Isolation and identification of *Escherichia coli* from cloacal swab of ornamental pet birds in Chattogram, Bangladesh

Submitted by

Sabrina Rahman Sabrin

Roll No: 18/44

Reg. No: 03006

Intern ID: 42

A Clinical report submitted in partial satisfaction of the requirement for the

Degree of Doctor of Veterinary Medicine (DVM)

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

November, 2023

Isolation and identification of *Escherichia coli* from cloacal swab of ornamental pet birds in Chattogram, Bangladesh

Supervised by

Dr. Eaftekhar Ahmed Rana

Assistant Professor

Department of Microbiology and Veterinary Public Health, CVASU

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

November, 2023

Table of Contents

List of Tables

List of figures

Abstract

Pet birds are basically housed for ornamental use and as good companions. *Escherichia coli* is a common bacterial pathogen in pet birds, residing in their intestinal tract as a commensal organism. Avian Pathogenic *E. coli* can cause avian colibacillosis. The increase of antimicrobial resistance in companion animals has already been reported worldwide. However, there is limited data and studies focusing on the resistance profiles of *Escherichia coli* isolates from Passeriformes and Psittaciformes, which regarded to be among the most common and popular pet/companion bird species. The current cross-sectional study was conducted to isolate and identify *E. coli* from apparently healthy companion birds and their antimicrobial resistance profiles. Conventional bacteriological techniques were employed to isolate and recognize *E. coli*. The confirmation of *E. coli* identification was established through Gram staining, TSI and Culture Sensitivity test. The isolated strains were assessed for their susceptibility to 6 different antimicrobial agents to determine their resistance patterns. Among the 21 samples tested, *E. coli* isolates were identified in 12 samples, accounting for 57% of the total. Notably, these twelve isolates exhibited resistance to multiple antimicrobial drugs. This study provides evidence of *E. coli* presence in pet birds and their antibiotic resistance profiles. This information can serve as a basis for further large-scale research into *E. coli* colonization in pet birds in Bangladesh.

Keywords: Pet birds, *E. coli*, Antimicrobial resistance, Isolates.

Chapter 1

Introduction

A "Pet bird" refers to wild or exotic birds that possess desirable genetic qualities and are bred in captivity for their aesthetic qualities. People have been keeping birds as pets and companions for thousands of years. The ancient Egyptians, Greeks, Romans, and many other older civilizations kept birds as pets. Birds are wonderful choices as a child's first pet since they can be smoothly incorporated into family life and help children learn the responsibilities of caring for an animal. They are considered as cherished companions to humans and hold a significant role in human life (Cong *et al*., 2014).

The growing trend of keeping pet birds has heightened the importance of understanding pet bird-related diseases that can be transmitted to humans (pet bird zoonosis) and the field of pet bird healthcare (Veladiano *et al*., 2016). The majority of caged birds belong to two main orders: Passeriformes, which includes canaries and finches, and Psittaciformes, which encompasses parrots, parakeets, and lovebirds (Boseret *et al*., 2013).

Household pets are acknowledged as significant origins of zoonotic diseases and bear multidrug-resistant (MDR) bacteria. Several authors have emphasized the potential transfer of drug-resistant bacteria and genes for antimicrobial resistance between animals and humans through contaminated food, the surroundings, or direct contact (Damborg *et al*., 2016, Argudín *et al*., 2017, and Pompa *et al*., 2017). The close interaction between humans and their household pets creates numerous opportunities for such transmission. Additionally, the use of empiric treatments for pet birds might inadvertently contribute to the development of antimicrobial resistance (Giacopello *et al*., 2015).

E. coli, a member of the Enterobacteriaceae family, commonly resides in the gastrointestinal tracts of both humans and animals as a commensal organism. While it typically maintains a mutually beneficial relationship with its host and seldom causes illness, it can also act as a pathogen responsible for a wide range of diseases. Moreover, commensal *E. coli* in birds can serve as reservoirs for drug-resistant bacteria and related genes (Machado *et al*., 2018). The adaptability of *E. coli* to changing environments offers numerous resistance mechanisms, making commensal *E. coli* a potential source of antimicrobial resistance (Szmolka and Nagy *et al*., 2013).

