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Detection of some virulence and antibiotics resistance genes in *Campylobacter* isolated from turkeys

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ABSTRACT

A total of 69 cloacal swabs were collected from turkey from different turkey farms in Giza and Sharkia governorates in Egypt for isolation of *Campylobacter* species. The results revealed that *Campylobacter* was isolated from 16 out of 69 (23.2%) cloacal samples. The samples were identified biochemically revealed that 4 out of 16 (25%) were identified as *Campylobacter jejuni* (*C. jejuni*) while 12 out of 16 (75%) were *Campylobacter coli* (*C. coli).* *Campylobacter* isolates were confirmed by multiplex PCR using *hipO* gene which responsible for Na hippurate hydrolysis for confirmation of *C. jejuni* and *ceuE* gene which designed for detection of *C. coli*. All isolates were examined for presence of tetracycline resistance gene *tetM* was detected in 8 out of 16 (50%) *Campylobacter* isolates. The *cdtB* virulence gene was detected in all isolates. From mentioned results we recommended the use of multiplex polymerase chain reaction (PCR) for identification of *Campylobacter* species. This study may indicate the extent of the existence of *campylobacter* species in some turkey farms in Giza & Sharkia governorates. In conclusion, biosecurity programs must be applied inside slaughter houses to avoid carcass contamination. Applying more efforts in surveillance programs in turkey farms for epidemiological mapping of *Campylobacter* existence and antimicrobial resistance distribution which obligate the stop of uncontrollable use of antibiotics in poultry farms.

INTRODUCTION

Poultry production considered an important source of protein in Egypt and all over the world. It represents about 20% of daily consumption of animal's protein in Egyptian society. Turkey production has a smaller scale of production in comparison to poultry production due to the cost of breeding and the high price of turkey in Egypt. Moreover, turkey need a special condition in production due to its sensitivity to infection with many diseases, its expensive costs of feed, vaccines and treatment.

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(**Moawad et al. 2017).**

Poultry and poultry by-products causes about of 20-30 % of human *Campylobacter* infection **(EFSA 2021)** through consumption of under cooked food or contaminated food with *Campylobacter*, the carcass may be contaminated through the production chain of poultry inside slaughter houses **(Hamed et al. 2014).**

Thermophilic *Campylobacter* species are Gram negative motile bacilli by single polar flagella, S shape spirally curved bacilli. Mostly colonized normally in intestinal tract of poultry specially chicken and turkey without any pathogenic lesions, but sometimes it causes what is called vibronic hepatitis forming focal spots in the liver which related to infection with *Campylobacter jejuni,* and *Campylobacter coli*. Recently, a new species of *Campylobacter* with name *Campylobacter* hepaticus was found to be responsible of vibronic hepatitis, a disease which causes high morbidity, mortality, and drop in egg production in layer chickens. *Campylobacter jejuni* also may cause a gastrointestinal inflammation in chickens and turkeys (**Zhage and Sahin 2020).**

Campylobacter has four types of virulence genes which play an important role of its pathogenicity inside the host. Moreover, it carries invasion genes (*cadF, ciaB, and pldA*) genes, adherence genes (*cadF)*, colonization genes (*caiB*, *pldA* and *dnaJ*) which play an important role in colonization in the intestinal tract of host, and the cytolethal toxin genes which are encoded in (*cdtA, cdtB, and cdtC)* genes responsible for appearance of the lethal symptoms of *Campylobacter* infection. **(Reddy and Zshiri 2018**).

Misuse of antibiotics as growth promoters or random use in treatment of any bacterial disease may affect the commensal bacteria in gastrointestinal tract like *Campylobacter* spp.and may lead to the production of generations carrying antibiotic resistance genes. (**Peterson and Kaur 2018; Singh et al. 2019; Hamed et al. 2021).** For many years, *Campylobacter* spp. were considered susceptible to various antimicrobial agents, while in the recent years, both animals and human isolates of this bacterium have shown resistance to several antibiotics such as fluoroquinolones and tetracycline (**Iovine 2013)**.

Due to limited information about presence of *Campylobacter* spp. in turkey farms, the present study was aimed to detect the incidence of their occurrence, using both conventional method and multiplex PCR , in addition to the presence of *cdtB* & *tetM* genes.

