Original Article

Bacterial identification and antibiotic sensitivity profiles from captive birds at Taipei Zoo

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Abstract

Bacterial infections are common in zoo birds. They may be caused by pathogenic bacteria or opportunistic bacteria due to trauma or stress-induced immune compromise. Improper use of antibiotics contributes to the development of resistant bacteria, making treatment complicated or ineffective. However, comprehensive clinical investigations into bacterial prevalence and antibiotic susceptibility in zoo-captive birds remain limited. This study identified the bacterial species present in captive diseased birds at the zoo and conducted a 12-year analysis of their antibiotic susceptibility profiles. The specimens from 48 birds at Taipei Zoo from 2006 to 2017 were submitted to a commercial reference laboratory for bacterial identification. Among the total of 48 birds, 36 were live diseased individuals designated for further antibiotic susceptibility testing. A total of 105 bacteria were identified, and 75 were processed for antibiotic susceptibility. The most frequently isolated bacterium was *Escherichia coli* (24.76%, Gram-negative facultative anaerobe). Further antibiotic susceptibility testing using 33 different antibiotic discs revealed that only amikacin and ceftazidime exhibited over 50% susceptibility against the Gram-negative facultative anaerobes. In addition, this study observed a significant upward trend in *E. coli* resistance to kanamycin and neomycin, both of which belong to the aminoglycosides. The prevalence of *E. coli*, a commensal bacterium, in captive birds at the zoo is a significant concern due to the potential for horizontal transmission of antimicrobial resistance genes through contact between birds and zoo personnel or visitors. Since aminoglycosides, either alone or in combination with other classes of antibiotics, are commonly employed in wildlife treatment, these findings may provide valuable insights for future clinical applications and the development of effective zoo management strategies.

Keywords: antibiotic, bacteria, bird, *Escherichia coli*, zoo

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Introduction

Zoo birds can become diseased due to pathogenic bacteria, as well as infections resulting from opportunistic environmental microorganisms (Witte *et al.*, 2021). When the birds are under stress or immunosuppression, the normal flora may overgrow and cause an opportunistic infection (Akhter *et al.*, 2010). Many bacterial species might lead to disease in birds through either primary or secondary infection. Moreover, some bacteria, such as *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, have zoonotic potential (Abd El-Ghany, 2021; Hu *et al.*, 2021; Mellata, 2013). Therefore, it is important to identify the bacteria and assess their antibiotic sensitivity to ensure effective treatment when necessary.

The antibiotic-resistant bacteria have become an emerging concern in recent decades. Due to the high risk for widespread antibiotic resistance, *E. coli*, a Gram-negative bacterium of the *Enterobacteriaceae* family, has been classified as a priority pathogen by the World Health Organization (WHO, 2017). Evidence also suggests a reduction in antibiotic effectiveness against Gram-negative bacteria due to the spread of quinolone- and cephalosporin-resistant *Enterobacteriaceae*, as well as strains producing carbapenemases (Livermore, 2012). In addition, antibiotic resistance in *E. coli* has been increasingly prominent, especially in the classes of cephalosporins, penicillins, fluoroquinolones, and sulfonamides (Roth *et al.*, 2019). Identifying the resistance is essential for selecting optimal antibiotics to which the bacteria are sensitive. This is particularly critical in serious cases requiring antibiotic treatment, as it can significantly impact morbidity and mortality.

Furthermore, bacterial infection in captive birds at zoos is more likely to be treated. If antibiotics are overused or misused, bacteria are prone to develop resistance or evolve into multidrug-resistant (MDR) strains. Many studies have shown that avian pathogenic *E. coli* isolates act as a reservoir of antimicrobial-resistant (AMR) genes that could be transmitted to other bacteria by horizontal gene transfer (Giufre *et al.*, 2012; Overdevest *et al.*, 2011). The AMR genes may further transfer to humans through contact, causing a public health issue (Jeong *et al.*, 2021; Sem *et al.*, 2024; Wibisono *et al.*, 2022). More than 25 human infectious disease outbreaks occurred due to visiting animal exhibits from 1990 to 2000. Petting zoos have been linked to several zoonotic outbreaks, including *E. coli* (Bender and Shulman, 2004; Chomel *et al.*, 2007). To prevent the spread of AMR bacteria to humans, it is crucial to treat animal infections with appropriate antibiotics. In addition, effective zoo management practices also play a role in mitigating the dissemination of AMR strains. However, despite the importance of addressing bacterial infections and resistance, data on bacterial identification and antibiotic susceptibility in zoo birds remain limited.

