





ORIGINAL ARTICLE

Isolation of multidrug-resistant *Escherichia coli* from turkeys in Dinajpur, Bangladesh, and their antibiogram profile

Tonmoy Kundu¹ , Nazmi Ara Rumi¹ , Md. Khaled Hossain¹ , Md. Shajedur Rahman² ,
Mirdha Md. Kamal Hossain³, Joyanta Halder³

¹Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Rangpur, Bangladesh

²Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University, Rangpur, Bangladesh

³Animal Biotechnology Division, National Institute of Biotechnology, Ashulia, Bangladesh

ABSTRACT

Objective: The study was carried out for molecular characterization and antibiotic resistance analysis of *Escherichia coli* isolated from different turkey farms in Dinajpur, Bangladesh.

Materials and methods: A total of 45 samples comprising feces ($n = 23$) and cloacal swabs ($n = 22$) were collected randomly from turkeys. The samples were subjected to isolation and identification of *E. coli* by cultural and biochemical characteristics, followed by polymerase chain reaction and sequencing. An antibiogram of the isolated *E. coli* isolates was carried out by following the Kirby–Bauer disk diffusion method.

Results: Out of the 45 samples, 28 (62.21%) were positive for *E. coli*, of which 16 (35.55%) fecal samples were positive and 12 (26.66%) cloacal swabs were positive. The antibiotic sensitivity analysis revealed that all the *E. coli* isolates were 100% sensitive to levofloxacin, norfloxacin, neomycin, gentamicin, and nitrofurantoin. On the other hand, all the isolates were 100% resistant to amoxicillin, azithromycin, erythromycin, tetracycline, bacitracin, cephalixin, nalidixic acid, vancomycin, methicillin, piperacillin, pefloxacin, novobiocin, cefepime, trimethoprim, netilmicin, and aztreonam.

Conclusion: This study's results uncover the occurrence and antibiotic resistance pattern of *E. coli* in the study area's turkeys.

ARTICLE HISTORY

Received September 10, 2020

Revised November 19, 2020

Accepted December 05, 2020

Published March 05, 2021

KEYWORDS

Antimicrobial resistance; cloacal swabs; *Escherichia coli*; fecal swabs; Turkey



© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

Introduction

Turkey (*Meleagris gallopavo*) production has gained special attention among Bangladesh's people considering its progressive commercial value and comprehensive mandate for its products [1]. The reasons behind such consideration are that turkeys have less disease risk, elevated market value, lesser feed cost, and lower mortality than the rest of the poultry species [2]. In the last few years, the acceptance of turkeys in Bangladesh has increased through triggering alternative protein supplementation, income generation, and poverty alleviation. Furthermore, Bangladesh's climate pattern supports the production system of different poultry species positively [3]. It is noticeable that poultry meat alone provides a significant amount

of animal protein through meat production, accounting for 37% of total meat requirements in Bangladesh [4].

In a decade, globally, turkey meat production increased significantly, which is 5.6 million tons in 2012, whereas it was 5.1 million tons in 2003 [5]. Thus, turkey can be a useful alternative source of protein as the broiler meat has problems with higher disease incidence and lower taste. The drawbacks of broiler meat production tend to move farmers' attention to turkey production in developing countries like Bangladesh. As an excellent forager, a turkey helps eradicate insects that are harmful to crops and vegetables [6]. This poultry species is more suitable to raise in any climatic condition if they are provided proper feeding and management, such as disease and predator

Correspondence Nazmi Ara Rumi ✉ rumi_dvm@yahoo.com 📧 Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Rangpur, Bangladesh.

How to cite: Kundu T, Rumi NA, Hossain MK, Rahman MS, Hossain MMK, Halder J. Isolation of multidrug-resistant *Escherichia coli* from turkeys in Dinajpur, Bangladesh, and their antibiogram profile. J Adv Vet Anim Res 2021; 8(1):64–69.

protection [2]. Although there are many points in favor of turkey production over other poultry species, they still have some limitations that need to be addressed appropriately. It should be noted that farmers are not aware enough regarding feed efficiency, which needs particular attention because feed represents 70% of the total cost of the poultry production system [7].

