RESEARCH ARTICLE

Isolation, Identification and Antibiogram of Cloacal Flora from Apparently Healthy Caged Zoo Birds

Akash V. Golaviya[1], Suchit S. Pandya[1]*, Pratyush G. Patankar[1], Harshrajsinh B. Joddha[2], Chirag M. Chavda[2], Bharat B. Bhanderi[2] and Mayurdhvaj K. Jhala[2]

Abstract

Antibiotics are routinely used in animals and birds to prevent and cure bacterial infections. Various multidrug resistance bacteria have been detected in caged zoo birds, however there is a little information about their resistance pattern. In current study we isolated and identified cloacal bacteria from 50 zoo birds and compared their antimicrobial susceptibility. A total of 28 cloacal samples (56%) were found positive for different bacteria by selective culture. The bacteria isolated from different types of caged birds were *Escherichia coli* (26%), *Staphylococcus* spp. (8%), *Streptococcus* spp. (4%) and some unidentified Gram-positive and Gram-negative bacteria (14%). Of these isolates, *E. coli* was the most frequent isolate. The results of antibiotic sensitivity tests revealed that cefotaxime and sulphadiazine were highly (>60%) resistant to isolated cloacal microflora of zoo birds. However, the antibiotics such as ceftriaxone and amikacin showed moderate to high sensitivity against almost all the bacterial isolates. Of these, co-trimoxazole was found to be consistently highly effective (100%) on all the *E. coli* isolates. Overall, the results showed that zoo birds can be carrier of multidrug resistant organisms including *Escherichia coli*.

Key words: Antibiotic sensitivity, Cloacal flora, *Escherichia coli*, Zoo birds.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.1.09

Introduction

Zoo birds come in close contact with humans as a result of their handling, management, and treatment, and hence could be a serious source of infection for both humans and animals. Zoo birds are susceptible to several diseases such as colibacillosis, salmonellosis, chlamydiasis and mycoplasmosis that affect poultry and human (Abdallah and Khalil, 2016). Zoo birds are frequently infected with *Escherichia coli, Staphylococcus, Klebsiella, Enterobacter, Salmonella, Pasteurella, Pseudomonas and Campylobacter* in response to stress and immunosuppression (Legadevi *et al*., 2019). In zoo birds, *Escherichia coli* is the most common opportunistic enterobacteria, and it has been linked to systemic diseases such as bacterial enteritis, colisepticemia, coligranuloma, coliform cellulitis, peritonitis, salpingitis, and omphalitis (Nolan *et al*., 2020).

In the recent years, significant increase in antibiotic-resistant has been noted not only of pathogenic, but also among commensal bacteria of zoo birds (Nowaczek *et al*., 2021). The occurrence of resistant bacteria in wild birds, including multidrug resistant strains, has been demonstrated by several authors around the world (Literak *et al*., 2010; Radhouani *et al*., 2012; Pinto *et al*., 2015; Legadevi *et al*., 2019: Sigirci *et al*., 2020). Human being contract several zoonotic diseases through direct or indirect contact with diseased or carrier birds in cages or as pets. Zoo visitors, zookeepers, and veterinarians are more likely to contract zoonotic illnesses from zoo cage birds (Akhter *et al*., 2010). The information on normal gut flora and antibiotic resistance pattern is limited for the majority of wild bird species . For this, proper isolation and characterization of the pathogenic and non-pathogenic bacteria are essential to control diseases and maintain health of zoo and wild birds. Few studies have been done in India on the isolation and identification of cloacal microflora from zoo birds (Legadevi *et al*., 2019). So the goal of this study was to isolate, identify as well as determine the antibiotic sensitivity patterns of bacteria isolated from caged zoo birds.

[1]Sri Sayajibaugh Zoo, Vadodara Municipal Corporation, Vadodara-390018, Gujarat, India

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India

Corresponding Author: Suchit S. Pandya, Sri Sayajibaugh Zoo, Vadodara Municipal Corporation, Vadodara-390018, Gujarat, India, E-mail: pandyasuchit@gmail.com

How to cite this article: Golaviya, A. V., Pandya, S. S., Patankar, P. G., Joddha, H. B., Chavda, C. M., Bhanderi, B. B., & Jhala, M. K. (2023). Isolation, Identification and Antibiogram of Cloacal Flora from Apparently Healthy Caged Zoo Birds. Ind J Vet Sci and Biotech. 19(1), 38-42.