In response to the outbreaks of avian influenza H5N6 in 2017 and H5N1 in 2023 in Taiwan, Taipei Zoo has implemented stringent preventive and control measures. Moreover, the aviary section of the zoo was temporarily closed in 2020 due to a chlamydia infection among birds belonging to the family *Columbidae*. Veterinarians also exercised considerable caution in the administration of antibiotics. As a result, opportunities for direct contact between zoo birds and visitors have been significantly reduced since 2017, thereby decreasing the likelihood of transmission of AMR genes or the emergence of resistant bacterial strains. However, after the avian influenza outbreak in 2023, the aviary section resumed stable public access and remained continuously open thereafter. Consequently, the prevention of AMR bacteria has emerged as an issue warranting increased attention. Therefore, in this research, we examined microbial databases to investigate the identified bacterial species and analyze the trends in antibiotic sensitivity profiles during the years preceding the H5N6 epidemic and H5N1 pandemic in Taiwan. The study findings may serve as an important reference for future clinical practice and the formulation of management strategies in the post-epidemic/pandemic era.

Materials and Methods

Sample collection: Our study collected 53 samples from 48 birds covering 19 species at Taipei Zoo, Taiwan, from May 2006 to November 2017. Details regarding the bird species, sample sizes, sampling locations, submission year for isolation, the causes of illness, and the cause of death are summarized (Table 1). Among the 53 samples, 36 were collected from 36 live diseased birds. Sterile swabs were used to sample various lesions (n = 35) and vomiting material (n = 1) from these birds. Of the lesion-derived samples, 74.29% originated from wounds (n = 26), 8.57% from the larynx-trachea (n = 3), 8.57% from the oral cavity (n = 3), 5.71% from the conjunctiva (n = 2), and 2.86% from the infraorbital sinus (n = 1). The remaining 17 specimens were collected from 12 deceased birds during necropsy procedures. The specimens collected from the 36 live diseased birds were designated by veterinarians for antibiotic susceptibility testing.

Bacterial identification and antibiotic sensitivity testing: The samples were submitted to the Reference Technology Limited Company (Taichung, Taiwan) for bacterial identification and antibiotic sensitivity testing. The bacteria were identified using API system (BIOMÉRIEUX, Marcy-l'Étoile, France), and the antibiotic sensitivity was examined using Kirby-Bauer disk diffusion susceptibility test with BBL™ (Baltimore Biological Laboratory) Sensi-Disc™ Antimicrobial Susceptibility Test Discs (Becton, Dickinson and Company, NJ, USA). Thirty-three different antibiotic discs were employed. The disc concentrations were specified in the official documentation provided by the manufacturer. The interpretative criteria for bacterial susceptibility were based on the current guidelines available at the time from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and with reference to the official technical documentation provided by the antimicrobial disc manufacturer during the testing period. Antimicrobial susceptibility results were interpreted as susceptible (S), intermediate (I), or resistant (R) based on the

diameter of the inhibition zones, in accordance with established interpretive criteria as described above. For each bacterial species, the number of isolates falling into each S/I/R category was divided by the total number of isolates of that species to calculate the corresponding percentage for each antibiotic (Supplementary Table). Only the bacterial species with more than three isolates were included in the calculation of antimicrobial susceptibility percentages. Antimicrobial resistance percentage of individual bacterial species to each antibiotic was calculated by adding the number of resistant isolates to half the number of intermediate isolates and dividing this sum by the total number of isolates (Table 3 and Fig. 1). All bacterial isolates were tested using the same antimicrobial panel. However, on a rare occasion, one or two discs were unavailable during susceptibility testing due to a temporary inventory shortage from the overseas supplier.

