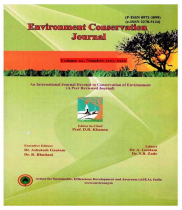
Environment Conservation Journal 23 (1&2): 65-71, 2022

Journal homepage: https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Detection of antibiotic resistance in *Escherichia coli* isolates from Egyptian vultures from arid regions of India

Khushboo Panwar ✉
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India.

Taruna Bhati
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India.

Sanju Ritod
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India.

B. N. Shringi
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India.

ARTICLE INFO

Received : 26 July 2021
Revised : 15 October 2021
Accepted : 08 November 2021

Available online: 11 February 2022

Key Words:
Antibiotic resistance
Bacteria
Egyptian vultures
Escherichia coli
Rajasthan
16S rRNA

ABSTRACT

Egyptian vultures (*Neophron percnopterus*) wintering in north-western India remains for several months (October to March) and with due course they have become an inhabitant of a synanthropic site in Jorbeer, a livestock and other animal carcasses dumping and disposing site in the outskirts of Bikaner city of Rajasthan. The main purpose of this study was to isolate and identify *E. coli* from critically endangered Egyptian vultures through conventional and molecular methods along with determining their antibiotic resistance profile. Bacteriological analyses were conducted on 38 freshly voided fecal samples, leading to the isolation of *E. coli* in 30 samples which were identified by biochemical tests and 16S rRNA sequencing. In the antibiogram study, out of 12 antibiotics two antibiotics namely norfloxacin and co-trimoxazole were highly effective against most (93.33%) of the isolates. Highest resistance was against cephotaxime (56.65%) followed by amoxyclav (43.33%). Antibiogram showed a moderate spread of *E. coli* strains showing antibiotic resistance among Egyptian vultures at Jorbeer, Bikaner.

Introduction

Egyptian vultures wintering in north-western India remain for several months (October to March), with some of them assembling at a synanthropic site in Jorbeer, located in the outskirts of Bikaner city and used to dump livestock and other animal carcasses. The key source of food for these vultures in shared dumps is farm animal carcasses and stray animal carcasses, which contain high levels of antimicrobial resistance (Kang *et al.,* 2004). This species has been listed as critically endangered by IUCN and in India, there has been a rapid decline in the population with a 35% decrease each year since 1999 which has been attributed mainly to the use of NSAIDs (diclofenac sodium in particular) (Cuthbert *et al.,* 2006). All bacteria including *E. coli* isolated from vultures, internally or externally, can potentially be the cause of diseases both in wildlife, domestic animals and humans (Hubalek, 2004).

Escherichia coli (*E. coli*) is a common colonizer of the gastrointestinal tract which belongs to the family *Enterobacteriaceae* and is a short gram negative, non-spore forming, peritrichous and fimbriate bacillus which may have a capsule or a microcapsule. *Escherichia coli* has been extensively studied in various taxonomic classes of mammals and bird species. The majority of acquired virulence factors that differentiate

Corresponding author E-mail: vetcvas.bhati@gmail.com
https://doi.org/10.36953/ECJ.021-2115

pathogenic *E. coli* from harmless *E. coli* are encoded on mobile genetic elements capable of horizontal gene transfer or on elements that were once mobile but now have become a stable part of the genome with time (Kaper *et al.,* 2004). The selective pressure exerted by antibiotic exposure has been proposed as a key factor in the development and spread of antimicrobial resistance in commensal *E. coli* which is a reliable indicator of antimicrobial selection pressure (Fluckey *et al.,* 2007). The fact that antibiotic use increased by 30% globally between 2000 and 2010 demonstrates the rise in antibiotic use. A survey conducted in India between 2005 and 2009 revealed a 40% rise in the use of antibiotics (Ganguly *et al.,* 2011). Due to its natural history and habits, Egyptian vultures are suitable for testing both virulence and antibiotic resistance trends, but very little microbial studies and antibiotic sensitivity profiling using this host species have been conducted to date in this region (India). Hence the present study was conducted to detect the antibiotic resistance pattern of *Escherichia coli* isolates from Egyptian vultures from Bikaner, Rajasthan, India.