MATERIALS AND METHODS

Sampling

A total of 69 cloacal swabs were collected from apparent healthy turkey in turkey farms in Sharkia and Giza governorates. All samples were kept on Cary-Blair Medium (Oxoid) as transport media and submitted to Reference lab for veterinary quality control on poultry production for isolation of *Campylobacter* spp.

Isolation and identification of *Campylobacter*

Campylobacter spp. isolation and identification was done by standard methods according to (**ISO 10272-1:2017)**. Samples were added to Bolton broth (1:9 v/v) as 1g to 9 ml of Bolton broth, incubated at 37℃ for 4 hrs then at 44℃ for 44 hrs then inoculated in selective agar plates mCCDA incubated at 41.5℃ for 48 hrs. The select the suspected colonies (metallic gray colonies) were subjected to Gram staining and examined under Microscope to see the specific shape of *Campylobacter* (curved gram negative bacilli).

Biochemical identification

For differentiations of thermophilic *Campylobacter* spp. biochemical tests of Na hippurate hydrolysis test, oxidase test and catalase test were used according to (**ISO 10272-1:2017)**.

Molecular Assessment

DNA was extracted from culture broth using a QIAamp DNA Mini Kit (Qiagen, Germany, GmbH Catalogue No. 51304). The extracted DNA was used in subsequent (PCR) assays for species confirmation and to detect genes respon-sible for virulence and antimicrobial agent resistance. PCR was performed in a final

volume of 25 µL that contained 12.5 µL of EmeraldAmp MAX PCR Master Mix [EmeraldAmp GT (2× premix), Japan], 1 µL of each primer at concentrations of 20 pmol, 4.5 µL of diethyl pyrocarbonate water, and 6 µL of the DNA template. The reaction was performed in a Biometra thermal cycler, T3000 (Germany). The oligonucleotide primers (Table 1) were supplied by Metabion, Germany.

The PCR products were separated by electro-phoresis according to **(Sambrook et al. 1989)** on a 1% agarose gel (AppliChem, Germany, GmbH) in 1× TBE buffer at room temperature using a gradient of 5 V/cm. Each well was loaded with 15 µL of the PCR product. A GelPilot 100 bp (Qiagen) ladder was used to determine the fragment sizes. The gel was photo-graphed by a gel documentation system (Biometra BDA digital, Germany), and the data were analyzed using a gel documentation system (Alpha Innotech, Biometra, Germany) and a piece of computer software (auto-matic image capture software, Protein Simple, formerly Cell Bioscience, USA). The temperature and time conditions of the primers during PCR are shown in Table (1).

The amplification efficiency was verified for positive field samples that might have the tested genes, which were previously examined in a veterinary quality control reference laboratory for poultry production, Animal Health research institute.

PCRAmplification:

The extracted DNA was further tested by Thermo two step PCR kit (Thermo scientific) for the presence of *(cdtB, hipO, ceuE, and tetM)*genes. The polymerase chain reactions were done according to the manual instruction of the PCR kit as following: 12.5ul PCR master mix, 1 ul of each primer with concentration 20 pmol, 5ul of DNA then complete the total volume to 20 ul with PCR grade water. The amplification condition ran as initial denaturation at 95 ˚C for 5 min for one cycle, 40 cycles for 3 following steps: denaturation at 95 ˚C for 45 sec.,annealing for 40 seconds at 54°C for *cdtB*, 59°C for *hipO*, 47°C for *ceuE*, 55°C for *tet(M)*, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. A generuler 100 bp ladder (Fermentas, Thermofisher) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1. Oligonucleotide sequences and thermal profiles used in PCR Test target Tested gene Primer

Test target	Gene	Sequence	Amplicon size	Reference
Tetracycline resistance	*tet(M)*	F: ACAGAAAGCTTATTATATAAC R: TGGCGTGTCTATGATGTTCAC	171bp	(Aminov et al., 2004)
Virulence gene of *Campylobacter*	*cdtB*	F: CAC GGT TAA AAT CCC CTG CT R:GCA CTT GGA ATT TGC AAG GC	495bp	(González-Hein et al., 2013)
Campylobacter jejuni	*(hipO)*	F:GACTTCGTGCAGATATGGATGCTT R : GCTATAACTATCCGAAGAA-GCCATCA	344bp	(Persson and Olsen, 2005)
Campylobacter coli	*ceuE*	F: ATG AAA AAA TAT TTA GTT TTT GCA R: ATT TTA TTA TTT GTA GCA GCG	894bp	(Nayak et al., 2005)

RESULTS:

Campylobacter spp. Isolation and Identification

Campylobacter isolates were isolated from 16 out of 69 (23.2%) examined cloacal swabs which were collected from turkey farms in Giza and Sharkia governorates during the period from 2021 to 2022. All samples were biochemically identified and confirmed by PCR , the highest prevalence was *C. coli* which constituted 12 out 16 *Campylobacter* isolates (75%), while *C. jejuni* represented 4 out of 16 (25%) *Campylobacter* isolates as shown in Table (2).

Table 2. prevalence of *C. jejuni* and *C. coli* in turkey cloacal swabs in Giza and Sharkia governorates .

Governorates	No. of the examined samples	No. of positive samples		Positive samples by using Conventional cultural methods			
				C. jejuni		*C. coli*	
		No.	%*	No.	%**	No.	%**
Sharkia	55	4	7.2	2	50	2	50
Giza	14	12	85.7	2	16.7	10	83.3
Total	69	16	23.2	4	25	12	75

* % according to the total number of examined samples
** % according to the total number of positive *Campylobacter* isolates .

2-Molecular confirmation, typing and detection of *cdtB* and *tetM* of *Campylobacter* isolates.

All *Campylobacter* isolates were confirmed and typed by using multiplex PCR technique. The amplification of the DNA for *hipO* gene which used for detection *C. jejuni* and was found in 4 out 16 *Campylobacter* isolates (25%) . On the other hand, *ceuE* gene which used for detection of *C. coli* was found in 12 out of 16 (75%) of *Campylobacter* isolates, as shown in Table (2)

All *Campylobacter* strains showed positive amplification of *cdtB* virulent gene, while only 8 out of 16 (50%) of total *Campylobacter* isolates harboured *tetM* gene . The *tetM* gene was detected in 1 out of 4 (25%) of *C. jejuni* isolates and 7 out of 12(58.3%) of *C. coli* isolates as shown in Table (3).

Table 3. Distribution of tetracycline resistance gene (*TetM*) and *cdtB* gene in isolated *Campylobacter* strains.

No. of Strain	Type	*tetM* gene	*cdtB* gene
1	*C .coli*	Positive	Positive
2	*C . jejuni*	Positive	Positive
3	*C .coli*	Negative	Positive
4	*C .coli*	Negative	Positive
5	*C .coli*	Negative	Positive
6	*C .coli*	Positive	Positive
7	*C .coli*	Positive	Positive
8	*C .coli*	Positive	Positive
9	*C . jejuni*	Negative	Positive
10	*C .coli*	Negative	Positive
11	*C . jejuni*	Negative	Positive
12	*C .coli*	Positive	Positive
13	*C .coli*	Negative	Positive
14	*C .coli*	Positive	Positive
15	C . *jejuni*	Negative	Positive
16	C .*coli*	Positive	Positive

DISCUSSION

Turkey meat is one of the consumed poultry meat in Egypt especially in occasion, which encourage us to identify potential microorganisms as *Campylobacter*. It is one of the most important food poisoning microorganism related to public health hazards in the last 20 years, as it has different ways to produce diseases through animals, contaminated food and one to one communication (**Hakeem and Lu 2021**).

In the present study, the investigation of the prevalence of *Campylobacter* in turkeys from turkey farms was done during the period from 2021 to 2022 in Giza and Sharkia governorates. On examination of a total of 69 cloacal swabs, 16 (23.2%) were positive for *Campylobacter* isolation, our results were in accordance with previous findings which recorded 22.5% *Campylobacter* from Delta governorates, Egypt (**Khalil et al. 2020)**, and higher than that detected by **Eid et al. (2018**) who isolated *Campylobacter* in percentage of 16% of examined turkey farms in Sharkia, Egypt.

In this study the percentage of detection of *C. coli* was higher than the percentage of *C. jejuni* in examined turkey farms, this finding is contrary to that reported by **Eid et al. (2018) and Khalil et al. (2020)** who reported *that C. jejuni i*n high percentage than *C.coli* in examined Turkey farms in Sharkia and Delta governorates in Egypt .

Campylobacter can be differentiated by multiplex PCR through using *hipO* gene which is responsible for hippurate hydrolysis activity of *C. jejuni* **(Linton et al. 1997**), and *ceuE* which designed encoding a 34.5 to 36.2 KDa lipoprotein compound of binding-protein dependent transport system for sidrophore enterochelin characterized for *C. coli* (984bp) (**Park and Richardson 1995; Richardson and Park 1995**). Also *ceuE* gene has two primer (COL1 and COL2) were designed for identified *C.coli* only. (**Gonzalez et al. 1997**). Multiplex PCR is a rapid and accurate technique in *Campylobacter* detection and identification (**El-Adawy et al. 2012).** In this study we use *hipO* gene for confirmation of *C. jejuni*. Also, **Khalil et al., (2020) and Karmi (2019)** used the same gene in their studies for detection *C. jejuni* while **Eid et al. (2018) and Gahamanyi et al. (2021)** were used *CJ* gene for detection of *C. jejuni* in their studies. In the present investigation *ceuE* gene designed for detection of *C. coli* at 984bp was used. That was similar to the results of **He et al. (2010); Rajagunalan et al. (2014); and Eid al al. (2018).** On the other hand, **Karmi (2019); and El Baaboua et al. (2022)** used *glyA and*, *cadF* genes for detection of *C. coli* respectively.

Our study detected the *cdtB* gene which is one of virulence genes of *Campylobacter* spp. which responsible for production of *Campylobacter* cytolethal distending toxin. This gene was detected in 100% of *Campylobacter* isolates that nearly accord the result of **Bang et al. (2004)** who detected *cdtB* gene in 87.1% in *Campylobacter* species that isolated from turkeys while **Kavan et al. (2015)** detected the presence of *cdtB* gene in 6% of *Campylobacter* spp. isolated from turkeys in Iran.

The ribosomal protection genes (*tetM, tetO, tetQ*) are encoded on conjugative elements and many are encoded on transposons, but the vast majority is present on transferable plasmids. They have the widest host range and are found in a number of Gram-positive and Gram-negative bacteria **(Chopra and Roberts, 2001; Roberts, 1996)**. Tetracycline resistance in *Campylobacter* spp. is primarily mediated by ribosomal production protein (*tetO*), which is transferred as plasmid-encoded gene **(Gibreel et al. 2004).** In the present tudy, the identification of a new class of tetracycline-resistant determinants in *Campylobacter* spp. like *tet(M*) gene was done. This gene might be transferred to *Campylobacter* spp. by means of a plasmid, by conjugative transposons **(Hormeño et al. 2020).** Our results are supportive of this finding and indicate a need for closer investigation of these interactions.

In conclusion

The prevalence of *Campylobacter* spp. in turkey farms exists in a proportion which can't be underestimated.

In addition to the dissemination of antimicrobial resistance such as *tetM* gene, which directs us to apply more efforts in surveillance programs in turkey farms for epidemiological mapping of *Campylobacter* spp. existence and

antimicrobial resistance distribution, it emphasizes the need for cautious use of tetracycline in turkey production to decrease the extension of tetracycline-resistant *Campylobacter* spp.

Presence of *tetM* gene, which is responsible for tetracycline resistance in the family *Enterobactiaceae* and gram positive bacteria, not for *Campylobacter* spp. That leads us to give more focus on the possibility of transmission of antibiotic resistance genes from bacteria to others, so it is recommended to stop uncontrollable use of antibiotics in poultry farms.

CONCLUSION

Multiplex PCR is a more reliable rapid technique than conventional methods in *Campylobacter* detection and identification.

REFERENCES

Aminov RI, Chee-Sanford JC, Garrigues N, Mehboob A, Mackie RI, 2004. Detection of tetracycline resistance genes by PCR methods. Public Health Microbiology: Methods and Protocols, 3-13.

Bang DD, Borck B, Nielsen EM, Scheutz F, Pedersen K, and MADSEN M, 2004 Detection of Seven Virulence and Toxin Genes of Campylobacter jejuni Isolates from Danish Turkeys by PCR and Cytolethal Distending Toxin Production of the Isolates. Journal of Food Protection, 67 (10): 2171–2177

Chopra I, Roberts M, 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiology and molecular biology reviews (65): 232-260

EFSA 2021, European food safety authority https://open.efsa.europa.eu/

Eid S, Al-Atfeehy NM, Samir H, and Hefny YH. 2018. Implication of Turkey Broiler Flocks in Prevalence of Antibiotic Resistance Campylobacter spp. Journal of Researcher. 10(3):60-67 doi:10.7537/marsrsj100318.08

El-Adawy H, Hotzel H, Tomaso H, Neubauer H, Hafez HM 2012 Elucidation of colonization time and prevalence of thermophilic Campylobacter species during turkey rearing using multiplex polymerase chain reaction. Journal of poultry science 91(2): 454-459

El Baaboua A, El Maadoudi M, Belmehdi O, Kounnoun A, Bouyahya A, Abrini J. 2022. Development and evaluation of real time PCR for Detection and Identification of C. jejuni and C. coli from human food. Journal of Research Square, pp. 1:16, Doi. https://doi.org/10.21203/res.3.rs-1758442/v1

Gahamanyi N, Song D-G, Yoon K-Y, Mboera LEG, Matee MI, Mutangana D, Amachawadi RG, Komba EVG, Pan C-H. 2021 Antimicrobial Resistance Profiles, Virulence Genes, and Genetic Diversity of Thermophilic Campylobacter Species Isolated From a Layer Poultry Farm in Korea. Frontiers in Microbiology, 12:622275

Gibreel A, Tracz MD, Lisa Nonaka L, Ngo TM, Connell SR, Taylor DE. 2004 Incidence of antibiotics resistance in Campylobacter jejuni isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet (O)-mediated tetracycline resistance. Journal of Antimicrobial Agent Chemotherapy, 48(9) 3442-3450, DOI: 10.1128/AAC.48.9.3442–3450.2004

Gonzalez I, Grant KA, Richardson PT, Park SF, Collins MD. 1997 Specific Identification of the Enteropathogens Campylobacter jejuni and Campylobacter coli by Using a PCR Test Based on the ceuE Gene Encoding a Putative Virulence Determinant. journal of clinical microbiology. 35(3): 759–763

González-Hein G, Huaracán B, García P, Figueroa G. 2013. Prevalence of virulence genes in strains of Campylobacter jejuni isolated from human, bovine and broiler. Brazilian Journal of Microbiology (44): 1223-1229.

Hakeem MJ, Lu X. 2021 Survival and Control of Campylobacter in Poultry Production Environment. Frontiers in Cellular and Infection Microbiology 10, 615049. doi: 10.3389/fcimb. 2020.615049,

Hamed EA, Abdel Rahman MAA, Shalaby AG, Morsy, MM, Nasef SA. 2014. Detection of antibiotic resistant genes of some Campyl-

obacter species isolated from Egyptian ducks. Journal of Suez Canale veterinary medicine XIX (2) (193):206

Hamed EA, Abdelaty MF, Sorour HK, Roshdy H, AbdelRahman MAA., Magdy O, Ibrahim WA, Sayed A, Mohamed H, Youssef MI, Hassan WM, Badr H. 2021. Monitoring of Antimicrobial Susceptibility of Bacteria Isolated from Poultry Farms from 2014 to 2018, Veterinary Medicine International Volume 2021, Article ID 6739220, 8 pages https:// doi. org/10.1155/2021/6739220

He Y, Yao X, Gunther NW, Xie Y, Tu S-I, Shi X. 2010. Simultaneous Detection and Differentiation of Campylobacter jejuni, C. coli, and C. lari in Chickens Using a Multiplex Real-Time PCR Assay. Food Anal. Methods 3, 321–329 https://doi.org/10.1007/s12161-010-9136-6

Hormeño L, Campos MJ, Vadillo S, Quesada A. 2020. Occurrence of tet (O/M/O) mosaic gene in tetracycline-resistant Campylobacter. Microorganisms (8): 1710.

ISO 10272-1 2017. Microbiology of food and animal feeding stuffs; Horizontal method for the detection of thermotolerant Campylobacter. International Standards Organization, Geneva

Iovine NM. 2013 Resistance mechanisms in Campylobacter jejuni. Journal of Virulence. 4(3): 230-240

Karmi M. 2019 Prevalence of Campylobacter spp. And it's pathogenic genes in poultry meat, human and environment in Aswan, upper Egypt. Assiut Veterinary medical Journal. 65(161):151-158

Kavan RS, Hassanzadeh M, Bozorgmehri Fard MH, Pourbakhsh SA, Akhondzadeh Basti A, Barin A, Ashraf I. 2015. Detection of cytolethal distending toxin (cdt) Genes in Campylobacter jejuni and Campylobacter coli isolated from the intestinal Of commercial broiler chickens, turkey and quail of Iran. Iranian Journal of Veterinary Medicine 9(2): 109-116

Khalil MR, Moawad A, Kafafy MH, Fahmy H, SobhyM. 2020 Molecular Characterization of Campylobacter species from turkey flocks in delta governments.. Assiut Veterinary Medical Journal 66(164)111-117. DOI: 10.21608/avmj.2020.1672555

Linton D, Lawson AJ, Owen RJ, Stanley J. 1997 PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples, Journal of Clinical Microbiology, 35(10): 2568-2572

Moawad AA, Hotzel H, El-Awady H, Neubauer, H, Hafez MH, 2017. Turkey production in Egypt: current situation and challenge. Turkey production and health:challenges and opportunities proceeding 9th "Hafez" international Symposium. Meeting of working group 10(Turkey) of WPSA. Held in Berlin, Germany18- 20May 2017, 12-17.

Nayak R, Stewart TM, Nawaz MS. 2005. PCR identification of Campylobacter coli and Campylobacter jejuni by partial sequencing of virulence genes. Journal of Molecular and Cellular Probes (19):187-193

Park SF, Richardson PT 1995. Molecular characterization of a Campylobacter jejuni lipoprotein with homology to periplasmic siderophore-Binding proteins. Journal of Bacteriology, 177(9): 2259–2264. doi: 10.1128/ jb.177.9.2259-2264.1995

Persson S, Olsen KE. 2005. Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. Journal of medical microbiology (54): 1043-1047.

Peterson E, Kaur, P. 2018. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producer, Environmental Bacteria, and Clinical Pathogens. Journal of Frontiers in Microbiology, 9, 2928, 1-21, doi:10.3389/ fmicb.2018.02928, 2018.

Rajagunalan S, Bisht G, Pant S, Singh SP, Singh R, . Dhama K. 2014 Prevalence and molecular heterogeneity analysis of revalence Campylobacter jejuni and Campylobacter coli isolated from human, poultry and cattle, in Pantnagar, India. Veterinarski

ARHIV 84 (5): 493-504

Reddy S, Zishiri, OT. 2018 Genetic characterisation of virulence genes associated with adherence, invasion and cytotoxicity in Campylobacter spp. isolated from commercial chickens and human clinical cases', Onderstepoort Journal of Veterinary Research 85 (1) 1-9 https://doi. org/10.4102/ ojvr.v85i1.1507

Richardson PT, Park SF 1995. Enterochelin acquisition in Campylobacter coli: characterization of components of a dependent transport system. Journal of Microbiology 141(12): 3181–3191.

Roberts MC. 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS microbiology reviews (19): 1-24.

Sambrook J, Fritscgh EF, Mentiates T. 1989 Molecular Cloning. A Laboratory Manual. Vol. 3. Laboratory Press, Cold Spring Harbor, New York 55-57.

Singh A, Daljeet C, Sharda R, Shukla S, Audarya SD, Sikrodia R, Gangil R, Singh N. 2019 Antibiotic Resistance in E. coli Isolated from Poultry. international journal of current Microbiology of applied science, 8(10) 89-94,https:doi.org/10.20546ijcmas.2019.810.010,

Zhang O, Sahin O. 2020 Diseases of poultry. Swayne DE, Boulianne M, Logue CM, McDougald LR., Nair V, Suarez DL. Editorial Board for the American Association of Avian Pathologists, 374 Blackwell Publishing .14th ed., Chapter 17 Campylobacter infection. pp. 754:769, ISBN-9781119371151.