Antibiotic resistance trends of E. coli: To assess trends in antibiotic resistance over the 12-year period, the dataset was divided into two subperiods at the temporal midpoint, and resistance rates were compared between the two intervals. The antibiotic resistance percentages of E. coli to antibiotics during the period from 2006 to 2010 (n = 11) and the period from 2011 to 2017 (n = 12) were compared, including those of the cephalosporins, the penicillins, the aminoglycosides, enrofloxacin, and trimethoprim-sulfamethoxazole. The tested cephalosporins in this study included ceftazidime, cefuroxime, cephalexin, cephalothin, and ceftiofur. Those belonging to the penicillins were amoxicillin, ampicillin, clavulanic ticarcillin, penicillin, piperacillin, carbenicillin, and clavulanic amoxicillin. Those within the aminoglycosides were amikacin, gentamycin, kanamycin, streptomycin, neomycin, and tobramycin. Statistical analyses were performed using the *t*-test, with a *P*-value less than 0.05 considered statistically significant.

Results

Bacteria identification: A total of 105 bacteria covering 24 different bacterial species were identified from the 53 samples, as shown in Table 2. The five most frequently isolated bacterial species were *E. coli* (24.76%), *Enterococcus* spp. (17.14%), *K. pneumoniae* (11.43%), *Pseudomonas* spp. (7.62%), and *Staphylococcus* spp. (4.76%). It is noteworthy that approximately one-quarter of the bacterial isolates were *E. coli* (n = 26).

Antibiotic sensitivity testing: Seventy-five out of the 105 identified bacterial isolates underwent further antibiotic susceptibility testing. The antimicrobial resistance rates of individual bacterial species to each antibiotic are presented in Table 3. Detailed data on the percentage distribution of each S/I/R category are provided in the Supplementary Table. The bacterial species in the Supplementary Table could be grouped into four classifications, including Gram-negative strict aerobe, Gram-positive facultative aerobe, Gram-positive facultative anaerobe, and Gram-negative facultative anaerobe. Acinetobacter baumannii, which is a Gram-negative, strict aerobe, was completely susceptible to carbenicillin, amikacin, kanamycin, tobramycin, ceftazidime, and polymyxin B. On the other hand, it was completely resistant to ampicillin, penicillin, chloramphenicol, cephalexin, cephalothin, clindamycin, vancomycin, and metronidazole. *Staphylococcus* spp., which is a Gram-positive facultative aerobe, was completely susceptible to amoxicillin, ampicillin, carbenicillin, and vancomycin, whereas completely resistant to sulfadiazine, colistin sulphate, nalidixic acid, and metronidazole. *Enterococcus* spp., which is a Gram-positive facultative anaerobe, was completely susceptible to clavulanic amoxicillin and completely resistant to neomycin, sulfadiazine, colistin sulphate, nalidixic acid, and metronidazole. The Gram-negative facultative anaerobic bacteria accounted for 65.3% of these 75 isolates, including *Enterobacter Cloacae*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, and *P. aeruginosa*. The antibiotics showing over 50% susceptibility were amikacin and ceftazidime. On the contrary, the antibiotics exhibiting over 50% resistance in common were amoxicillin, ampicillin, penicillin, clindamycin, cephalexin, cephalothin, erythromycin, vancomycin, and metronidazole. For *E. coli*, only amikacin and colistin sulphate demonstrated complete effectiveness.

Antibiotic resistance trends for E. coli: Given that *E. coli* accounted for nearly one-quarter of the identified bacterial species, its temporal trends in antibiotic resistance rates were further examined. Among the 26 isolates, 23 were derived from live bird specimens and were submitted for antimicrobial susceptibility testing at the request of veterinarians. The resistance trends for *E. coli* to different antibiotic classes are shown in Figure 1, including cephalosporins (Fig. 1A), penicillins (Fig. 1B), enrofloxacin and trimethoprim-sulfamethoxazole (Fig. 1C), and aminoglycosides (Fig. 1D). The results showed that none of the 23 *E. coli* isolates exhibited resistance to amikacin throughout the entire analyzed period (2006–2017). There were no significant resistance differences to cephalosporins ($P \geq 0.69$), penicillins ($P \geq 0.21$), enrofloxacin ($P = 0.17$), and trimethoprim-sulfamethoxazole ($P = 0.31$). However, resistance to kanamycin and neomycin of the aminoglycosides showed a significant increase with *P*-values of 0.03 and 0.007, respectively (Fig. 1D). In addition, although *E. coli* showed either a noticeable increase or decrease in resistance tendency to piperacillin, enrofloxacin, gentamicin, and tobramycin, there was no statistical significance between these two subperiods ($P > 0.5$).