Material and Methods

Sampling

A total of 38 freshly voided faecal samples were collected from Egyptian vultures from Jorbeer conservation area, Bikaner (Raj.). The faecal droppings were collected aseptically with the help of sterile spatula in sterile collection bottles. For further processing, the samples were transported to the laboratory as soon as possible and incubated overnight in nutrient broth for 24 hours.

Isolation and Identification of *E. coli*

Escherichia coli strains from faecal samples were isolated and identified as described by Edward and Ewing (1986) and Quinn *et al.* (2000). Identification of the *E. coli* isolates was based on culture and biochemical characteristics. For genotypic confirmation, amplification of the 16S rRNA genes was performed. Bacterial DNA was extracted using method of Chen and Kuo (1993) and 5'-GCTTGACACTGAACATTGAG-3' was used as the forward primer and 3'-GCACTTATCTCTTCCGCATT-5' as the reverse primer in a polymerase chain reaction (PCR) as per Khaled *et al.* (2010).

Antimicrobial susceptibility testing

The antibiotic susceptibility of 30 *E. coli* isolates was determined by the disc diffusion method on Mueller–Hinton agar (Hi Media) by Bauer *et al.* (1966). A set of 12 antibiotics representative of the main classes used in human and veterinary medicine were used in the antibiogram study. The surface of Mueller–Hinton agar plates was inoculated by swabbing overnight broth cultures of *E. coli* with turbidity adjusted to 0.5 McFarland standards. Seven antibiotic discs were carefully placed on the surface of one MHA plate with enough space around each disc to allow diffusion of the antibiotic and incubated at 37 °C for 16 to 18 h. The inhibition zone around each disc was measured in millimetres and results were interpreted according to CLSI guidelines (CLSI, 2013). Multiple antibiotic resistance (MAR) index was determined according to the method described by Krumperman (1983).

Results and Discussion

In the present study, out of 38 faecal samples collected from Egyptian vultures from Jorbeer conservation area, Bikaner (Raj.), 30 (78.94%) *E. coli* isolates were identified using conventional and molecular tests (Figure1, 2). The antibiotic sensitivity test using 12 antibiotics was determined for 30 isolates and the results of the antibiograms are presented in Table 1. Two antibiotics namely norfloxacin and co-trimoxazole were highly effective against most (93.33%) of the isolates followed by tetracycline (86.67%), ciprofloxacin (83.33%), gentamicin (76.66%) and chloramphenicol (73.33%) (Figure 3,4). The highest resistance was found against cephotaxime (56.65%) followed by amoxyclav (43.33%) and streptomycin (33.33%). *Escherichia coli* may have a multi-resistant phenotype because of distant use of antibiotics, which lead to evolution of a multi-resistant organism which has spread to various ecological niches. The sensitivity of *E. coli* isolates to ciprofloxacin was recorded as 83.34% in present study which is similar to observation made by Sharada *et al.* (2008) who reported 83% *E. coli* isolates from poultry in Bangalore sensitive to ciprofloxacin. In the present study, no *E. coli* was resistant to ciprofloxacin which is similar to the observations made by Shrestha *et al.* (2011), Borges *et al.* (2012) and Saidi *et al.* (2012). This

Table 1: Antibiogram of *E. coli* isolated from Egyptian Vultures

SN	Antibiotic disc	Antibiogram pattern (%)		
		Sensitive	Intermediate	Resistant
1.	Norfloxacin(NX)	93.33	6.67	-
2.	Co-Trimoxazole(COT)	93.33	-	6.67
3.	Tetracycline(TE)	86.67	13.33	-
4.	Ciprofloxacin (CIP)	83.33	16.67	-
5.	Gentamicin(HLG)	76.66	16.67	6.67
6.	Chloramphenicol (C)	73.33	20	6.67
7.	Ampicillin(AMP)	23.33	56.67	20
8.	Streptomycin(S)	23.33	43.33	33.34
9.	Nalidixic acid(NA)	20	66.67	13.33
10.	Kanamycin(K)	16.66	76.67	6.67
11.	Amoxyclav (AMC)	6.67	50	43.33
12.	Cephotaxime (CTX)	-	43.35	56.65

may be due to less use of ciprofloxacin and other fluoroquinolones at farms or hospitals in the study area since ciprofloxacin resistance in gram negative bacilli is coincident with increased use of fluorquinolones. In contrary, high level of resistance was reported from China against ciprofloxacin by Gyles (2008). Kibret and Abera (2011) in a study on *E. coli* from clinical sources in Ethopia found high degree of sensitivity to norfloxacin (90.6%) and gentamicin (79.6%) which is almost similar to our results. Similarly Akond *et al.* (2009) found 80% *E. coli* strains from poultry and poultry environment in Bangladesh susceptible to gentamicin and none of the isolates showed resistance to norfloxacin. Similar to present study Miles *et al.* (2006) reported 2.9% isolates from avian samples resistant to chloramphenicol.