Additionally, the farmers' feeding system deserves special attention, as the turkey poult has higher protein requirements during their first 7 weeks [8]. On the other hand, farmers are not knowledgeable about turkey breeder ration, which was also observed in other parts of the world [9]. Only a few organisms are causing turkey diseases that need intensive observation to address commercially viable turkey production. *Escherichia coli* is the most abundant disease-producing strain in the domestic turkey production system among different microorganisms, leading to decreased egg and meat production and ultimately leading to the bird's death [10]. *E. coli* infections and observable consequences include egg peritonitis, omphalitis, coligranuloma, swollen head syndrome, cellulitis, colisepticemia, and death of the birds [11]. Thus, *E. coli* affects the turkey production system through decreased feed efficiency, slow growth rate, and higher morbidity and mortality, inclining the turkey to other diseases [12]. Furthermore, the treatment of bacteria using different antibiotics gives the turkey antibiotic resistance, which further affects the turkey's performance against diseases. Therefore, the present work was designed to isolate and characterize the avian *E. coli* in Bangladesh's turkey population and formulate a control strategy against the isolates by selecting the best antibiotics [13].

Materials and Methods

Ethical approval

The Hajee Mohammad Danesh Science and Technology University's ethical committee approved this study's methodology [approval number: Hajee Mohammad Danesh Science and Technology University (HSTU)/IRT/3084].

Selection of study area

The present research work was carried out on different selected turkey farms in the Dinajpur district for 6 months from January 2019 to June 2019. Samples were collected and further research was conducted at the Department of Microbiology, Faculty of Veterinary and Animal Science, HSTU, Dinajpur-5200.

Collection of samples

A total of 45 samples were collected randomly from healthy and sick turkeys from different turkey farms (five from

HSTU farm, eight from Ma Turkey farm, eight from Habib turkey farm, eight from Anowara turkey farm, eight from Liton turkey farm, and eight from Nazrul turkey farm) in Dinajpur district. Among the samples, 23 were fecal and 22 cloacal samples. According to age, 25 samples were collected from 4-week-old turkeys, and 20 samples were collected from 10-week-old turkeys.

Isolation of bacteria

In nutrient agar (NA) and nutrient broth media, the primary culture was conducted. Then, different bacteriological agar media such as MacConkey agar (MA), eosin methylene blue (EMB) agar, and tryptic soy agar were used for subculturing of suspected bacteria. The aseptic condition was maintained and incubated at 37°C for 24 h. For further investigation, the NA plates were maintained at 4°C. Pure cultures were obtained following the protocols published previously [14].

Identification of bacteria

To identify the bacterial flora, cultural, morphological, and biochemical characteristics were studied. Then, the morphology and staining characteristics of bacteria were evaluated by Gram stain [14]. Furthermore, biochemical tests, such as sugar fermentation, catalase, methyl red (MR), Voges-Proskauer (VP), Simmon's citrate, triple sugar iron, and indole tests, were carried out using standard methods [15].

Genomic DNA extraction and purification

Genomic DNA isolation and purification from *E. coli* was initiated according to the protocol developed in our laboratory [16]. Briefly, genomic DNA was extracted using the chloroform-isoamyl alcohol method from *E. coli* isolates cultured in a sodium thioglycolate broth. Initially, cells were harvested by centrifugation in an IEC CL31R multispeed centrifuge (Thermo Scientific) at 2,400 g for 10 min. The pellets were then resuspended in 400 µl Tris-EDTA buffer, containing 0.01 M Tris-HCl pH 7.4, 0.001 M EDTA with gentle agitation. After that, 10% SDS (Fisher Scientific), followed by proteinase K (Ambion), was supplemented to the suspension. The suspension was then incubated at 65°C for 1 h in a hybridization oven (Biomtra OV2, Anachem, UK). While incubation was completed, 100 µl of 5M NaCl, followed by 100 µl CTAB/NaCl (prewarmed at 70°C), was added to the solutions. The solution was then gently inverted and was subjected to incubation for 20 min at 65°C and cooling at room temperature for 5 min. A ratio of 24:1 chloroform isoamyl alcohol (Sigma-Aldrich) was used in the solution and centrifuged at 1,300 g for 15 min. The supernatants were then collected in a new tube and treated with 5 µl RNase A (5 mg/ml in RNase A buffer

containing 0.5M NaCl, 0.01M EDTA), incubated at 37°C for 30 min. Quantification and quality were examined using a nanodrop spectrophotometer, and the DNA was stored at -20°C for further use.