Various *E. coli* strains can result in both intestinal and non-intestinal illnesses due to the presence of virulence factors that affect a range of cellular processes (Kaper *et al*., 2004). According to the findings of Croxen and Finlay *et al*., 2010, there has been a significant global upsurge in the number of cases of these diseases, impacting hundreds of millions of individuals each year. *E. coli* can also give rise to diseases in animals, and colibacillosis stands out as a primary cause of bird mortality, leading to considerable economic losses worldwide (Schouler *et al*., 2012). A study carried out by Trampel et al., 2007 in the United States found that, *E. coli* was detected in 14 out of 15 birds that appeared to be healthy, indicating that these animals harbor the bacterium without exhibiting clinical symptoms, potentially contributing to the disease's spread.

Many bacterial infections often result from the contamination of water or food by the feces of infected animals, and direct contact between susceptible and infected animals can also lead to contamination (Kuroki *et al.,* 2013). Birds play a significant role in spreading these diseases, even if they don't display clinical symptoms. Birds can potentially carry various bacteria, in addition to viruses and parasites (Dovc *et al.,* 2004). According to Berchieri *et al*., 2001, the duration of fecal shedding and the extent of tissue invasion (pathogenicity) vary depending on the age of the bird at the time of infection. Consequently, a bird may transmit the infection to other animals or humans over an extended period.

Historically, veterinarians often used antibiotics to treat sick birds, especially when bacterial infections were the root cause (Doneley *et al*., 2009). Antimicrobial resistance refers to bacteria's ability to resist the effects of drugs meant to combat them. The World Health Organization (WHO) has connected the recent surge in antibiotic-resistant bacterial infections to the excessive and unregulated use of these drugs in both human and veterinary medicine (Siqueira *et al*., 2017). As a result, this research aimed to investigate the prevalence, virulence, and patterns of antimicrobial resistance in bacteria that could potentially transmit diseases from commonly kept pet birds.

Numerous studies have investigated the isolation of *E. coli* from cloacal swabs of pet birds to identify antimicrobial resistance against specific drugs and determine the prevalence of *E. coli* in healthy birds. However, a comprehensive understanding of *E. coli* in pet birds is still a subject of ongoing analysis, and this research will undoubtedly contribute significantly to enhance our knowledge in this area. Moreover, Thus this study was designed to form the following objectives:

a) Isolation and identification of *E. coli* from cloacal swabs of pet birds.

b) Characterization of *E. coli* using bacteriological culture media and biochemical tests.

Chapter 2

Materials and methods

2.1 Study location

The study was conducted between March to September 2023, involving visits to various pet bird shops, Shahedul Alam Quadery Teaching Veterinary Hospital (SAQTVH), CVASU and some houses with pet birds in Chattogram. Majority of the pet birds observed during this period were healthy despite a few clinical cases.

2.2 Sample collection

A total of 21 samples were collected from cloacal swab of pet birds. For this purpose, buffered peptone water was prepared as nutrient broth in felcon tube for transportation of samples. Swabs were taken by sterilized cottons according to body size of birds. After collection, all the tubes were kept in incubator overnight for further process. Autoclaving of instruments was maintained as a regular activity in these research.