In the present study, 13.33% of *E. coli* isolates were found resistant to nalidixic acid which is similar to the observations made by Costa *et al.* (2008) and Umar *et al.* (2018) who recorded 14% and 18.03%. *E. coli* isolates obtained from wild animals and Indonesian zoo birds respectively, resistant to nalidixic acid. Contrary to present observation Salehi and Bonab (2006) found 100% *E. coli* isolates from broiler chickens with colisepticemia resistant to nalidixic acid. Blanco *et al.* (2016) detected the residues of fluoroquinolones in the plasma of 92% nestling vultures feeding on domestic livestock carcasses. In the present study, 20% *E. coli* isolates were resistant to ampicillin which is similar to results obtained by Miles *et al.* (2006) who reported 20.6% *E. coli* isolates from broiler chickens and humans and Aksoy *et al.* (2007) reporting 13.1% *E. coli* isolates from cattle and sheep resistant to ampicillin. Contrarily, 100% resistance was observed by Matin *et al.* (2017) from faecal, liver and spleen samples of chicken and by Sarker *et al.* (2019) in *E. coli* from broilers. About 50% of *E. coli* isolates from cloacal samples of Canarian Egyptian vultures were resistant to ampicillin and tetracycline which is in contrast to present study. Whereas susceptibility to ampicillin (23.33%) among *E. coli* isolates was almost similar to that observed by Gyles (2008) (38%) from avian population from Canada.

In present study, 56.62% of *E. coli* isolates were found resistant to cephotaxime which is contrary to observation made by Kar *et al.* (2015) who observed 100% *E. coli* isolates from poultry faecal and cattle milk samples resistant to cephotaxime. Resistance to cephotaxime and ampicillin is also suggestive of relatively high proportion of extended spectrum β-lactamases (ESBL) producing *E. coli* strains in Egyptian vultures in the present study which could contribute to the spreading of such ESBL producing strains as observed by Alcalá *et al.* (2016). No resistance was found in *E. coli* isolates for tetracycline in given study which is similar to observation made by Allen *et al.* (2011) from samples of landfills and natural habitats. On contrary high percentage of resistance against

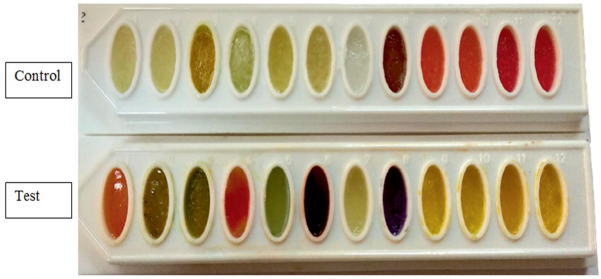


Figure 1: Metabolic and biochemical reactions by Hi *E. coli*TM Identification Kit.

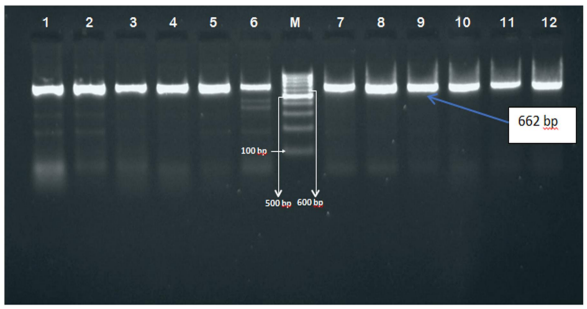


Figure 2: 16S rRNA ribotyping of *Escherichia coli* isolates from Egyptian vultures

