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Occurrence of *Escherichia coli* in cloacal samples of broiler chicken from Kollam and Kottayam districts#

S. Afsal*[1], C. Latha[2], C. Sethulekshmi[3], M. Binsy[3], C. J. Beena[4] and V.L. Gleeja[5]
Department of Veterinary Public Health College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651, Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Foodborne pathogens like E. coli are considered as the major causes of foodborne illness in humans worldwide. The present study was undertaken to determine the occurrence of E. coli in cloacal samples of broiler chicken from Kollam and Kottayam districts. The occurrence of E. coli in cloacal samples from broiler chicken was 76.5 per cent from Kollam and 79 per cent from Kottayam through culture techniques. Out of the total 400 cloacal swab samples collected from broiler chicken, 77.8 per cent were positive for E. coli. The samples which were subjected to conventional culture techniques were further analysed for PCR confirmation. The study revealed that, 56.5 and 67 per cent samples were positive for E. coli from Kollam and Kottayam, respectively. An overall occurrence of 61.8 per cent out of 400 samples were confirmed for E. coli by PCR. One Health approach can be used as a suitable tool to combat the foodborne zoonotic diseases, since it is an integrated, multidisciplinary, holistic approach. Proper implementation of biosecurity measures in farms is mandatory to control foodborne zoonotic diseases.

Keywords: *E. coli, broiler chicken, one health*

Running title: Occurrence of *Escherichia coli* in cloacal samples

Foodborne illnesses are usually infectious or toxic in nature caused by bacteria, viruses, parasites or chemical substances, entering the body through contaminated food or water. It is often caused by ingestion of eggs, raw or undercooked meat, fresh produce and dairy products

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1. *MVSc Scholar and *Corresponding author. Email-afsalsudheer007@gmail.com*
2. *Professor and Head*
3. *Assistant Professor*
4. *Assistant Professor and Head, AICRP, KVASU*
5. *Assistant Professor, Department of Statistics*

contaminated by norovirus, *Campylobacter* spp., non-typhoidal Salmonella and pathogenic *E. coli* (WHO, 2015). *Escherichia coli* causes diarrheal diseases, which account for more than four per cent of the total daily global disease burden every day. It also leads to 1.8 million deaths every year, among which 90 per cent were children (Yulistiani and Praseptiangga, 2019). They are the most important foodborne pathogens of *Enterobacteriaceae* family, which causes foodborne illness transmitted mainly through poultry products. Hence, the present study was designed with an objective to find the occurrence of *E. coli* in cloacal samples of broiler chicken.

Materials and methods

The present study was undertaken to determine the occurrence of *E. coli* in cloacal samples of broiler chicken and molecular confirmation of the positive isolates. A total of 400 cloacal swab samples from broiler chicken were collected from different farms of Kollam and Kottayam districts of Kerala. From each district 200 samples were collected for a period of 12 months from October 2019 to November 2020.

After sampling, the cloacal swabs were aseptically placed in tubes containing peptone water. The samples were brought to the laboratory in a thermocool container within 24 h for further isolation and identification by conventional culture techniques. The samples in peptone water were streaked on to MacConkey agar (MCA) incubated at 37°C for 24 h. Followed by plating of lactose fermenting colonies onto EMB agar and incubated at 37°C for 24 h. Colonies with typical characteristics of greenish metallic sheen with dark centre were selected for further confirmation by PCR. The boiling and snap chilling technique was used for the preparation of DNA template (Lee *et al*., 2009).

The molecular reagents and chemicals used in the study were procured from Sigma (Bangalore), Thermo Scientific (Mumbai) and Sigma- Aldrich (USA). The reagents and chemicals used for the PCR were PCR reaction buffer (10X), Taq DNA polymerase (1U/µL), dNTP mix (2.0 mM), $MgCl_2$ (25mM), forward and reverse primer set (100 pmoles/µL) and sterilised milliQ water. The materials used for submarine agarose gel electrophoresis were Tris Boric acid EDTA (TBE), electrophoresis buffer (1X), agarose gel (1.5 %), gel loading buffer (6X), safe DNA staining solution and molecular weight marker (50 bp ladder).

Oligonucleotide primers targeting the *uid*A for *E. coli* were used in the study. The target gene that was detected by PCR and the primer sequences used (Alqahtani *et al.,* 2015) in the study with some modifications are

Table 1. Primers used for the PCR identification of *E. coli*

Sl. No	Primers	Sequence	Size (bp)
1.	*uid*A F	5' TGGTAATTACCGACGAAAACGGC 3'	162 bp
	*uid*A R	5'ACGCGTGGTTACAGTCTTGCG 3'	

Table 2. Components of PCR mixture for amplification of *uid*A gene

Sl. No	Name of reagents	Quantity (µL)
1.	Template DNA	2.0
2.	PCR buffer (10X)	2.5
3.	$MgCl_2$ (25mM)	1.0
4.	*Taq* polymerase (1U/µL)	0.5
5.	dNTP mix (2.0 mM)	1.0
6.	Forward primer (*uid*A) (100 pmoles/µL)	1.0
7.	Reverse primer (*uid*A) (100 pmoles/µL)	1.0
8.	Nuclease free water	16.0
Total		25.0

Table 3. Temperature and cyclic conditions for *uid*A gene

Sl. No	Steps	Conditions	No. of cycles
1.	Initial denaturation	94°C for 10 min	
2.	Denaturation	94°C for 40 sec	35 cycles
3.	Annealing	55°C for 60 sec	
4.	Extension	72°C for 50 sec	
5.	Final extension	72°C for 5 min	