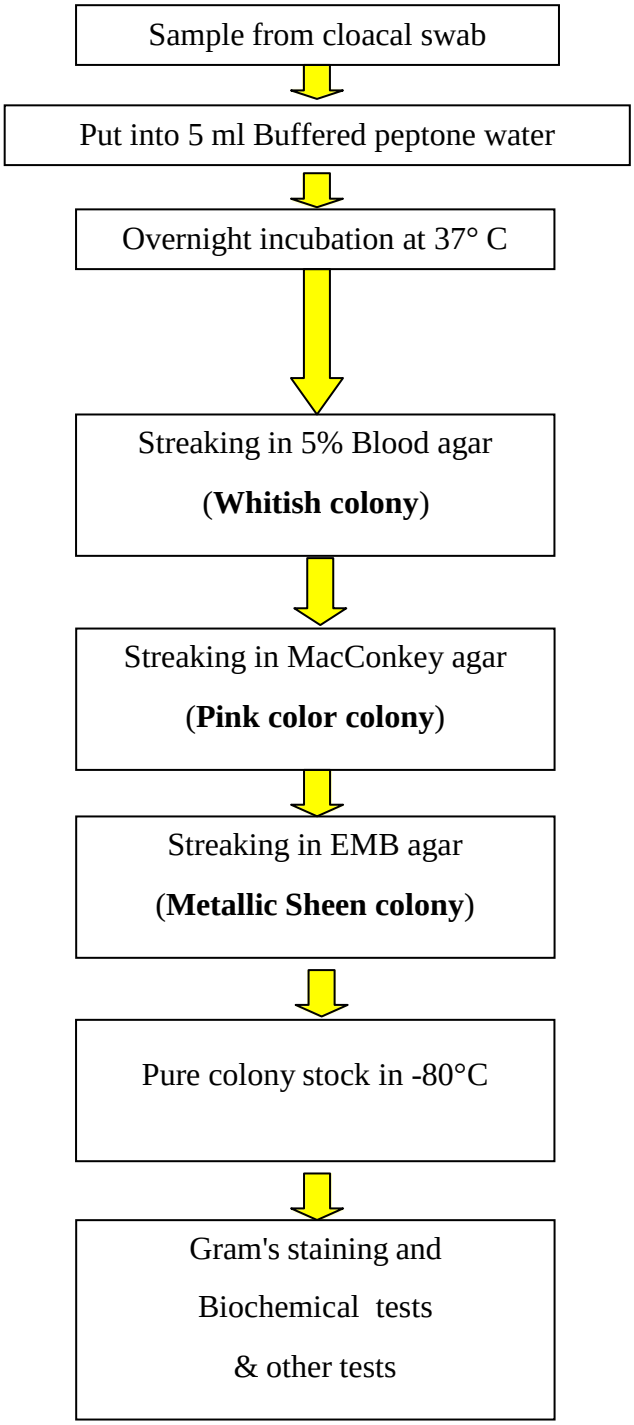
Figure 1: Samples collected in Buffered Peptone Water

2.3 Study population/Target species:

Samples taken from the birds were, Budgerigar (6)**,** Pigeon (3)**,** Parrot (4; red breasted parakeet, red lory, rump parrot, ring neck)**,** Dove (3)**,** Conure (2 green set)**,** Cockatiel (2 lutino)**,** Love bird (1)**,** Java (1).

Figure 2: Birds from which samples were collected

2.4 <u>Protocol in brief:</u>



2.4.1 Isolation of *E. coli*:

To isolate *E. coli* bacteria from collected cloacal swab samples, the process began by taking 0.5 grams of cloacal swab material and placing it into test tubes with Buffered Peptone Water. These mixtures were then left to incubate at 37°C overnight for the initial enrichment step. Following the initial enrichment, the samples were inoculated onto Blood agar with the help of sterilized loop and placed in the incubator at 37°C for 24 hours. On the following day, the cultures revealed large, greyish, thick and opaque colonies which is specific for *E.coli*. Subsequently, in the next day streaked on MacConkey agar medium and placed incubated at the same temperature for 24 hours. Interestingly, the cultures displayed unique characteristics as they grew on the MacConkey agar plates.

The colonies that formed were easily identifiable due to their vivid pink color, large size, and lack of a mucoid appearance. These specific colony traits led to suspicion that the microorganism might be *E. coli* based on its colony morphology. Subsequently, individual colonies from the MacConkey agar plates were streaked onto EMB (Eosin Methylene Blue) agar plates. These EMB agar plates were also incubated for 24 hours at 37°C. Confirmation of the organism's identity as *E. coli* was established by observing the distinctive "greenish metallic sheen" colony morphology on the EMB agar plates, in addition to performing other biochemical tests and examining Gram's staining properties.

2.4.2 Gram staining procedure:

A small colony was taken with a bacteriological loop, spread on a clean, grease-free microscope slide, and gently heated to fix it. Next, the smear was treated with crystal violet solution for two minutes and then rinsed with running water. Gram's iodine was applied as a mordant for one minute and excess fluid was removed. Acetone alcohol was briefly used as a decolorizer, followed by washing with running water. Safranin was applied as a counterstain for 2 minutes. The slides were then rinsed, blotted, air-dried, and examined under a microscope with a high-power objective (100X) using immersion oil. The smear displayed pink, rod-shaped colonies, indicating Gram-negative characteristics.