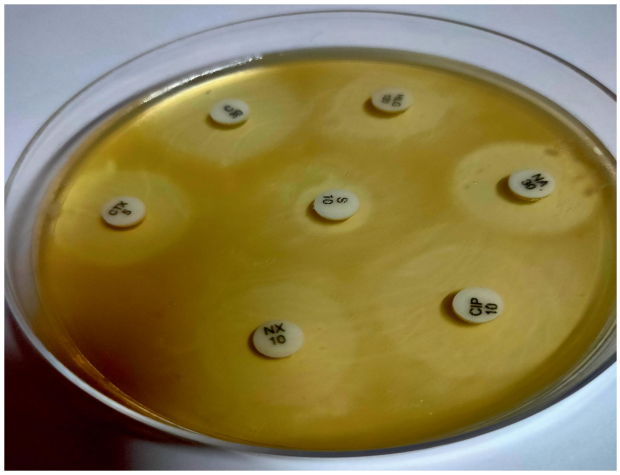


Figure 3: Sensitivity pattern of *E. coli* isolates against various tested antibiotics

tetracycline was observed by other scientists *viz.* 100% by Sarker *et al.* (2019) in *E. coli* from broilers. A high level of resistance to tetracycline (83.08%) and co-trimoxazole (76.92%) reported by Sharada *et al.* (2008) and resistance to co-trimoxazole (88%), ciprofloxacin (83%), norfloxacin (78%) and tetracycline (77%) noted by Kar *et al.* (2015) in *E. coli* isolates from poultry faecal and cattle milk samples is in contrast to present study. The *E. coli* isolates from poultry are resistant to these drugs because of regular usage in poultry industry for control of pathogenic avian colibacillosis which is not commonly done in wild birds like vultures.

Ammar *et al.* (2015) observed *E. coli* isolates from avian samples in Egypt to be 100% resistant to amoxyclav which is way higher than our observation (43.33%). The resistance observed in our study is much higher than the observation made by Costa *et al.* (2008) in which resistance of *E. coli* isolates from wild animal was in range of 4.5-7%.

Except for gentamicin an overall high resistance (intermediate + resistant) was recorded for other aminoglycosides *i.e.* streptomycin (76.68%) and kanamycin (83.34%), in the present study. A higher resistance of 60.4% in *E. coli* isolates from turkey to streptomycin was seen by Cunha *et al.* (2014) which is similar to present study. Salehi *et al.* (2007) found out susceptibility to kanamycin for *E. coli* isolates to be 11% which is almost similar to our observation (16.67%). Horn *et al.* (2015) determined that *E. coli* strains isolated from necropsy samples and cloacal swabs of canaries were resistant to gentamicin (4%) and 40% to streptomycin. De Pontes *et al.* (2018) observed a high resistance to aminoglycosides (74%), and streptomycin (67%) in *E. coli* isolates from cockatiels kept in captivity. Aminoglycosides, such as amikacin or gentamicin, are clinically important therapeutic agents for treatment of infections particularly severe infections by Gram-negative bacteria in human and veterinary medicine, but they are less preferred in birds because of the toxicity disadvantage (Flammer, 2006). However, carcasses and other remains, frequently from diseased animals treated with aminoglycosides, can be consumed by vultures leading to the ingestion of antimicrobial residues or resistant bacteria such as *E. coli*. The difference in the prevalence of antimicrobial resistance in wildlife living in natural habitats in different geographic sites may reflect different levels of general pollution in the local environment. All the multidrug resistant *E. coli* isolates were subjected to determination of MAR (Multiple Antibiotic Resistance) index. Five antibiotic resistant patterns with MAR index ranging from 0.08-0.41 were obtained. In addition, the results obtained showed the presence of eight multidrug-resistant strains (MDR), which were

Table 2: Multiple antibiotic resistance (MAR) index of *E. coli* isolates

S N	MAR index value type	Isolate I.D.	No. of isolates	No. of antibiotics to which isolates were resistant	MAR Index value	Significance
1	MAR1	E_1,E_3,E_4,E_6,E_7,E_8,E_9,E_{10}, E_{11}, E_{12},E_{14},E_{15}	12	1	0.08	22 (73.3%) isolates had less than 0.2 MAR index value with less risk source of MDR strains
2	MAR2	E_{16},E_{17},E_{19}, E_{20},E_{21},E_{23}, E_{24},E_{27},E_{29}, E_{30}	10	2	0.16	
3	MAR3	E_{22},E_{25},E_{26},E_{28},E_{18}	5	3	0.25	08 (26.7%) Isolates had 0.2 or more than 0.2 MAR index value with high risk potential source of spread of MDR strains
4	MAR4	E_5,E_{13}	2	4	0.33	
5	MAR5	E_2	1	5	0.41	