Table 1 Bird species, numbers, sampling location, submission year for isolation, and the cause of illness and death.

Bird Species	Bird No.	Sampling location (No. of samples)	Submission year	The cause of illness and the cause of death
Accipiter nisus (Eurasian sparrowhawk)	1	Wing wound swab (1)	2014	A live bird with trauma.
Accipiter trivirgatus (Asian crested goshawk)	1	Foot wound swab (1)	2007	A live bird with trauma.
Anodorhynchus hyacinthinus (Hyacinth macaw)	1	Heart (1) Lung (1)	2009	A necropsied bird with an uncertain leading cause of death.
Anthracoceros malayanus (Black hornbill)	4	Esophagus (1) Cecum (1) Liver abscess (1) Liver mass (1)	2016 2006 2007 2011	Four necropsied birds. The leading causes of death were esophagitis, cecitis, liver abscess, and hepatic tumor, respectively.
Aptenodytes patagonicus (King penguin)	7	Liver (1) Oviduct (1)	2008 2015	Two necropsied birds. The leading causes of death were liver abscess, gastritis, and salpingitis, respectively.
		Oral swab (3) Larynx swab (1) Vomit material (1)	2006, 2011, 2013 2010 2017	Five live birds with respiratory signs.
Ara chloroptera (Green-winged macaw)	1	Trachea swab (1)	2013	A live bird with respiratory signs.
Balearica pavonina (Black crowned crane)	1	Pericardium (1) Blood from the heart (1)	2010	A necropsied bird. The leading cause was Pericarditis.
Bugeranus carunculatus (Wattled crane)	5	Digital mass pus swab (1) Right hock wound swab (1)	2012 2013	Two live birds with pododermatitis (bumblefoot).
		Liver (1) Lung (1) Kidney (1)	2015 2006 2011	Three necropsied birds. The leading causes of death were hepatitis, pneumonia, and nephritis, respectively.
Chrysolophus pictus (Golden pheasant)	1	Infraorbital sinus (1)	2017	A live bird with Infraorbital sinusitis.
Cygnus atratus (Black swan)	1	Left digital mass pus swab (1)	2009	A live bird with pododermatitis (bumblefoot).
Cygnus cygnus (Whooper swan)	3	Right paw pus swab (1) Left digital mass pus swab (1) Left paw pus swab (1)	2014 2008 2012	There live birds with pododermatitis (bumblefoot).
Otus bakkamoena (Collared scops owl)	4	Intramedullary pin wound swab (1) Wound swab (1) Larynx swab (1) Right conjunctiva swab (1)	2010 2007 2016 2009	Four live birds. The first two were with a surgical wound and trauma, respectively. The other two had respiratory signs.
Pernis ptilorhynchus (Crested honey buzzard)	1	Wing wound swab (1)	2015	A live bird with trauma
Phoenicopterus ruber (Greater flamingo)	4	Right leg wound swab (1) Right hock swab (2) Left leg swab (1)	2013 2008, 2008 2012	Four live birds with pododermatitis (bumblefoot).
Probosciger aterrimus (Palm cockatoo) *3*	1	Ascites (1) Liver (1) Lung (1) Kidney (1)	2012	A necropsied bird with an uncertain leading cause of death.
Spheniscus demersus (Jackass penguin)	4	Right conjunctiva pus swab (1) Paw pus swab (1) Secretion from right paw (1) Left paw swab (1)	2011 2016 2009 2014	Four live birds. The first one was with conjunctivitis, and the other three were with pododermatitis (bumblefoot).
Spilornis cheela (Crested serpent eagle)	1	Right wing pus swab (1)	2017	A live bird with trauma.
Spizaetus nipalensis (Hodgson's hawk eagle)	6	Wound swab (2) Left shoulder pus swab (2) Left wing pus swab (1) Right shoulder pus swab (1)	2014, 2015 2008, 2016 2007 2010	Six live birds with trauma.
Uroxissa caerulea (Taiwan blue magpie)	1	Skin pus (1)	2015	A live bird with trauma.
Total	48	53		36 live birds and 12 deceased birds.