2.4.3 Biochemical test:

We performed **Triple Sugar Iron (TSI)** test. It checks for the bacterium's ability to ferment glucose, sucrose, and/or lactose, resulting in the production of acid and gas, and also its potential to create hydrogen sulfide.

By using a sterile straight inoculation needle to gently make contact with the upper part of a clearly isolated colony. Next, the inoculation needle were introduced into the TSI agar by initially piercing through the middle of the medium until it reaches the bottom of the tube, and then streaking it along the surface of the agar slant. The tube cap was kept slightly and placed in an incubator at 35°C for 18 to 24 hours in the surrounding air.

2.4.4 Antibiogram study:

The isolates of the samples which showed positive result for *E. coli* were subjected to an antibiotic sensitivity test using the disc diffusion method. The procedure for the cultural sensitivity (CS) test began by cultivating the preserved organism on blood agar and then incubating it at 37°C for 24 hours to ensure the growth of pure colonies. Following this, three or four individual colonies from the blood agar were transferred into a tube containing 3ml of sterile phosphate buffer saline solution (0.85% w/v NaCl solution). To prevent clumping of cells, the inoculum was mixed using a vortex machine. The bacterial suspension was adjusted to match the 0.5 McFarland standard, representing a bacterial concentration of 1-2×10^8 CFU/ml. Within 15 minutes of preparing the inoculum, a pre-sterile cotton swab was dipped into it and swirled against the tube's side to remove excess fluid. This swab was used to streak the entire dry surface of Mueller Hinton agar three times, rotating the plate about 60 degrees each time. After 15 minutes of inoculation, sterile discs were placed on the agar surface using sterile forceps. The agar plates were then incubated at 37°C for 18 hours. The size of the clear area (in millimeters) around each disc, including the disc's diameter, was measured using a ruler, and the results were interpreted as sensitive, intermediate, or resistant following the CLSI 2011 guidelines.

Table 1. Ranges for determination of pattern of antibiotic susceptibility

Antimicrobial agent	Disc code	Zone diameter (mm)		
		sensitive	**intermediate**	**resistant**
Ciprofloxacin	CIP-5	>21	16-20	<16
Ceftriaxone	CRO	>21	14-20	<14
Azithromycin	AZM	>18	14-17	<13
Erythromycin	E	>23	14-22	<13
Tetracycline	TE-30	>15	12-14	<11
Sulfamethoxazole-Trimethoprim	SXT	>16	11-15	<10

Chapter 3

Results

3.1 Samples found positive for *E.coli* in cloacal swab of healthy pet birds

A total of 12 samples were identified as *E. coli* out of 21 samples based on their cultural growth properties, morphology, biochemical characteristics and CS test.

Table 2. Number of *E.coli* positive bird

Variant	Co-variant	No. of birds	*E.coli* positive
Age (month)	24	3	3
	18	2	1
	12	2	1
	10	2	0
	9	2	0
	8	3	2
	7	3	2
	6	2	2
	3	2	1 **(total 12)**
Sex	Male	14	9
	Female	7	3 **(total 12)**

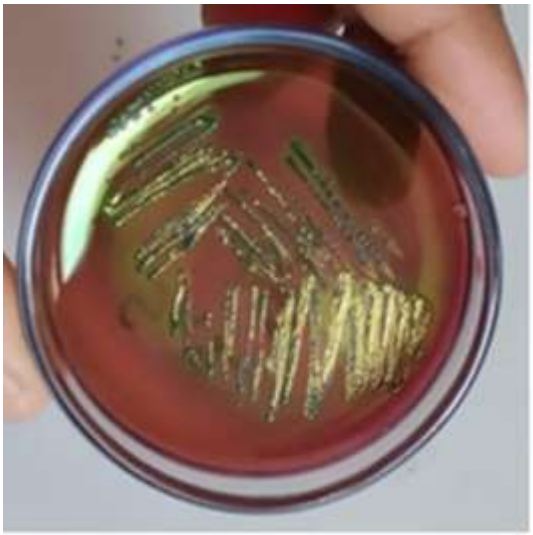
Most prevalence was observed among the age of 2 years and less prevalence occurred in the ages of below 1 years. That indicates adult birds have more possibility of carrying *E. coli*. Besides, highest prevalence is also observed in male birds than females.