Polymerase chain reaction (PCR)

The reaction scale used for PCR was 25 ml consisting of 12.5 µl of 2× master mix [GENE Amp Fast PCR Master Mix (2×)], 2 µl template DNA, 0.2 µl Taq DNA polymerase, 0.5 µl forward primer, 0.5 µl reverse primer, and 9.3 µl molecular grade water. The samples were then subjected to initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 53°C for 1 min; extension at 72°C for 1 min, and a final extension at 72°C for 10 min [17].

Sequencing and phylogenetic analysis

PCR product of fecal origin *E. coli* was sequenced by the Genetic Analyzer 3,130 (Applied Biosystems) using the dideoxy chain termination method (Sanger and Coulson method) at the National Institute of Biotechnology (NIB), Savar, Dhaka. Sequencing was carried out using both forward and reverse directions. Obtained sequences were edited and analyzed by Molecular Evolutionary Genetics Analysis software [18]. A phylogenetic tree was made using the neighbor-joining method with 1,000-bootstrap replication [19,20]. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site [21]. The nucleotide sequence was submitted to GenBank under the accession number MW165527 (Feces/Turkey/2019)

Antibiogram study of isolated *E. coli*

Commonly used antibiotics were used to test the antibiotic sensitivity and resistance patterns of the isolated *E. coli*. Two lines, namely resistant and sensitive, were used to evaluate the sensitivity against antibiotic disks. Disk

diffusion method or the Kirby-Bauer method [18] and guidelines of Clinical and Laboratory Standards Institute [20] were used to interpret the antimicrobial drug sensitivity on newly prepared and dried-up Mueller Hinton agar. A total of 20 antibiotics were used in this study. The antibiotics include amoxicillin (11 µg), aztreonam (11 µg), azithromycin (12 µg), bacitracin (5 µg), cefepime (7 µg), cephalixin (10 µg), erythromycin (10 µg), gentamicin (10 µg), kanamycin (14 µg), levofloxacin (25 µg), methicilin (5 µg), nalidixic acid (30 µg), neomycin (15 µg), netilmicin (9 µg), nitrofurantoin (22 µg), norfloxacin (20 µg), novobiocin (8 µg), pefloxacin (10 µg), tetracycline (30 µg), and trimethoprim (10 µg). The disks were purchased from Himedia, India.

Results

Isolation of *E. coli*

Out of the 45 samples (23 fecal and 22 cloacal swabs), 28 (62.21%) were positive for *E. coli*, of which 16 (35.55%) were positive for fecal samples and 12 (26.66%) were positive for cloacal swabs (Table 1). According to the age group, 17 (37.77%) samples were positive in 4-week-old turkeys and 11 (24.44%) were positive in 10-week-old turkeys. All the isolated organisms were positive in MA, EMB agar, MR, VP, Simmon's citrate test, triple sugar iron test, motility indole urease test, sugar fermentation test, and catalase test.

Antibiotic sensitivity test of *E. coli*

The antibiotic sensitivity pattern of *E. coli* revealed that all isolates were sensitive to levofloxacin, neomycin, gentamycin, and nitrofurantoin (Table 2). All *E. coli* were resistant to amoxicillin, azithromycin, erythromycin, tetracycline, bacitracin, cephalixin, nalidixic acid, vancomycin, methicilin, piperacillin, pefloxacin, novobiocin, cefepime, trimethoprim, netilmicin, norfloxacin, and aztreonam (Table 2). Two different categories, namely resistant and sensitive,

Table 1. Results of *E. coli* isolated from fecal and cloacal swabs of turkeys.

Name of farms	Samples collected (n = 45)		<i>E. coli</i> positive samples(28)		Occurrence rate (%)
	Fecal	Cloacal	Fecal	Cloacal	
HSTU Farm	3	2	3	1	80.00%
Borogurgola	4	4	2	2	50.00%
Basherhat	4	4	2	1	37.50%
Farmerhat	4	4	4	2	75.00%
Bottoli	4	4	2	3	62.50%
Gopalganj	4	4	3	3	75.00%
Sub-total	23	22	16	12	
Total	45		28		62.21

Table 2. Resistant and sensitive percentage of isolated *E. coli* ($n = 28$).