resistant to three or more antibiotics having MAR index more than 0.2. The majority of the *E. coli* (12 isolates) were resistant to only one antibiotic (MAR index of 0.08). The majority of isolates (73.3%) had a MAR index of less than 0.2 while 26.7% isolates had MAR index value more than 0.2 and were considered as multiple drug resistant (Table 2) suggesting exposure of the vultures to bacteria from significantly contaminated sites. These results are almost identical to the findings of Kelsey *et al.* (2003), who found that 97% of *E. coli* isolates from surface water were resistant to one or two antibiotics, with only one isolate resistant to more than two antibiotics. Multidrug resistance (MDR) was detected in 18.8% of *E. coli* isolates from healthy wildlife and livestock by Kabali *et al.* (2021) which corroborates present study. On the contrary, Adzitey, 2015 had only four isolates with MAR index less than 0.2 out of 45 isolates of *E. coli*. All *E. coli* isolates from fecal matter samples of migratory birds in Bangladesh were found to be multidrug resistant (MDR) by Islam *et al.* (2021) which is in contrast to present study. Szmolka and Nagy, 2013 described the role of antimicrobial therapy in animals in the selection of multidrug resistant populations of commensal *E. coli* that could transfer those resistance genes in vivo to pathogenic strains of *E. coli* or to *Salmonella.*

Conclusion

In conclusion, considerable number of MDR *E. coli* strains from Egyptian vultures in arid regions of Rajasthan was observed that raises concern regarding the use of antimicrobials in human and veterinary medicine. Hence to protect the populations of this threatened species it is necessary to prevent the vultures from feeding largely on the carcasses of farm animals or from different clinical settings which contain high rates of antimicrobial resistance as they can serve as a source of antimicrobial-resistant bacteria and can spread them to other species and into the environment, and consequently may pose a risk to human and animal health. The proper disposal of diseased and subsequently medicated livestock carcasses through burial or incineration can prevent the spillover of veterinary antimicrobials in the scavenging vultures.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences Rajasthan University of Veterinary and Animal Sciences, Bikaner for providing necessary facilities and funds to carry out this research work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Adzitey, F. (2015). Antibiotic resistance of *Escherichia coli* isolated from beef and its related samples in Techiman municipality of Ghana. *Asian Journal of Animal Sciences, 9*(5): 233-240.

Akond, M.A., Alam, S., Hassan, S.M.R., & Shirin, M. (2009). Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *International Journal of food safety, 11,* 19-23.

Aksoy, A., Yildirim, M., Kaçmaz, B., Apan, T.Z., & Göçmen, J.S. (2007). Verotoxin production in strains of *Escherichia coli* isolated from cattle and sheep, and their resistance to antibiotics. *Turkish Journal of Veterinary and Animal Sciences, 31*(4), 225-231.

Alcalá, L., Carla, A.A., Carmen, S., Chabier, G.E., Jesús, O., Antonio R., Carmelo, O., & Carmen, T. (2016). Wild Birds, Frequent Carriers of Extended-Spectrum β-Lactamase (ESBL) Producing Escherichia coli of CTX-M and SHV-12 Types. *Microbial Ecology, 72*(4), 861-869.

Allen, S.E., Boerlin, P., Janecko, N., Lumsden, J.S., Barker, I.K., Pearl, D.L., & Jardine, C. (2011). Antimicrobial resistance in generic *Escherichia coli* isolates from wild small mammals living in swine farm, residential, landfill, and natural environments in southern Ontario, Canada. *Applied and environmental microbiology, 77*(3), 882-888.

Ammar, A.M., El-Hamid, M.I., Eid, S.E.A., & El Oksh, A.S. (2015). Insight into antimicrobial resistance and virulence genes of emergent multidrug resistant avian pathogenic *Escherichia coli* in Egypt: How closely related are they. *Revue de Medecine Veterinaire, 166*(9-10), 304-314.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493-496.

Blanco, G., Alexandra, J., David, S., José, B., & Dolores, B. (2016). Wildlife contamination with fluoroquinolones from livestock: Widespread occurrence of enrofloxacin and marbofloxacin in vultures. *Chemosphere, 144,* 1536-43.