3.2 Cultural properties of *E. coli:*

In selective culture media, Among all the samples 57% of the birds (12) contains *E.coli* showing bright pink, transparent, smooth and raised colonies on MacConkey agar, on EMB agar plates it showed characteristic colonies with greenish metallic sheen.

***E. coli* on Blood agar**

***E. coli* on MacConkey agar**



***E. coli* on EMB agar**

Figure 3: Isolation of *E. coli* in selective agar media

3.3 Gram staining:

In gram staining, *E. coli* were gram negative, small, rod shaped, and often seen in single, paired or short chain arrangements.

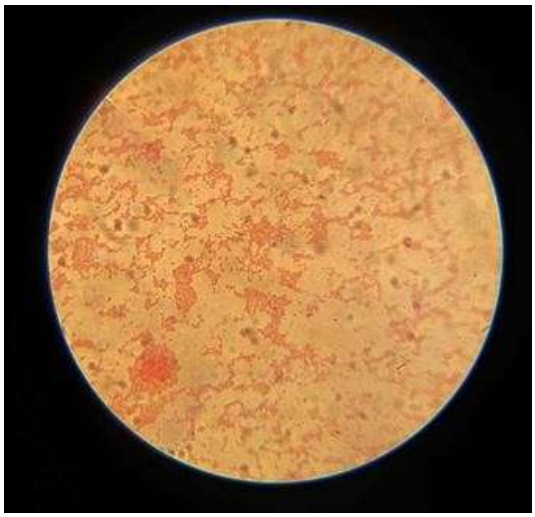


Figure 4: Identification of *E. coli* by gram staining

3.4 Results of Biochemical test

In TSI test, *E. coli* ferments glucose so bottom will turn yellow. *E. coli* produces gas which visible in cracks or bubbles, the slant may remain red or turn slightly pink indicating alkaline reaction. In our study, all the *E. coli* isolates were TSI positive.

Figure 5: Identification of *E. coli* by TSI test

3.5) Antimicrobial susceptibility profiles of *E. coli*

After performing cultural isolation, all the suspected *E. coli* positive isolates (12) were selected for Culture sensitivity (CS) test. Antibiotic resistance of *E. coli* was identified by zone of inhibition, however maximum 6 different antimicrobial discs were used.

Figure 6: CS test positive

Table 3. Percentage of antimicrobial resistance pattern

Antimicrobials	Number	Susceptibile	Intermediate	Resistant
Ciprofloxacin	12	75%	-	-
Azithromycin	12	50%	-	-
Tetracycline	12	-	-	92%
Erythromycin	12	-	-	100%
Ceftriaxone	12	92%	-	-
Sulfamethoxazole-Trimethoprim	12	-	-	83%

The highest susceptibility was observed in Ciprofloxacin (75%), Ceftriaxone (92%) and Azithromycin (50%). The highest resistance was observed in Erythromycin (100%), Tetracycline (92%), Sulfamethoxazole-Trimethoprim (83%).

CIP=Ciprofloxacin,AZM=Azithromycin,TE=Tetracycline,E=Erythromycin,
CRO=Ceftriaxone,SXT=Sulfamethoxazole-Trimethoprim

S=Susceptible, R=Resistant

Chapter 4

Discussion

In pet birds, zoonotic concerns are raised by several key bacterial pathogens. *Escherichia coli*, is one of them as indicated in studies by Youssef and Mansour *et al*., 2014 and Zahoor *et al*., 2018. The role of *Esherichia coli* as a beneficial member of the regular microbiota in healthy psittacine birds following a typical diet of grains, fruits, vegetables, and sprouts remains a matter of ongoing controversies. In this study, twelve bacterial isolates were obtained from pigeons, parrots, budgerigars, cockatiels, Love bird, Java, and Doves. These isolates have the potential to transmit diseases to humans and are resistant to multiple antibiotics.