Table 2 The prevalence of bacterial species.

Bacterial species	Number of isolates	Prevalence (%)
Escherichia coli	26	24.76
Enterococcus spp.	18	17.14
Klebsiella pneumoniae	12	11.43
Pseudomonas spp.	8	7.62
Staphylococcus spp.	5	4.76
Acinetobacter baumannii	4	3.81
Enterobacter cloacae	4	3.81
Proteus mirabilis	4	3.81
Micrococcus spp.	3	2.86
Morganella morganii	3	2.86
Acinetobacter lwoffi	2	1.90
Bacillus spp.	2	1.90
Corynebacterium spp.	2	1.90
Salmonella spp.	2	1.90
Aeromonas hydrophila	1	0.95
Aggregatibacter actinomycetemcomitans	1	0.95
Citrobacter freundii	1	0.95
Citrobacter koseri	1	0.95
Enterobacter intermedium	1	0.95
Klebsiella ornithinolytica	1	0.95
Klebsiella oxytoca	1	0.95
Serratia marcescens	1	0.95
Streptococcus spp.	1	0.95
Viridans streptococcus	1	0.95
Total	**105**	**100**

Table 3 Antimicrobial resistance percentage of individual bacterial species to each antibiotic.

Antibiotic class	Antibiotic	Bacterial species (No. of identification)							
		Acinetobacter baumannii (4)	*Staphylococcus* spp. (5)	*Enterococcus* spp. (17)	*Enterobacter Cloacae* (4)	*Escherichia coli* (23)	*Klebsiella pneumoniae* (11)	*Proteus mirabilis* (4)	*Pseudomonas aeruginosa* (7)
Aminoglycosides	Amikacin	0%	20%	64.71%	0%	0%	18.18%	0%	0%
	Gentamicin	25%	20%	76.47%	50%	33.33%	50%	12.5%	14.29%
	Kanamycin	0%	40%	35.29%	50%	34.78%	27.27%	NT*	42.86%
	Streptomycin	50%	40%	94.12%	50%	76.07%	81.82%	0%	92.86%
	Neomycin	25%	40%	100%	87.5%	60.87%	81.82%	37.5%	100%
	Tobramycin	0%	50%	88.24%	50%	28.26%	72.73%	12.5%	14.29%
Amphenicols	Chloramphenicol	100%	80%	26.47%	75%	34.78%	81.82%	25%	100%
Penicillins	Amoxicillin	75%	0%	5.88%	100%	54.35%	63.64%	62.5%	100%
	Ampicillin	100%	0%	5.88%	100%	71.74%	100%	100%	100%
	Clavulanic ticarcillin	37.5%	20%	58.82%	50%	50%	95.45%	0%	21.43%
	Penicillin	100%	40%	29.41%	87.5%	100%	90.91%	100%	85.71%
	Piperacillin	0%	0%	14.71%	50%	47.83%	72.73%	0%	14.29%
	Carbenicillin	0%	0%	17.65%	50%	54.35%	100%	0%	42.86%
	Clavulanic amoxicillin	62.5%	10%	0%	100%	54.35%	81.82%	37.5%	100%
Cephalosporins	Ceftazidime	0%	50%	97.06%	50%	6.52%	18.18%	0%	0%
	Cefuroxime	87.5%	20%	97.06%	62.5%	21.74%	40.91%	75%	100%
	Cephalexin	100%	20%	97.06%	25%	67.39%	77.27%	100%	100%
	Cephalothin	100%	20%	97.06%	25%	67.39%	68.18%	100%	100%
	Ceftiofur	NT*	NT*	64.71%	NT*	15.38%	0%	NT*	25%
Lincosamides	Clindamycin	100%	90%	94.12%	100%	100%	100%	100%	100%
Tetracyclines	Oxytetracycline	62.5%	80%	52.94%	50%	77.27%	80%	50%	50%
	Tetracycline	75%	80%	52.94%	50%	78.26%	81.82%	50%	100%
	Doxycycline	25%	80%	47.06%	50%	78.26%	81.82%	50%	100%
Sulfonamides	Trimethoprim/sulfamethoxazole	25%	80%	17.65%	50%	47.83%	81.82%	37.5%	100%
	Sulfadiazine	25%	100%	100%	50%	61.90%	70%	50%	28.57%
Macrolides	Erythromycin	50%	80%	47.06%	100%	95.65%	100%	100%	100%
Fluoroquinolones	Enrofloxacin	50%	70%	58.82%	50%	21.74%	63.64%	12.5%	14.29%
Polymyxins	Colistin sulphate	25%	100%	100%	0%	0%	0%	75%	0%
	Polymyxin B	0%	80%	85.29%	0%	2.17%	0%	100%	0%
Nitrofuran	Nitrofurantoin	75%	20%	5.88%	25%	2.17%	54.55%	75%	100%
Quinolone	Nalidixic acid	50%	100%	100%	50%	47.83%	63.64%	25%	100%
Glycopeptides	Vancomycin	100%	0%	2.78%	100%	100%	100%	100%	85.71%
Unclassified	Metronidazole	100%	100%	100%	100%	97.83%	100%	100%	100%