Borges, C.A., Beraldo, L.G., Maluta, R.P., Cardozo, M.V., Guth, B.E.C., Rigobelo, E.C., & de Avila, F.A. (2012). Shiga toxigenic and atypical enteropathogenic *Escherichia coli* in the Faeces and carcasses of slaughtered pigs. *Foodborne Pathogens and Disease, 9*(12), 1119-1125.

Chen, W., & Kuo, T. (1993). A simple and rapid method for the preparation of Gram negative bacterial genomic DNA. *Nucleic Acids Research, 21*(9), 2260.

CLSI, (2013). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third informational Supplement (M100-S23). Clinical and Laboratory Standards Institute, Pennsylvania, USA. pp: 72-81.

Costa, D., Poeta, P., Sáenz, Y., Vinué, L., Coelho, A.C., Matos, M., & Torres, C. (2008). Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. *Microbial Drug Resistance, 14*(1), 71-77.

Cunha, M.P., de Oliveira, M.G., de Oliveira, M.C., da Silva, K.C., Gomes, C.R., Moreno, A.M., & Knöbl, T. (2014). Virulence profiles, phylogenetic background, and antibiotic resistance of Escherichia coli isolated from turkeys with airs acculitis. *The Scientific World Journal*, 289024. https://doi.org/10.1155/2014/289024

Cuthbert, R., Green, R.E., Ranade, S., Saravanan, S., Pain, D.J., Prakash, V., & Cunningham, A.A. (2006). Rapid population declines of Egyptian vulture (*Neophron percnopterus*) and red-headed vulture (*Sarcogyps calvus*) in India. *Animal Conservation, 9*(3), 349–354.

De Pontes, P.S., Coutinho, S.D.A., de Oliveira Iovine, R., Cunha, M.P.V., Knöbl, T., & de Carvalho, V.M. (2018). Survey on pathogenic *Escherichia coli* and Salmonella spp. in captive cockatiels (*Nymphicus hollandicus*). *Brazilian Journal of Microbiology, 49* Suppl(Suppl 1), 76-82.

Edward, P.R., & Ewing, W.H. (1986). Identification of Enterobacteriaceae. (4th edn.), pp. 1-107. Elsevier Science Publishing Co. Inc., New York.

Flammer, K. (2006). Antibiotic Drug Selection in Companion Birds. *Journal of Exotic Pet Medicine*, *15*(3), 166-176.

Fluckey, W.M., Loneragan, G.H., Warner, R., & Brashears, M.M. (2007). Antimicrobial Drug Resistance of *Salmonella* and *Escherichia coli* Isolates from Cattle Feces, Hides, and Carcasses. *Journal of Food Protection, 70*(3), 551–556.

Ganguly, N.K., Arora N.K., Sujith, J.C., Mohamed, N.F., Gill, J.P.S., Gupta, U., Shah, H., Sadhna, J., Joshi, P.C., Kakkar, M., Kotwani, A., Rattan, A., Sudarshan, H., Kurien, T., Chand, W., Alice E., & Ramanan, L. (2011). Rationalizing antibiotic use to limit antibiotic resistance in India *Indian Journal of Medical Research, 134*(3), 281-294.

Gyles, C.L. (2008). Antimicrobial resistance in selected bacteria from poultry. *Animal Health Research Reviews, 9*(2), 149-158.

Horn, R.V., William, M., Cardoso, E., Lopes, S., Régis, S.C., Teixeira, Á. H., Albuquerque, R.C., Rocha-e-Silva, D.N., Machado, W., & Bezerra, G.A. (2015). Identification and antimicrobial resistance of members from the Enterobacteriaceae family isolated from canaries (Serinus canaria) *Pesquisa Veterinary Brasileira, 35*(6): 552-556.

Hubalek, Z. (2004). An annotated checklist of pathogenic microorganisms. *Journal of Wildlife Diseases, 40,* 639–659.

Islam, S., Md, M.H.N., Md, A. S., Samina, I., Md, A.I., Saifur, R., Md Abdul, K., Hossam, M., and Md, Tanvir, R. (2021). Virulence Determinants and Multidrug Resistance of *Escherichia coli* Isolated from Migratory Birds. *Antibiotics*, 10:190.