In our study, we found that the prevalence of *E. coli* was 57% among 21 cloacal samples of healthy pet birds. The prevalence were in 25% of Budgeriger, 25% of Dove, 16.7% of parrot, 16.7% of pigeon,8.3% of Love bird and 8.3% of Cockatiel. Almost similarly 54.5% prevalence was recorded in cloacal swab on canary birds in Brazil as stated in Beleza *et al*., (2019). Besides in Gholami-Ahangaran *et al*., 2021, it was observed that *E. coli* was detected in 18.9% of cloacal swab samples from 195 healthy pet birds. More specifically, among different types of healthy pet birds, *E. coli* was found in 10% of canaries, 8% of finches, 40.9% of parrots, 33.3% of parakeets, 26.6% of budgerigars, and 20% of lovebirds. Similarly, 37.7% prevalence was observed in sigirci *et al*., 2020. The differences between rates could be based on multiple criteria, including geographical differences, sampling techniques, and detection procedures.

For biochemical test, we performed TSI (Triple Sugar Iron) test where all the isolates exhibited positive result with yellow color in both the bottom and the slanted portions of the test tube, along with the production of gas. Similar findings are also observed in the study of Akbari *et al*., 2022 and SEN *et al*., 2020.

Our research demonstrated complete resistance of *E. coli* to Erythromycin, mirroring the results of Rahman *et al*., 2020, who observed over 80% of *E. coli* isolates being resistant to Erythromycin and Tetracycline. Furthermore, similar findings of E. coli's antimicrobial resistance to Erythromycin can be found in other relevant studies, such as Racheck *et al*., 2000.

Trimethoprim-sulfamethoxazole shows broad-spectrum activity through oral efficacy, and it is particularly proper used for therapy of birds. Diren Sigirci *et al*., 2019 reported that 38% of the isolates from cloacal swabs of the synanthropic birds were resistant to SXT. Relatively, the high resistance rate to sulphamethoxazole/trimethoprim (83%) was observed in this study.

Tetracycline and Sulfamethoxazole-Trimethoprim also showed resistance to *E. coli*. The prevalence of tetA and tetB genes among isolates strongly indicates that these genes play a crucial role in conferring resistance to tetracycline in this particular environment (Olowe *et al*., 2013). Simultaneously, a high proportion of *E. coli* exhibited resistance to the SXT drug which agree with the findings of Ali *et al*., 2022.

Several authors have also observed a resistance rate to tetracycline; 41% from the cloacal swabs of captive cockatiels (De Pontes *et al*., 2018), and 28.6% from the cloacal swabs of free-living grey-breasted parakeets (Machado *et al*., 2018). Regarding previous reports in Turkey (65–100%), our results are consistent (Yılmaz and Dolar *et al*., 2017, Diren Sigirci *et al*., 2019. However, tetracycline resistance rate in Turkey is higher than the other countries. In our country, the wide uncontrolled use of antimicrobial compounds in human and veterinary practices, animal production, agriculture and industrial technology, and an increase in population mobility and the circulation of food might be the reason for this crucial problem. Factors responsible for the emergence and dissemination of resistant and MDR strains establish risk for human and animal health due to an increase in morbidity, mortality and the cost associated with the treatment of infections (Clemente *et al*., 2015).

This research emphasizes that pet birds could serve as significant carriers of antimicrobial resistance genes, which have the potential to be transmitted to both humans and animals, directly or indirectly. From a One Health standpoint, it's crucial not to underestimate this potential risk.

Limitation

The study conducted with a limited sample size due to time constraints, may not provide an entirely accurate representation of the actual circumstances. Therefore, additional bacteriological research is required to gain a comprehensive understanding of *E. coli* in pet birds.

Chapter 5

Conclusion

The current research indicates that *E. coli* is commonly found in cloacal swab samples from pet birds, with a prevalence of 57% in our study. This makes pet birds susceptible to infections. Additionally, the study reveals the presence of multi-drug resistant *E. coli* in these samples, suggesting indiscriminate use of antibiotics in pet birds. The study indicates that close interaction between these birds and humans poses a public health risk. Therefore, the findings can assist veterinarians in understanding microbiological culture and sensitivity results in pet birds.

References

Ahangaran, M. G., Zinsaz, P., Pourmahdi, O., Ahmadi-Dastgerdi, A., Ostadpour, M., & Soltani, M. (2022). Tetracycline Resistance Genes in *Escherichia coli* Strains Isolated From Biofilm of Drinking Water System in Poultry Farms. Acta Veterinaria Eurasia, 48(1), 64-69.