*NT: No test

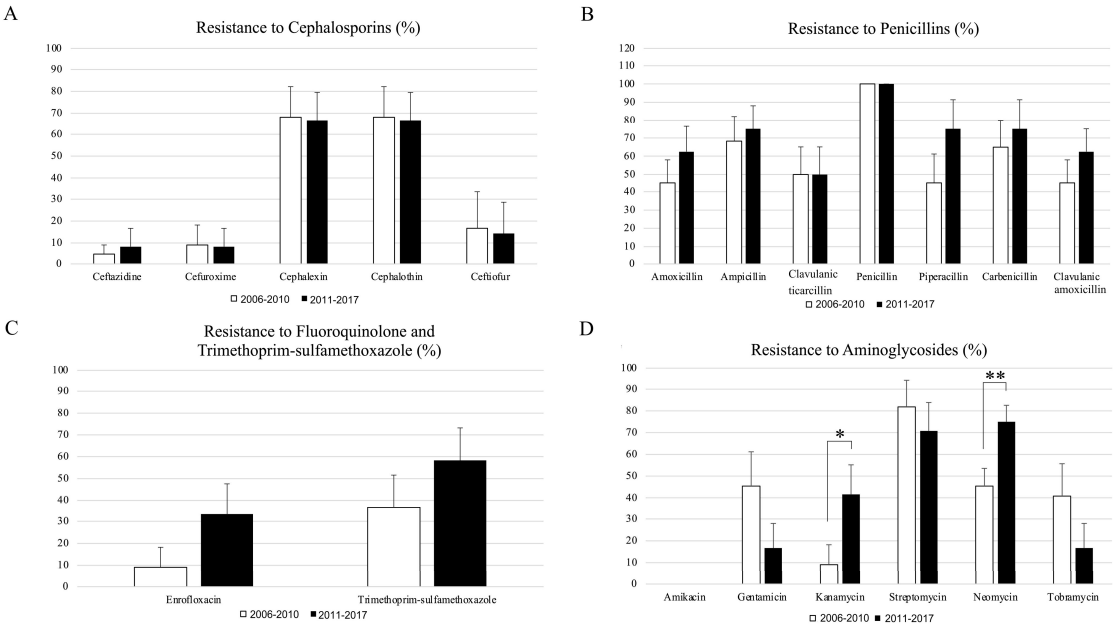

Figure 1 Resistance trends of *E. coli* to (A) cephalosporins, (B) penicillins, (C) enrofloxacin, trimethoprim-sulfamethoxazole, and (D) the aminoglycosides. **P* < 0.05, ***P* < 0.01.