Source of support: Nil

Conflict of interest: None

Submitted: 23/11/2022 **Accepted:** 24/12/2022 **Published:** 10/01/2023

Materials and Methods

Study Area and Zoo Birds

A total 50 cloacal swab samples were collected aseptically using sterile swab from captive zoo birds at the Shri Sayajibaugh Zoo, Vadodara, Gujarat, India during January 2022. Sampled zoo birds included Cockatiel (07), Golden Pheasant (02), Budgerigar (10), Peacock (03), Silver Pheasant (03), Ring Neck Pheasant (03), Red Mollucan Lorry (01), Senegal Parrot (01), Alexandrine Parakeet (04), Violet Turaco (02), Conure Golden Fronted (02), Love Bird (02), Guinea Turaco (01), African Grey (02), Green Chick Conure (02), Black Cap Lorry (01), Sun Conure (02), and ConureJindiya (02). The cloacal swab samples were transferred to specimen vial and labelled properly according to the species of zoo birds. The samples were transferred to the Microbiology Laboratory of the University under cold chain condition.

Cultural Isolation and Identification of Bacterial Isolates

Cloacal swab samples were inoculated as per Barrow and Feltham (1993) on 5% sheep blood agar (SBA) and MacConkey agar and incubated overnight at 37 °C. From each plate of blood agar, isolated *Staphylococcus* spp. colony was inoculated on Mannitol salt agar (MSA) while from each plate of MacConkey agar, isolated lactose fermenting colony was inoculated on eosin methylene blue (EMB) agar and incubated overnight at 37 °C for preliminary characterization. Colony growth on MSA agar (yellow or red colonies) and colonies on EMB agar showing greenish metallic sheen were considered as presumptive *Staphylococcus* spp. and *E. coli,* respectively. The pure culture of *Staphylococcus* spp. *Streptococcus* spp. and *E. coli* isolates was stored in Brain heart infusion (BHI) slants for further identification and biochemical tests.

Pure bacterial isolates were identified conventionally based on culture characters, staining reactions, and microscopical morphology according to Cruickshank *et al*. (1975). The presumptive *Staphylococcus* spp, *Streptococcus* spp. and *E. coli* isolates were identified by primary biochemical tests*, viz.,* catalase, KOH and oxidase. The *E. coli* isolates were further characterized for their biochemical and haemolytic activity by sugar fermentation test and on 5% sheep blood agar as per method described by Edwards and Ewing (1972) and Markey *et al.* (2013), respectively. Haemolytic patterns of the *E. coli* isolates were categorized according to the types of haemolytic zone they produced on SBA plates.

Antibiotic Susceptibility Testing of Isolates

The bacterial isolates were subjected to *in vitro* antibiotic sensitivity testing using the disk diffusion method (CLSI, 2022). The antibiotic discs were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai (India). Isolates were tested against commonly used antibiotics, *viz.,* amikacin (30 µg), amoxiclav (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), co-trimoxazole (25 µg), sulphadiazine (300 µg), tetracycline (30 µg). The zone diameter was compared with zone size interpretative chart supplied by the manufacturer. The inhibition zones were measured and scored as susceptible (S), intermediate (I) or resistant (R) (CLSI, 2022).

Results and Discussion

The increase in antibiotic-resistant bacteria in wild zoo birds is a global threat, since it can cause high morbidity and mortality, as well as higher healthcare expenditures in zoo. Majority of zoo birds' studies of resistant bacterial isolates have been based on phenotype characterization of these isolates (Literak *et al.,* 2010). In order to expand the available information on antibiotic resistance in zoo birds, we have investigated this topic on caged zoo birds.

Overall Incidence of Types of Bacterial Isolates

In the present study, out of total 50 cloacal swab samples screened, 28 (56%) samples were found positive for bacterial isolates by selective cultural and biochemical examination of single colony per plate. Eleven isolates (22%) were identified as *E. coli,* two as *Staphylococcus* spp. (4%), two for *Streptococcus* spp. (4%), two as mixed infection (*E. coli* and *Staphylococcus* spp., 4%), while remaining seven isolates (14%) included unidentified Gram-positive (6%) and Gram-negative bacteria (8%).