Antimicrobial agents	Sensitive (%)	Resistant (%)
Amoxicillin (11 µg)	0 (0%)	28 (100%)
Azithromycin (12 µg)	0 (0%)	28 (100%)
Aztreonam (11 µg)	0 (0%)	28 (100%)
Bacitracin (5 µg)	0 (0.0%)	28 (100%)
Cefepime (7 µg)	0 (0%)	28 (100%)
Cephalexin (10 µg)	0 (0.0%)	28 (100%)
Erythromycin (10 µg)	0 (0.0%)	28 (100%)
Gentamicin (10 µg)	28 (100%)	0 (0.0%)
Kanamycin (14 µg)	0 (0%)	28 (100%)
Levofloxacin (25 µg)	28 (100%)	0 (0%)
Methicilin (5 µg)	0 (0%)	28 (100%)
Nalidixic acid (30 µg)	0 (0.0%)	28 (100%)
Neomycin (15 µg)	28 (100%)	0 (0%)
Netilmicin (9 µg)	0(0%)	28 (100%)
Nitrofurantoin (22 µg)	28 (0.0%)	0 (100%)
Norfloxacin (20 µg)	28 (100%)	0 (0.0%)
Novobiocin (8 µg)	0 (0%)	28 (100%)
Pefloxacin (10 µg)	0 (0%)	28 (100%)
Tetracycline (30 µg)	0 (0.0%)	28 (100%)
Trimethoprim (10 µg)	0 (0%)	28 (100%)

S = Sensitive, R = Resistant.

were used to evaluate the results of sensitivity against antibiotic disks according to the guidelines of Clinical and Laboratory Standard Institute [22].

PCR, sequencing, and phylogenetic analysis

About 584-bp product from *E. coli* DNA was amplified with specific E_1 and E_2 primers [23] (Fig. 1). Sequencing of the PCR product was conducted at the NIB. Blast analysis revealed that our sequence had 100% homology with the sequences available at GenBank (Fig. 2). For example, bacteria with accession numbers MT649857 and MT649847 were isolated from a patient in China, CP054236 from urinary tract infection of a woman in Texas, USA, CP053281 from human, MT453873 from the superficial surgical incision of a patient from Iraq, MW116771 from the saliva of a cat from Bangladesh. These findings indicate the sequence of our *E. coli* isolated from turkeys clustered with the pathogenic *E. coli*. Hence, we assumed that the isolated *E. coli* of our study could be pathogenic.

Discussion

The study was conducted for the isolation, identification, molecular characterization, and antibiotic sensitivity study

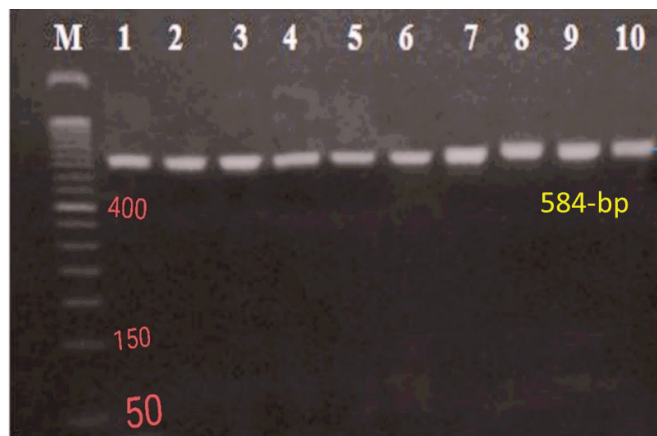


Figure 1. Amplification of DNA from *E. coli*. Lane M: Marker, Ladder is 50-bp, Lane 1–10: test samples.

of the *E. coli* isolated from turkey's fecal and cloacal swabs. Among the 45 samples, 28 (62.22%) were positive for *E. coli*. *E. coli* isolated from fecal and cloacal swab samples collected from turkey farms were compared with the previous results [24–26]. The isolates of *E. coli* showing the results in biochemical tests, including MR, VP, and indole tests, were similar to the previous reports [27–29].