Discussion

The most frequently isolated bacterium in diseased birds in this study was *E. coli*. Absolutely, *E. coli* is a commensal bacterium in the intestine and can be a causative organism of many diarrheal illnesses. It also causes other extraintestinal illnesses, including cystitis, pneumonia, bacteremia, and bacterial peritonitis (Mueller and Tainter, 2023). In diseased free-living raptors, *E. coli* has been reported to exhibit high levels of resistance to clindamycin (95%), ampicillin (75%), and tetracycline (75%) (Vidal et al., 2017). These are aligned with our findings. In addition, all *E. coli* exhibited resistance to vancomycin, and 95.65% showed resistance to metronidazole in this study. This reflects antibiotic abuse, making *E. coli* acquire antimicrobial resistance mechanisms to evolve its resistance (Szmolka and Nagy, 2013). Avian-derived *E. coli* has been reported as reservoirs of AMR genes, which are capable of horizontal transfer to other bacterial species (Giufre *et al.,* 2012; Overdevest *et al.,* 2011). Additionally, zoo birds harboring multidrug-resistant *E. coli* may readily transmit these strains to veterinarians, zoo personnel, or visitors through contact, raising intensive concerns regarding zoonotic spread and public health implications (Ahmed *et al.,* 2007; Sem *et al.,* 2024; Szmolka and Nagy, 2013). Based on our findings, amikacin, colistin sulfate, polymyxin B, nitrofurantoin, ceftazidime, and ceftiofur could be the options for the treatment of *E. coli* infections (Table 3).

The second prevalent bacterial species in this study was *Enterococcus* spp., which is part of the normal intestinal flora. Ellerbroek *et al.* reported that *Enterococcus* spp. have intrinsic resistance to the cephalosporins, aminoglycosides (low-level type), lincosamides, and polymyxins. Furthermore, it can acquire antibiotic resistance to the macrolides and the tetracyclines, the glycopeptides, trimethoprim-sulfonamides, as well as ampicillin (*E. faecium*) and chloramphenicol (Ellerbroek *et al.,* 2004). In this study, *Enterococcus* spp. also showed considerable resistance to the cephalosporines (94.12%, except 66.66% for ceftiofur), lincosamides (clindamycin, 94.12%), and polymyxins (≥ 82.35%). Interestingly, resistance to various aminoglycoside antibiotics in our study ranged widely, from 35.29% to 100%. The resistance to the macrolides (erythromycin) and tetracyclines was medium (41.18% to 52.94%). According to our findings, potential treatment options for *Enterococcus* spp. infections were ampicillin, amoxicillin, clavulanic amoxicillin, piperacillin, nitrofurantoin, and vancomycin (Table 3).

K. pneumoniae was the third frequently identified strain in this study. It was commonly isolated from the feces and oropharynx of clinically healthy passerines and parrots (Gibbs *et al.,* 2007). However, *K. pneumoniae* could cause primarily systemic infections, leading to renal failure, chronic respiratory conditions, and encephalomyelitis (El Fertas-Aissani *et al.,* 2013; Gerlach, 1994). Local infections affecting birds' sinuses, oral cavity, skin, and crop were also observed (Gerlach, 1994). It was reported that *K. pneumoniae* isolated from passerine and psittacine exhibited high resistance to sulfonamides, ampicillin, nalidixic acid, and tetracycline (Davies *et al.,* 2016). Other research revealed its resistance to β-lactam antibiotics and the tetracyclines (Kim *et al.,* 2005; Wu *et al.,* 2012). Similar findings were also seen in diseased free-living raptors, showing resistance to clindamycin (100%), ampicillin (68%), tetracycline (67%), cefuroxime (58%), enrofloxacin (57%), and trimethoprim/sulphamethoxazole (55%) (Vidal *et al.,* 2017). Our results demonstrated similar resistance to enrofloxacin