Kang, H.Y., Jeong, Y.S., Oh, J.Y., Tae, S.H., Choi, C.H., Moon, D.C., & Lee, J.C. (2004). Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *Journal of Antimicrobial Chemotherapy, 55*(5): 639-644.

Kabali, E., Pandey, G.S., Musso, M., Kapila, P., Mukubesa, A.N.,bNdebe, J., Muma, J.B., Mubita, C., Muleya, W., Elizabeth, M., …et al (2021). Identification of *Escherichia coli* and Related Enterobacteriaceae and Examination of Their Phenotypic Antimicrobial Resistance Patterns: A Pilot Study at A Wildlife–Livestock Interface in Lusaka, Zambia. *Antibiotics, 10,* 238.

Kaper, J.B., Nataro, J.P., & Mobley, H.L. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology*, *2*(2): 123.

Kar, D., Bandyopadhyay, S., Bhattacharyya, D., Samanta, I., Mahanti, A., Nanda, P.K., & Bandyopadhyay, S. (2015). Molecular and phylogenetic characterization of multidrug resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. *Infection Genetics and Evolution*, *29,* 82-90.

Kelsey, R.H., Scott, G.I., Porter, D.E., Thompson, B., & Webster, L. (2003). Using multiple antibiotic resistance and land use characteristics to determine sources of fecal coliform bacteria pollution. *Environmental Monitoring and Assessment, 81*, 337–348.

Kibret, M., & Abera, B. (2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Sciences, 11*(1): 40-45.

Khaled, A., Razik, E.I., Abdel, R., Youssef, F., Alaa, M., & Hazem, A. (2010). Direct identification of major pathogen of bubaline subclinical mastitis in Egypt using PCR. *American journal of Science, 6*(10), 652-660.

Krumperman, P.H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Applied and Environmental Microbiology, 46*, 165-170.

Matin, M.A., Islam, M.A., & Khatun, M.M. (2017). Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. *Veterinary world, 10* (1): 29

Miles, T.D., McLaughlin, W., & Brown, P.D. (2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. *BMC Veterinary Research*, *2*(7):1-9.

Quinn, P.J., Carter, M.E., Markey, B.K., & Carter, G.R. (1994). Wolfe Publishing, Mosby-Year Book Europe Ltd. Lynton House, 7-12. Tavistock Square, London WCH 9LB, England. *Clinical Veterinary Microbiology.*

Saidi, B., Mafirakureva, P., & Mbanga, J. (2012). Antimicrobial resistance of *Escherichia coli* isolated from chickens with colibacillosis in and around Harare, Zimbabwe. *Avian diseases, 57*(1), 152-154.

Salehi T.Z., Safarchi, A., Peighambari, S.M., Mahzounieh, M., & Rabbani Khorasgani, M. (2007). Detection of *stx1*, *stx2*, *eae, espB* and *hly* genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. *Journal of Veterinary Research, 62*, 37-42.

Salehi, T.Z., & Bonab, S.F. (2006). Antibiotics susceptibility pattern of *Escherichia coli* strains isolated from chickens with colisepticemia in Tabriz province, Iran. *International Journal of* Poultry *Science, 5*(7), 677-684.

Sarker, Md. S., Md. Shahriar, M., Md. Younus, A., Md. Bayzid, A.A., & Zamila, B.B. (2019). Antibiotic resistance of *Escherichia coli* isolated from broilers sold at live bird markets in Chattogram, Bangladesh. *Journal of Advance Veterinary and Animal Research*, *6*(3), 272–277.

Sharada, R.S., & Thiyageeswaran, R.M. (2008). Antibiotic Resistance Pattern Of *Escherichia Coli* Isolated From Poultry In Bangalore. *The Internenational Journal of Microbiology, 7*(1).

Shrestha, E.K., Dhakal, I.P., Sapkota, M., Manandhar, P., & Rijal, T.B. (2011). Antimicrobial resistance pattern of *Escherichia coli* isolates from chicken and human samples in Chitwan. *Nepalese Veterinary Journal*, 38.

Simões, R.R., Poirel, L., Da Costa, P.M., & Nordmann, P. (2010). Seagulls and beaches as reservoirs for multidrug-resistant *Escherichia coli*. *Emerging Infectious Diseases, 16*(1), 110.

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