The colony characteristics of *E. coli* observed in NA and EMB agar were similar to previous findings [30]. In Gram stain, the isolated bacteria's morphology exhibited Gram-negative, short-rods arranged in single or paired and motile, supported by an earlier study [31]. The *E. coli* isolates revealed a complete fermentation of five basic sugars by producing acid and gas supported by earlier research work [28,32]. About 80% of the samples were positive for *E. coli* from HSTU farms, 50% from Borogurgula turkey farms, 37.5% from Basherhat, 75% from Farmerhat turkey farms, 62.5% from Bottoli, and 75% from Gopalgonj. The incidence of *E. coli* Isolated was from fecal and cloacal swab samples collected from turkey, as reported previously [24–26]. The different isolates of *E. coli* showed identical results in different biochemical tests, including MR, VP, and indole test, and a similar type of biochemical reaction was reported [27,29]. Molecular characterization revealed that the target *E. coli* DNA gene band found at 584-bp was similar to a previous study [23]. Antibiogram profile reports showed various degrees of antibiotic resistance in *E. coli* strains isolated from poultry [33,34]. The antibiotic study revealed that all *E. coli* was 100% sensitive to nitrofurantoin, gentamicin, levofloxacin, norfloxacin, and neomycin. The isolates were 100% resistant to amoxicillin, azithromycin, erythromycin, tetracycline, bacitracin, cephalexin, nalidixic acid, vancomycin, methicilin, piperacillin, pefloxacin, novobiocin, cefepime, trimethoprim, netilmicin, and aztreonam. Several antibiotics were also

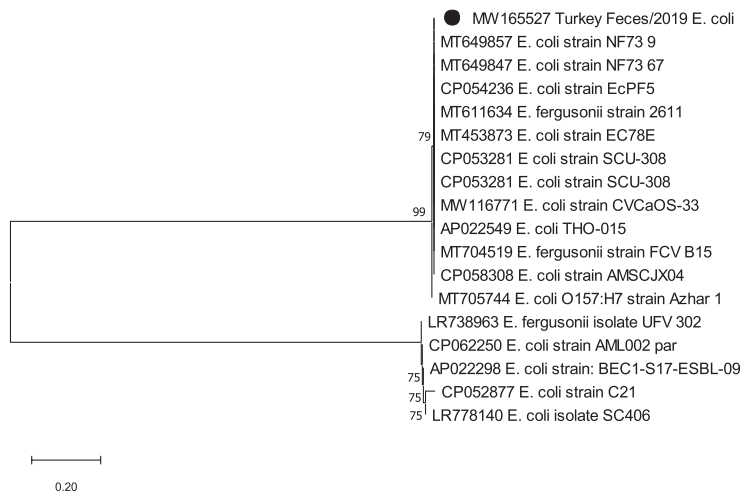


Figure 2. Phylogenetic analysis of the partial *16SrRNA* gene of *E. coli* isolated from turkeys. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with the sum of branch length = 2.46853609 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 18 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGAX. Our sequence is marked in the tree with a black dot.

used by Tawyabur et al. [35] and reported similar results. Moreover, Tawyabur et al. [35] studied the prevalence of multidrug-resistant *E. coli* in diseased and healthy turkeys. However, they did not find any significant difference in the antibiotics resistance pattern between these two groups.

Conclusion

In the context of this study, it may be concluded that both the fecal and cloacal samples of turkeys from different farms are reservoirs of *E. coli*. It also brings out the multidrug-resistant *E. coli* of the study area. Antibiotic drug resistance is a problem in treating turkey diseases associated with *E. coli* and a potential public health hazard to individuals that consume turkey meat.

List of abbreviations

MA = MacConkey Agar, NA = Nutrient agar, EMB = Eosin Methylene Blue, MR = Methyl Red, VP = Voges-Proskauer, HSTU = Hajee Mohammad Danesh Science and Technology University

Acknowledgments

Nothing to disclose.

Conflict of interest

The authors declared that they have no conflict of interest to disclose.

Authors' contribution

TK, NAR, and MKH designed the study. TK did the actual work. TK, NAR, and MSR drafted the manuscript. MMKH, MSR, and JH critically checked and improved the manuscript. All the authors read the manuscript and approved the final version for publication.