(63.64%), nalidixic acid (63.64%), tetracycline (81.82%), trimethoprim/ sulfamethoxazole (81.82%), and clindamycin (100%). However, the resistance to β-lactam antibiotics was quite variable, from 0% to 100%. Another study indicated *K. pneumoniae* susceptible to amikacin, tobramycin, and gentamicin (Vidal *et al.*, 2017). In our research, it also showed susceptibility to amikacin, but high resistance to tobramycin and variable resistance to gentamycin. Our results suggest that ceftiofur, colistin sulfate, polymyxin B, amikacin, and ceftazidime may serve as effective therapeutic options against *K. pneumoniae.*

Taiwan experienced a large-scale outbreak of highly pathogenic avian influenza virus (HPAIV) subtype H5N6 in 2017 and HPAIV type H5N1 in 2023. The H5N6 epidemic and H5N1 pandemic affected multiple poultry farms across various regions, prompting the government to implement emergency response measures. Taipei Zoo has initiated a series of biosecurity measures since 2017, including suspension of wild bird rescue and rehabilitation services, cessation of activities in the avian display zone of the children's area, installation of disinfectant footbaths at the entrances of the aviary and waterfowl zones, and strengthening avian health surveillance and environmental pathogen control strategies. The aviary section of the zoo was even closed in 2020 due to the outbreak of *Chlamydia psittaci* among birds of the family *Columbidae*. Since 2017, more stringent management practices have been implemented, encompassing rigorous regulation of human–avian interactions and a more judicious approach to antibiotic administration. Additionally, microbiological testing was transferred to another contracted commercial laboratory that employed a distinct detection system and antimicrobial testing panel. Due to the methodological and panel differences introduced after 2017, integrating all data for longer-term analysis has become challenging.

The aviary section of the zoo has been fully reopened to the public following the 2023 H5N1 outbreak. As a result, renewed attention has been drawn to its implications for public health and the safety of veterinary antibiotic use. Over the past two decades, the range of antibiotics applied at Taipei Zoo has remained largely consistent. Benefiting from the implementation of stringent biosecurity measures and management practices during and after the epidemic and pandemic periods, the emergence of AMR strains or the horizontal transfer of resistance genes seems unlikely to have undergone substantial changes beyond the study period that would compromise the validity of the findings. Nevertheless, future studies may be further informed by analysis of data collected after 2017 to further update the current conclusions, if feasible.

The present study has several limitations. Firstly, the identified bacteria may not be the primary etiology. The disease might be caused by nutritional deficiency or husbandry problems. In addition, most individuals among the necropsied bird population showed no pre-existing clinical symptoms, and the identified bacteria might not be the cause of death. Secondly, the antibiotic resistance profiles of bacteria may vary across different regions, as empirical antibiotic use by clinicians and environmental factors can influence resistance levels. Therefore, except in emergency cases, it is recommended to conduct antibiotic sensitivity testing and select the most appropriate antibiotics according to the results. However, proper husbandry and adequate nutrition should be prioritized to reduce the risk of bacterial infections in animals. These strategies contribute to mitigating bacterial antibiotic resistance while minimizing adverse pharmacological effects in birds. Finally, due to the small sample size, some uncommon but clinically significant pathogenic bacteria may have been overlooked. A broader, large-scale investigation covering a wider geographic area is recommended for future studies.

To sum up, this study presents data on the prevalent bacterial strains and their antimicrobial susceptibility profiles in zoo captive birds. *E. coli* was the predominant bacterium, exhibiting a rising trend of resistance to the aminoglycosides. It is recommended that sampling and submission for antimicrobial susceptibility testing should be conducted prior to each treatment to ensure appropriate therapeutic choices. Pending the availability of test results, veterinarians could have provisional antibiotic options by referring to Table 3, reducing the possibility of resistant strain development. Furthermore, stringent zoo management practices, including the implementation of effective animal isolation protocols and comprehensive disinfection procedures, also play a critical role in preventing the dissemination and transmission of pathogenic organisms. Collectively, all of these may significantly contribute to mitigating the issue of antimicrobial resistance within the zoo.

Data Availability Statement: Supplementary tables are available on request from the corresponding author.

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