The present result agreed with the report of Rogers (2006) and Hedawy and El-Shorbagy (2007) who isolated and identify bacterial isolates in 38% and 18% samples, respectively. Nearly similar findings were reported by Doneley (2009) and Akhter et al. (2010). *Enterobacteriaceae*, particularly *E. coli*, is one of the most common bacterial infections spread by zoo birds, causing food poisoning and providing a zoonotic risk (Abdallah and Khalil, 2016). In present study, the overall incidence of *E. coli* was 26% (13 samples) in the examined zoo birds. On the other hand, more than 60% prevalence of *E. coli* was reported by Akhter *et al.* (2010), Suphoronski *et al.* (2015) and Abdallah and Khalil (2016) in zoo birds. The variation in *E. coli* prevalence rate may be attributed to multiple factors such as age and health of birds, state of immunity and medication and environmental conditions (temperature, humidity, and air currents). Also, it may be related to degree of hygiene of feed, water, air and litter in zoo.

The results for the fermentation reactions of carbohydrates by all 13 *E. coli* isolates were found variable (Table 1). Result revealed that dulcitol, sucrose, salicin, adonitol and inositol were fermented by 8 (61.53%), 6 (46.15%), 3 (23.07%), 2 (15.38%) and 2 (15.38%) isolates, respectively. There were some of *E. coli* isolates, which were late fermenter and fermented the sugars after 48-72 h. The result in decreasing order indicated 4 (30.76%), 2 (15.38%), 2 (15.38%), 1 (7.69%) and 1 (7.69%) isolate slowly fermented the salicin, sucrose, dulcitol, adonitol and inositol, respectively. The haemolysis ability of *E. coli* isolates was observed on 5% sheep blood

agar. Out of 13 *E. coli* isolates, 5 (38.46%) isolates produced complete haemolysis (β) around the colonies.

Overall Antibacterial Resistant Pattern of Isolates

All the isolates in this study were tested against 07 antimicrobial agents. Considering the total of evaluated isolates, the highest rate of antimicrobial resistance occurred for sulphadiazine 42.85% (12/28). *Staphylococcus* spp. and *Streptococcus* spp. isolates were highly sensitive (> 60%) to most of the commonly used antibiotics. *E. coli* isolates were highly resistant to cefotaxime (61.55%), sulphadiazine (61.55%), tetracycline (53.84%) and amoxiclav (46.15%). The drugs with the best efficacy for *E. coli* were co-trimoxazole (100%), amikacin (76.92%) and ceftriaxone (46.15%) (Table 2). In present study, unidentified Gram-positive bacteria were highly sensitive to amoxiclav, ceftriaxone and tetracycline, while all unidentified Gram-negative bacterial isolates were sensitive to co-trimoxazole (100%).

Table 1: Results of samples found positive for *E. coli* with haemolytic & biochemical activity

Sr. No.	Name of zoo birds with sample code	Haemo-lysis on 5% SBA	Sugar utilization				
			Adonitol (Ad)	Dulcitol (Du)	Inositol (I)	Salicin (Sa)	Sucrose (Su)
	Silver Pheasant-1	-	-	+	-	+*	+
	Silver Pheasan-2	-	-	+	+*	-	+*
	Red Mollucan Lorry-1	+ (β)	-	+	-	+	-
	Budgerigar-5	+ (β)	+	-	+	-	+
	Peacock-1	-	-	+	-	-	+
	Peacock-2	+ (β)	-	+	-	+	-
	Budgerigar-9	-	-	+	-	+	+
	African Gray-1	-	+*	-	-	-	+
	Green Chick Connure-2	+ (β)	-	+*	-	+*	-
	Ring Neck Pheasant-1	-	-	+	+	-	+*
	Black Cap Lorry-1	-	+	-	-	-	-
	Budgerigar-3	+ (β)	-	+*	-	+*	-
	Budgerigar-8	-	-	+	-	+*	+

β : Beta haemolysis, +: Positive, -: Negative, +*: Late fermenter.

Table 2: Overall results of antibiotic susceptibility testing of bacterial isolates