Akbari, P., & Asadpour, L. (2022). An Evaluation of Antimicrobial Resistance and Virulence Potential of Escherichia coli Obtained from Feces of Ornamental Birds in Guilan, Iran. Iranian Journal of Medical Microbiology, 16(5), 405-411.

Badr, H., Reda, R. M., Hagag, N. M., Kamel, E., Elnomrosy, S. M., Mansour, A. I., ... & Ali, H. R. (2022). Multidrug-resistant and genetic characterization of extended-spectrum beta-lactamase-producing E. coli recovered from chickens and humans in Egypt. Animals, 12(3), 346.

Beleza, A. J. F., Maciel, W. C., Carreira, A. S., Bezerra, W. G., Carmo, C. C., Havt, A., ... & Teixeira, R. S. (2019). Detection of Enterobacteriaceae, antimicrobial susceptibility, and virulence genes of *Escherichia coli* in canaries (Serinus canaria) in northeastern Brazil. Pesquisa Veterinária Brasileira, 39, 201-208.

Clemente, L., Manageiro, V., Jones-Dias, D., Correia, I., Themudo, P., Albuquerque, T., ... & Canica, M. (2015). Antimicrobial susceptibility and oxymino-β-lactam resistance mechanisms in Salmonella enterica and *Escherichia coli* isolates from different animalsources. Research in microbiology, 166(7), 574-583.

Croxen, M. A., & Finlay, B. B. (2010). Molecular mechanisms of *Escherichia coli* pathogenicity. Nature Reviews Microbiology, 8(1), 26-38.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., & Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical microbiology reviews, 26(4), 822-880.

Giacopello, C., Foti, M., Fisichella, V., & Piccolo, F. L. (2015). Antibiotic-resistance patterns of Gram-negative bacterial isolates from breeder canaries (Serinus canaria domestica) with clinical disease. Journal of exotic pet medicine, 24(1), 84-91.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. Nature reviews microbiology, 2(2), 123-140.

Machado, M. A. M., Castro, V. S., da Cunha Neto, A., Carvalho, R. C. T., Stanford, K., Conte-Junior, C. A., & Figueiredo, E. E. D. S. (2023). Genomic features and heat resistance profiles of *Escherichia coli* isolated from Brazilian beef. Journal of Applied Microbiology, 134(1), lxac027.

Mansour, A., Shaheen, H. I., Amine, M., Hassan, K., Sanders, J. W., Riddle, M. S., ... & Frenck, R. W. (2014). Diarrhea burden due to natural infection with enterotoxigenic *Escherichia coli* in a birth cohort in a rural Egyptian community. Journal of Clinical Microbiology, 52(7), 2595-2603.

Marques, C., Gama, L. T., Belas, A., Bergström, K., Beurlet, S., Briend-Marchal, A., ... & Pomba, C. (2016). European multicenter study on antimicrobial resistance in bacteria isolated from companion animal urinary tract infections. BMC veterinary research, 12(1), 1-17.

Nawaz, Z., Aslam, B., Zahoor, M. A., Siddique, A. B., Rafique, A., Aslam, R., ... & Mubeen, M. (2021). Frequency of extended spectrum beta lactamase producing *Escherichia coli* in fresh and frozen meat. Pak Vet J, 41(1), 102-6.

Okunlade, A. O., Esan, O. O., & Ogunleye, A. O. (2021). Multidrug resistant *E. coli* recovered from household reared female budgerigar pet bird (Melopsittacus undulatus) in Ibadan, Oyo State Nigeria: a case report. Bulletin of the National Research Centre, 45, 1-4.

Olowe, O. A., Idris, O. J., & Taiwo, S. S. (2013). Prevalence of tet genes mediating tetracycline resistance in *Escherichia coli* clinical isolates in Osun State, Nigeria. European Journal of Microbiology and Immunology, 3(2), 135-140.

Pardon, B., Smet, A., Butaye, P., Argudín, M. A., Valgaeren, B., Catry, B., ... & Deprez, P. (2017). Nosocomial intravascular catheter infections with extended‐spectrum beta‐lactamase‐producing *Escherichia coli* in calves after strain introduction from a commercial herd. Transboundary and emerging diseases, 64(1), 130-136.

