeability, macrolide hydrolysis, and ribosomal modification (Amdan *et al*. 2024). Cefotaxime showed the highest resistance, as beta-lactamase hydrolyzes its β-lactam ring (Knies *et al*. 2017). Trimethoprim-sulfamethoxazole was most effective against bacteria from psittacine bird feces, matching the results of Tarek *et al*. (2024), who found that 64% of gram-negative bacteria were susceptible.

These results are relevant to antimicrobial resistance (AMR) within the One Health framework, as zoonotic bacteria may pose risks to animals, humans, and the environment of the same. These data provide insights into future antibiotic resistance surveillance in avian species. A key limitation of this study is the limited number of fecal samples, which restricts the generalizability of our findings.

■ CONCLUSION

Klebsiella sp., *Escherichia coli*, and *Proteus mirabilis* were identified from Psittacine fecal samples. Among the seven antibiotics tested, resistance was observed across most agents, with trimethoprim-sulfamethoxazole showing the lowest resistance rate.

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