Name of Bacteria	Sensitivity Pattern	Sensitivity (%) of the bacterial isolates to various antibiotics						
		AK*	AMC*	CTR*	CTX*	COT*	SZ*	TE*
E. coli (n=13)	Sensitive	76.90	15.38	46.15	15.38	100	38.45	46.15
	Intermediate	23.10	38.46	30.76	23.07	0.00	0.00	0.00
	Resistant	0.00	46.16	23.09	61.55	0.00	61.55	53.85
Staphylococcus spp. (n=4)	Sensitive	50.00	100	50.00	75.00	75.00	75.00	75.00
	Intermediate	25.00	0.00	50.00	25.00	0.00	0.00	0.00
	Resistant	25.00	0.00	0.00	0.00	25.00	25.00	25.00
Streptococcus spp. (n=2)	Sensitive	100	100	50.00	100	100	50.00	100
	Intermediate	0.00	0.00	50.00	0.00	0.00	0.00	0.00
	Resistant	0.00	0.00	0.00	0.00	0.00	50.00	0.00
Unidentified Gram Positive (n=3)	Sensitive	66.67	100	100	100	33.33	66.67	100
	Intermediate	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Resistant	33.33	0.00	0.00	0.00	66.67	33.33	0.00
Unidentified Gram Negative (n=4)	Sensitive	75.00	25.00	50.00	25.00	100	25.00	50.00
	Intermediate	0.00	0.00	25.00	25.00	0.00	75.00	0.00
	Resistant	25.00	75.00	25.00	50.00	0.00	0.00	50.00

*AK: Amikacin; AMC: Amoxycillin/Clavulanic acid; CTR: Ceftriaxone; CTX: Cefotaxime; CoT: Co-trimoxazole; SZ: Sulphadiazine; TE: Tetracycline.

Detection of Multiple Drug Resistance (MDR) of Bacterial Isolates

Multidrug resistance, which is defined as resistance against three or more classes of antimicrobials, was associated with 84.61% of the *E. coli* isolated from zoo birds. In the present study, 53.84% (7/13) *E. coli* isolates showed resistance to two drugs, 7.69% (1/13) to three drugs, 7.69% (1/13) to four drugs and 15.38% (2/13) showed resistance to five drugs. One *E. coli* isolate was susceptible to all antimicrobials. *Staphylococcus* spp. and *Streptococcus* spp. isolates had a very lower MDR index than *E. coli* isolates. One unidentified Gram-positive and two unidentified Gram-negative isolates showed resistance to three antimicrobial drugs used in this study.

About 35% of isolates were resistant to commonly used antibiotics such as sulphadiazine, cefotaxime and tetracycline. Beleza *et al*. (2021) in Mulungu, Brazil reported highest rate of antimicrobial resistance in free living wild birds against ampicillin (41.8%), followed by nalidixic acid (36.3%) and amoxicillin-clavulanic acid (32.7%), while tetracycline (90.9%) showed highest rate of sensitivity. *E. coli* isolates of wild birds were also reported to be more resistant to tetracycline, nalidixic acid, amikacin and amoxiclav (Borges *et al*., 2017). High levels of resistance to tetracycline by different *E. coli* serotype isolated from zoo birds have been described by Abdallah and Khalil (2016). The growth and rapid spread of multidrug-resistant bacteria is a major public health concern, as they can cause severe infections with very limited therapeutic options (Suarez-Perez *et al.,* 2020).

Conclusions

This study highlights 56% cloacal samples positive for different bacterial isolates. Isolated cloacal flora were highly resistant to sulphadiazine and cefotaxime, but were sensitive to co-trimoxazole. Our data show that captive zoo birds can be a reservoir for MDR bacteria that are potentially pathogenic for humans and poultry. Further molecular research is needed to clarify the presence of antibiotics resistance genes and the mechanisms of resistance to these antibiotics.

Acknowledgement

The authors are thankful to the curator and helper of Shri Sayajibaugh Zoo, Vadodara, Gujarat, India for assistance in sample collection from zoo birds.

References

Abdallah, S.M., & Khalil, S.A. (2016). Molecular studies on *Escherichia coli* serotypes in Zoo birds. *Alexandria Journal for Veterinary Sciences*, *51*(2), 7-14.

Akhter, J., Hossain, M.T., Islam, M.T., Siddique, M.P., & Islam, M.A. (2010). Isolation and identification of microflora from apparently healthy caged parrots of Dhaka Zoo of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, *8*(1), 05-10.

Beleza, A.J.F., Maciel, W.C., Carreira, A.S., Marques, A.R., Nogueira, C.H.G., Pascoal Filho, N.M., ... & de Castro Teixeira, R.S. (2021). Wild birds as reservoirs of multidrug-resistant enterobacteria in Mulungu, Brazil. *Bio Rxiv*., 1-29.

Borges, C.A., Beraldo, L.G., Maluta, R.P., Cardozo, M.V., Barboza, K.B., Guastalli, E.A.L., ... & Ávila, F.A. (2017). Multidrug-resistant pathogenic *Escherichia coli* isolated from wild birds in a veterinary hospital. *Avian Pathology*, *46*(1), 76-83.