References

- [1] Yakubu A, Abimiku K, Musa IS, Idahor AKO, Akinsola OM. Assessment of flock structure, preference in selection and traits of economic importance of domestic turkey (*Meleagris gallopavo*) genetic resources in Nasarawa state, Nigeria. *Livest Res Rural Dev* 2013; 25:18.
- [2] Asaduzzaman M, Salma U, Ali HS, Hamid MA, Miah AG. Problems and prospects of turkey (*Meleagris gallopavo*) production in Bangladesh. *Res Agric Livest Fish* 2017; 4(2):77–90; <https://doi.org/10.3329/ralf.v4i2.33719>
- [3] Polash CR, Hossain MK, Rumi NA, Rahman MS, Mohammad SM, Hossain I. Isolation and identification of bacterial pathogens from cloacal swab of turkeys and their antimicrobial sensitivity patterns. *Asian J Med Biol Res* 2017; 3(4):419–25. <https://doi.org/10.3329/ajmbr.v3i4.35331>
- [4] Begum IA, Alam MJ, Buysse J, Frija A, Huylens-broeck GV. A comparative efficiency analysis of poultry farming systems in Bangladesh: a data envelopment analysis approach. *Appl Econ* 2011; 44:3737–47; <https://doi.org/10.1080/00036846.2011.581216>
- [5] FAO/STAT. Livestock primary production data. Food and Agriculture Organization of the United Nations. 2012; Rome, Italy.
- [6] Grimes, J., J. Beranger, M. Bender and M. Walters. How to raise heritage turkey on pasture. American livestock Breeds conservancy. 2007; Pittsboro, NC27312 USA. Headquarters, 233 S. W. Ackes Drive, 11th floor Chicago, Illinois- 60606.
- [7] Wood BJ and OW Willems. Selection for improved efficiency in poultry, progress to date and challenges for the future. *Proceedings*

of the 10th World Congress on Genetics Applied to Livestock Production.2014; Communication No. 111.