Rahman, M. M., Ahmed, P., Kar, A., Sakib, N., Shibly, A. Z., Zohora, F. T., & Hasan, M. N. (2020). Prevalence, antimicrobial resistance, and pathogenic potential of enterotoxigenic and enteropathogenic *Escherichia coli* associated with acute diarrheal patients in Tangail, Bangladesh. Foodborne Pathogens and Disease, 17(7), 434-439.

Sequeira, A. F., Turchetto, J., Saez, N. J., Peysson, F., Ramond, L., Duhoo, Y., ... & Vincentelli, R. (2017). Gene design, fusion technology and TEV cleavage conditions influence the purification of oxidized disulphide-rich venom peptides in *Escherichia coli*. Microbial cell factories, 16, 1-16.

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.

Singh, A., Lynch, M. D., & Gill, R. T. (2009). Genes restoring redox balance in fermentation-deficient E. coli NZN111. Metabolic engineering, 11(6), 347-354.

Suphoronski, S. A., Raso, T. D. F., 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, Paraná, Brazil. African Journal Microbiology Research, 9, 1778-1782.

Szmolka, A., & Nagy, B. (2013). Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Frontiers in microbiology, 4, 258.

Yılmaz, E. Ş., & Dolar, A. (2017). Detection of extended-spectrum β-lactamases in *Escherichia coli* from cage birds. Journal of Exotic Pet Medicine, 26(1), 13-18

Acknowledgement

All praise is owed to the Almighty Allah, the creator and ultimate authority of the universe, who has granted me the strength and opportunities to successfully complete this report. The satisfaction that comes with finishing any task is truly uplifting. As I stand here, I am honored to acknowledge the individuals and organizations to whom I am deeply thankful.

I would like to express my appreciation to my supervisor, **Dr. Eftekhar Ahmed Rana**, Assistant Professor in the Department of Microbiology and Veterinary Public Health at Chattogram Veterinary and Animal Sciences University. My heartfelt gratitude goes to him for his valuable guidance, suggestions, supervision, and encouragement throughout the entire duration of this study, which enabled me to complete this clinical report.

I extend my sincere thanks to Professor **Dr. A. K. M. Saifuddin**, the Director of External Affairs, for his supervision and gracious cooperation during my internship.

I am grateful to the owners of pet birds and bird shops who assisted me in collecting samples and data for this study.

I also want to convey my gratitude to all my friends and respected seniors for their help, support and cooperation during the time I spent working on this report.

Last but certainly not least, I am deeply appreciative of my family members for their unwavering support, cooperation, sacrifices, and prayers.

Biography

I am **Sabrina Rahman Sabrin**, the daughter of **Md. Lutfur Rahman** and **Nurnahar Begum**. I successfully completed my Secondary School Certificate Examinations in 2014 and subsequently completed my Higher Secondary Certificate Examination in 2016. In the 2017-2018 academic session, I enrolled in the Doctor of Veterinary Medicine (DVM) program at Chattogram Veterinary and Animal Sciences University (CVASU). Currently, I am serving as an intern veterinarian within the Faculty of Veterinary Medicine at CVASU and I am passionate about contributing to the advancement of the veterinary sector in Bangladesh.

Appendix

Questionnaire

Title :- Isolation and Identification of *E. coli* from cloacal swab of ornamental pet birds.

1. Owner's information:

a) Name- b) Location/Area c) Mobile no-

2. Bird's information:

a) Species- b) Sex- c) Age- d) Color-

e) Body weight f) Temp- g) Health status-

h) Rearing system/Housing- i) Feeding- j) Anthelmintics used-

k) Vaccination status- l) How many pet birds do you have? m)For how long you have been rearing pet birds?

N) Sanitation and hygiene- o)Does it get exposure of outside?(yes/no)

p) Did your pet have any digestive problem before? (yes/no) If yes what was that and how was the management?

q) Any previous disease? (yes/no)

If yes,

3. Disease history:

clinical signs:

treatment and management protocol:

-Antibiotics:

-Antiemitics:

-Anthelmintics:

-Fluid therapy:

-Vitamin-mineral:

-NSAIDs:

-Others: