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Identification and antibiotic resistance testing of Gram-negative rod-shaped bacteria in birds of paradise (*Paradisaeidae* sp.)

Sadhira Paramesthya[1], Agus Wijaya[2], Safika[3,*]

[1] Program of Veterinary Medicine, School of Veterinary Medicine and Biomedical Science, IPB University, Bogor
[2] Division of Internal Medicine, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor
[3] Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor

ABSTRACT: Antibiotic resistance represents a critical global health challenge, and is characterised by the loss of effectiveness of antibiotics against bacterial growth. This phenomenon complicates treatment options for infections, underscoring the need to understand bacterial resistance mechanisms, particularly in protected species such as birds of paradise. This study aimed to identify the bacterial species present in faecal samples from three different species of birds of paradise and evaluate the efficacy of various antibiotics against these bacteria. Samples were collected from Taman Mini Indonesia Indah (TMII) in East Jakarta. Using MacConkey Agar for bacterial isolation and subsequent identification through Gram staining and biochemical tests, this study identified *Serratia* sp., *Klebsiella* sp., and *Citrobacter* sp. Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method against seven antibiotics: erythromycin, gentamicin, oxytetracycline, cefotaxime, sulfamethoxazole, doxycycline, and nalidixic acid. These findings revealed that sulfamethoxazole was notably more effective than the other antibiotics tested, highlighting its potential for treating infections in birds of paradise.

Keywords:
antibiotic resistance, biochemistry test, birds-of-paradise, *Enterobacteriaceae*, gram-negative bacteria

■ INTRODUCTION

Birds of paradise, renowned for their splendid plumage, are listed as protected species under the Indonesian Ministry of Environment and Forestry Regulation No. P.106/MENLHK/SETJEN/KUM.1/12/2018. This designation comes in response to their dwindling populations, which significantly threaten their survival. In wild pigeons, instances of *Chlamydophila psittaci* have been observed in Japan, highlighting a similar risk among avian species (Stokes *et al*. 2021). Furthermore, birds are susceptible to Salmonella infections, which cause gastrointestinal distress and can lead to systemic illnesses (De Lucia *et al*. 2018). The spread of such infections is facilitated by migratory birds and poor waste management practices in addition to wildlife conservation challenges (Ramey 2021). Moreover, the prevalence of gram-negative bacteria, such as *E. coli* and *Klebsiella* sp., presents additional concerns owing to their complex outer membrane, which impedes antibiotic effectiveness (Gauba & Rahman 2023). Addressing antibiotic resistance in parasites and other wildlife is crucial for the ongoing efforts to conserve these remarkable creatures.

■ MATERIALS AND METHODS

Faecal samples were meticulously collected from four birds of paradise, representing three distinct species: the lesser bird of paradise (*Paradisaea minor*), the magnificent bird of paradise (*Cicinnurus magnificus*), and the red bird of paradise (*Paradisaea rubra*). These collections took place in Taman Mini Indonesia Indah in Jakarta. The samples were subsequently used for bacterial isolation using MacConkey Agar (MCA). Bacterial strains were identified by Gram staining and serial biochemical tests after isolation. The Kirby-Bauer disk diffusion method was applied to a panel of seven antibiotics to assess antibiotic resistance. The Clinical Laboratory Standards Institute (CLSI) 2023 guidelines were used to interpret the results and ensure rigorous adherence to established standards.

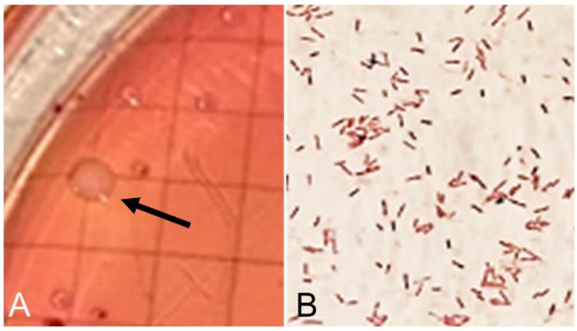

Figure 1 Bacterial isolation from birds of paradise on MacConkey Agar. (A) Depicts pink colonies (black arrow), characteristic of lactose-fermenting bacteria, primarily exhibiting bacilli-shaped morphology, (B) Shows a detailed view under 10x100 magnification, providing a closer inspection of the bacterial structure and arrangement.

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■ RESULTS AND DISCUSSION

In this study, six isolates from four faecal samples were identified as gram-negative bacteria, as evidenced by the growth of pink colonies on MacConkey Agar, which is indicative of lactose-fermenting bacteria. Microscopic analysis revealed that all isolates subjected to Gram staining appeared red and exhibited two morphological forms, bacilli and coccobacilli, as shown in Figure 1.

Biochemical tests identified the presence of Serratia sp. in an isolate from the lesser bird of paradise (isolate B1), with results similar to those reported by Brenner *et al*. (2005) and Cowan & Steel (2003). Similarly, isolates from the magnificent and red birds of paradise (isolates C1, C2, D1, and D2) were identified as *Klebsiella* sp., consistent with the findings of Cowan & Steel (2003). These isolates were characterised as *K. pneumoniae* subsp. rhinoscleromatis, as suggested by negative urea and citrate test results. Additionally, *Citrobacter* sp. was isolated from the red bird of paradise (isolate D3), which is known to cause gastrointestinal symptoms such as diarrhoea and vomiting in birds, which may lead to dehydration and lethargy (Wang *et al*. 2023).

Antibiotic resistance test results for the six isolates are summarised in Table 1. The test was conducted twice, revealing varied resistance patterns across the seven antibiotics used, which were selected based on their relevance to the complexity of bacterial infections and treatment efficacy. Erythromycin displayed mixed results, with three isolates sensitive and three showing intermediate resistance, challenging the findings of Brunton (2010), who reported the reduced efficacy of erythromycin in treating gram-negative bacteria. This discrepancy could be attributed to birds' lack of prior exposure to this antibiotic. Gentamicin was predominantly effective, although some resistance was also observed. All isolates were sensitive to both oxytetracycline and doxycycline. Cefotaxime showed varied results, some of which may be due to the production of extended-spectrum β-lactamase (ESBL) enzymes (Tian *et al*. 2009). Sulfamethoxazole was notably effective, as evidenced by the largest average inhibition zones compared to other antibiotics, corroborating the results of Alhumaid *et al*. (2020), who reported a sensitivity of 84.2% in their studies on gram-negative bacteria. Nalidixic acid exhibited resistance in three isolates, which was potentially linked to DNA gyrase mutations (Jacoby 2005).

■ CONCLUSION

Biochemical identification of gram-negative rod-shaped bacteria from faecal samples of paradise birds revealed the presence of *Serratia* sp., *Klebsiella* sp., and *Citrobacter* sp. Among the tested antibiotics, sulfamethoxazole demonstrated superior effectiveness, as evidenced by the largest average diameter of inhibition zones relative to the other antibiotics. In contrast, cefotaxime was less effective as it had the highest number of resistant isolates.

Table 1 Results of antibiotic resistance test of six isolates collected from four birds of paradise.

Antibiotic	Isolates Category						No. of Isolates		
	B1	C1	C2	D1	D2	D3	S	I	R
E 15 µg	S	I	S	S	I	I	3	3	0
CN 10 µg	S	R	S	S	I	S	4	1	1
OT 30 µg	S	S	S	S	S	S	6	0	0
CTX 30 µg	R	R	I	R	R	S	1	1	4
SXT 25 µg	S	S	S	S	S	S	6	0	0
DO 30 µg	S	S	S	S	S	S	6	0	0
NA 30 µg	S	R	S	R	R	S	3	0	3

Note 1 E = Erythromycin; G = Gentamycin; OT = Oxytetracycline; CTX = Cefotaxime; SXT = Sulfamethoxazole; DO = Doxycycline; NA = Nalidixic acid; S = Sensitive; I = Inter-mediate; R = Resistant

■ AUTHOR INFORMATION

Corresponding Author

* SS: safika@apps.ipb.ac.id

Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor. 16680, West Java, INDONESIA.

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