- [8] Robbins CT. Wildlife feeding and nutrition. Academic Press, Cambridge, MA, 1983, pp 33–7.
- [9] Ojewola GS, Udokainyang AD, Obasi V. Growth, carcass and economic response of localturkey poult to various levels of dietary energy. In: Aletor VA, Onibi GE (eds.). Increasing household protein consumption through livestock products. Proceedings of the 27th Annual Conference Nigeria Society of Animal Production, Akure, Nigeria, 2002, pp 167–9.
- [10] Sackey B, Mensah P, Collison E, Dawson ES. *Campylobacter*, *Salmonella*, *Shigella* and *Escherichia coli* in live and dressed turkey from metropolitan Accra. Int J Food Microbiol 2001; 71:21–8; [https://doi.org/10.1016/S0168-1605\(01\)00595-5](https://doi.org/10.1016/S0168-1605(01)00595-5)
- [11] Hofstad MS, Calnek BW, Heirnboldt CF, Reid WM, Yoder HW. Diseases of poultry. 2nd edition, Oxford and IBH Publishing Co., Delhi, India, 1992, pp 88–125.
- [12] Schmidt GP, Domermuth CH, Potter LM. Effect of oral *Escherichia coli* inoculation on performance of young turkeys. Avian Dis 1988; 32:103–7. <https://doi.org/10.2307/1590956>
- [13] McCarthy N, Reen FJ, Buckley JF, Frye JG, Boyd EF, Gilroy D. Sensitive and rapid molecular detection assays for *Salmonella enterica* serovars Typhimurium and Heidelberg. J Food Prot 2009; 72:2350–7; <https://doi.org/10.4315/0362-028X-72.11.2350>
- [14] Merchant IA, Packer RA. Veterinary bacteriology and virology. 7th edition, The Iowa University Press, Ames, IA, pp 286–306, 1967.
- [15] Cheesbrough M. Laboratory manual for tropical countries. Volume II. Microbiology. Tropical Health Technology, ELBS, London, UK, 2003, pp 214–20.
- [16] Rumi NA, Hosen MA, Kundu T, Rahman MS. Molecular characterization of *Salmonella* isolated from internal organs of dead turkey and its antimicrobial activity pattern. Asian J Med Biol Res 2019; 5(3):219–25; <https://doi.org/10.3329/ajmbrv5i3.43591>
- [17] Suresh KG, Batish VK, Grover S, Mohanty AK. Rapid and sensitive detection of *Escherichia coli* in milk by 16S rRNA gene targeted PCR. Int J Anim Biotechnol 2011; 1(1):107–10.
- [18] Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018; 35:1547–49; <https://doi.org/10.1093/molbev/msy096>
- [19] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39:783–91; <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- [20] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987; 4:406–25; <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- [21] Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci 2004; 101:11030–35; <https://doi.org/10.1073/pnas.0404206101>
- [22] CLSI. Performance standards for antimicrobial susceptibility testing: Twenty-third Informational Supplement M100-S23. Clinical and Laboratory Standards Institute. 2013; Wayne, PA, USA.
- [23] Tsen HY, Lin CK, Chi WR. Development and use of *16SrRNA* gene targeted PCR primers for identification of *Escherichia coli* cells in water. J Appl Microbiol 1998; 85(3):554–60; <https://doi.org/10.1046/j.1365-2672.1998.853535.x>
- [24] Tiffany DP. Bacterial profiles and ex vivo effects of *Salmonella* Heidelberg on leukocyte function in turkey purebred lines. The Faculty of the Virginia Polytechnic Institute and State University. Master of Science in Animal and Poultry Sciences, Blacksburg, VA, 2014, pp 45–8.
- [25] Jessica L, Danzeisen AJ, Calvert SL, Brian MC, Julie S, Sherwood CM, et al. Succession of the turkey gastrointestinal bacterial microbiome related to weight gain. Peer J 2013; 1:237; <https://doi.org/10.7717/peerj.237>
- [26] Bielke LR, Elwood AL, Donoghue DJ, Donoghue AM, Newberry LA, Neighbor NK, et al. Approach for selection of individual enteric bacteria for competitive exclusion in turkey. Poult Sci 2003; 82:1378–82; <https://doi.org/10.1093/ps/82.9.1378>
- [27] Menconi A, Wolfenden AD, Shivaramaiah SJ, Terraes C, Urbano T, Kuttel J, et al. Effect of lactic acid bacteria probiotic culture for the treatment of *Salmonella enterica* serovar Heidelberg in neonatal turkey chickens and turkey poults. Poult Sci 2011; 90:561–5; <https://doi.org/10.3382/ps.2010-01220>
- [28] Guthertz JT, Fruin DS, James LF. Microbiology of fresh comminuted Turkey meat. J Milk Food Technol 1976; 39: 823–9; <https://doi.org/10.4315/0022-2747-39.12.823>
- [29] Bryan FL. Factors associated with the dissemination of *Salmonellae* in turkey products and processing plants. Unpublished Ph.D. thesis. Library, Iowa State University, Ames, IA, 1965.
- [30] Khan MFR, Rahman MB, Khan MSR, Nazir KHMNH, Rahman M. Antibiogram and plasmid profile analysis of isolated poultry *Salmonella* of Bangladesh. Pak J Biol Sci 2005; 8:1614–9; <https://doi.org/10.3923/pjbs.2005.1614.1619>
- [31] Merchant IA, Packer RA. Veterinary bacteriology and virology. 7th edition, The Iowa State University Press, Ames, IA, pp 211–305, 1967.[32]. Naqi SA, Lewis DH, Hall CF. The intestinal microflora of Turkeys. Avian Dis 1970; 14(4):620–5; <https://doi.org/10.2307/1588633>
- [33] Yang H, Chen S, White DG, Zhao S, McDermott P, Walker R, et al. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J Clin Microbiol 2004; 42:3483–9; <https://doi.org/10.1128/JCM.42.8.3483-3489.2004>
- [34] Zhao S, Maurer JJ, Hubert S, De Villena JF, McDermott PF, Meng J, et al. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet Microbiol 2005; 107:215–24; <https://doi.org/10.1016/j.vetmic.2005.01.021>
- [35] Tawyabur M, Islam MS, Sobur MA, Hossain MJ, Mahmud MM, Paul S, et al. Isolation and characterization of multidrug-resistant *Escherichia coli* and *Salmonella* spp. from healthy and diseased turkeys. Antibiotics 2020; 9(11):770; <https://doi.org/10.3390/antibiotics9110770>