CLSI - Clinical Laboratory Standards Institute (2022). M100-S22: performance standards for antimicrobial susceptibility testing; thirty second informational supplement -M100S22E. pdf. M100Ed32 | Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition (clsi.org).

Cruickshank, R., Duguid, J., Marmion, B.P., & Swain, (1975). *Medical Microbiology*. The practice of medical microbiology, 12th Edn, Vol. II. Churchill. Edinburgh.

Doneley R.T. (2009). Bacterial and parasitic diseases of parrots. Veterinary Clinics of North America: *Exotic Animal Practice, 12*(3), 417-432.

Edwards, R. & Ewing, W.N. (1972). *Identification of Enterobacteriaceae.* 3rd edn., Burgess Publishing Co., Minnesota

Hedawy, K., & El-Shorbagy, M. (2007). Role of some wild birds in transmitting some Bacterial agents among poultry farms in Sohag Governorate. *Assuit Veterinary Medicine Journal*, *53,* 251-257.

Legadevi, R., Dillibabu, V., Karunakaran, N., Nagarajan, K., & Soundararajan, C. (2019). Isolation and identification of bacteria of normal flora in handfed exotic psittacine birds at Tamil Nadu, India. *Indian Journal of Veterinary & Animal Science Research*, *48*(2), 18-25.

Literak, I., Dolejska, M., Janoszowska, D., Hrusakova, J., Meissner, W., Rzyska, H., ... & Cizek, A. (2010). Antibiotic-resistant *Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and *QnrS*, in waterbirds on the Baltic Sea Coast of Poland. *Applied and Environmental Microbiology*, *76*(24), 8126-8134.

Markey, B., Leonard, F., Archambault, M., Cullinane, A., & Maguire, D. (2013). *Clinical Veterinary Microbiology*. 2nd Ed. UK, Elsevier Health. ISBN – 9780702055881.

Nolan, L. K., Barnes, H. J., Vaillancourt, J. P., Abdul-Aziz, T., & Logue, C. M. (2020). Colibacillosis. In: *Diseases of Poultry*, p. 770-830. Blackwell publishing, United States.

Nowaczek, A., Dec, M., Stępień-Pyśniak, D., Urban-Chmiel, R., Marek, A., & Różański, P. (2021). Antibiotic resistance and virulence profiles of *Escherichia coli* strains isolated from wild birds in Poland. *Pathogens*, *10*(8), 1059.

Pinto, A., Simões, R., Oliveira, M., Vaz-Pires, P., Brandão, R., & da Costa, P. M. (2015). Multidrug resistance in wild bird populations: importance of the food chain. *Journal of Zoo and Wildlife Medicine*, *46*(4), 723-731.

Radhouani, H., Poeta, P., Goncalves, A., Pacheco, R., Sargo, R., & Igrejas, G. (2012). Wild birds as biological indicators of environmental pollution: antimicrobial resistance patterns of *Escherichia coli* and *Enterococci* isolated from common buzzards (Buteo buteo). *Journal of Medical Microbiology*, *61*(6), 837-843.

Rogers, K.H. (2006). Prevalence of pathogenic enteric bacteria in wild birds associated with agriculture in Humboldt County,

California (Master's thesis, Humboldt State University). http://hdl.handle.net/2148/100

Sigirci, B.D., Celik, B., Halac, B., Adiguzel, M.C., Kekec, I., Metiner, K., ... & Kahraman, B. B. (2020). Antimicrobial resistance profiles of *Escherichia coli* isolated from companion birds. *Journal of King Saud University-Science*, *32*(1), 1069-1073.

Suárez-Pérez, A., Corbera, J.A., González-Martín, M., Donázar, J.A., Rosales, R.S., Morales, M., & Tejedor-Junco, M.T. (2020). Microorganisms resistant to antimicrobials in wild canarianegyptian vultures (*Neophronpercnopterus majorensis). Animals*, *10*(6), 970.

Suphoronski, S.A., de Freitas Raso, T., Weinert, N.C., Seki, M.C., & Carrasco, A.D.O.T. (2015). Occurrence of *Salmonella* sp. and *Escherichia coli* in free-living and captive wild birds from 2010-2013 in Guarapuava, Paran, Brazil. *African Journal of Microbiology Research*, *9*(29), 1778-1782.