Table 4. Occurrence of *E. coli* in broiler chicken by culture techniques

Sl. No.	District	Total samples analysed	Positive samples		*Chi*-square	p-value
			Number	Per cent		
1	Kollam	200	153	76.5	0 .361^{ns}	0.548
2	Kottayam	200	158	79.0		
Total		400	311	77.8		

p< 0.05 – significant, ^{ns}- Non significant

Table 5. Occurrence of *E. coli* in broiler chicken by PCR

Sl. No.	District	Total samples analysed	Positive samples		*Chi*-square	p-value
			Number	Per cent		
1	Kollam	200	113	56.5	4.668^{s}	0.031
2	Kottayam	200	134	67.0		
Total		400	247	61.8		

p< 0.05 – significant, ^{s}- significant

shown in Table 1. The components of reaction mixture for one reaction are shown in Table 2. The annealing temperature used for the primers of *uid*A gene was 55°C as depicted in Table 3. The PCR products were stained with SYBR safe dye and detected by submarine gel electrophoresis.

Results and discussion

Isolation and identification of E. coli

In the present study, isolation and identification of *E. coli* was done from cloacal swabs of broiler chicken. Similar type of samples were used in various studies conducted by Zinnah *et al*. (2007) from Mymensingh and Ejeh *et al.* (2017) from Nigeria. During the study, the samples were collected in peptone water and further streaked on EMB agar for the selective isolation of *E. coli.* The colonies with characteristic metallic sheen with black centre were identified as *E. coli.* Biochemical tests were used for further characterisation of the organism. This was same as that of the study conducted by Samanta *et al*. (2014) from West Bengal, India, and Akond *et al*. (2009) from Bangladesh.

Occurrence of E. coli in Broiler Chicken by Culture Techniques

The occurrence of *E. coli* in cloacal swabs of broiler chicken from Kollam district was 76.5 per cent and that of Kottayam district was 79 per cent. Altogether 77.8 per cent overall occurrence from both districts were observed. Statistical analysis using Pearson *Chi*-square test revealed that, there is no significant difference (p>0.05) between the occurrence of *E. coli* in broiler chicken from Kollam and Kottayam (Table 4). A study conducted by Kwoji *et al*. (2019) from Maiduguri reported 77.05 per cent occurrence of *E. coli* from broiler chickens, which is in accordance with the present study.

Eze *et al.* (2013) from Nigeria and Stella *et al.* (2016) from Brazil investigated the occurrence of *E. coli* in cloacal swabs from broiler chicken. The study reported 37 and 60 per cent *E. coli* occurrence, which was low when compared to the results of the present study. Also 100 per cent occurrence of *E. coli* in cloacal swabs of broiler chicken were recorded in a study by Wibisono *et al*. (2020) from Blitar, Indonesia.

Escherichia coli is a normal innocuous inhabitant of gastrointestinal tract of man and animals, hence the chance of occurrence is more. Even though they are the common inhabitant in gastrointestinal tract, some of them are pathogenic in nature and are responsible for causing various foodborne illness. Immunocompromised hosts and poultry are negatively affected with pathogenic *E. coli.* In humans *E. coli* responsible for various conditions like endocarditis, meningitis, urinary tract infection, septicemia, epidemic diarrhoea, whereas in case of poultry it causes omphalitis, cellulitis, yolk sac infection, swollen head syndrome, coligranuloma, and colibacillosis. Although most of them are non-pathogenic, they are used as indicators of faecal contamination (Akond *et al*., 2009). Unhygienic and poor management may contribute to the high occurrence of *E. coli* organisms in poultry.

Confirmation of E. coli by PCR

Molecular confirmation of *E. coli* in the current study was done by targeting *uid*A gene. Bej *et al*. (1991) from Connecticut used this gene as a suitable PCR target for *E. coli.* The study by Abdelrahman *et al*. (2008) also revealed that, PCR could be used as a routine diagnostic technique for rapid detection of *E. coli* using *uid*A gene primers.

In this study, PCR confirmed 56.5 and 67 per cent samples as positive for *E. coli* from Kollam and Kottayam districts, respectively. The occurrence of PCR confirmed *E. coli* isolate in broiler chicken from Kollam and Kottayam differ significantly ($p< 0.05$). Altogether, an occurrence of 61.8 per cent *E. coli* positive samples were detected from both districts by PCR (Table 5).

Hossain *et al*. (2008) and Nazir (2004) from Bangladesh reported 60 and 62.5 per cent occurrence of *E. coli,* which is in perfect tune with the present study. A low occurrence of 36.11 per cent noticed in cloacal swabs of broiler chicken by Andrews and Aswathy (2019) from Wayanad, Kerala. Compared to the present results, highest occurrence of 90.7 and 100 per cent were reported by Saidani *et al*. (2017) from Tunisia and Meguenni *et al*. (2019) from Central Algeria, respectively.

Escherichia coli is the most frequently seen pathogen associated with foodborne disease outbreaks, which is often identified by β-glucuronidase enzymatic activity or by detection of *uid*A gene by PCR. Since this house keeping gene found in almost all *E. coli* (Bej *et al*., 1991), it was considered as a suitable PCR target in this study. According to the report by Fratamico (2003), PCR based techniques was more sensitive than culture technique. This also revealed the importance of PCR in the present study.

Conclusion

From the study, it was concluded that an overall occurrence of 77.8 per cent samples were positive for *E. coli* by culture techniques. The samples which were subjected to conventional culture techniques were further analysed for PCR confirmation. The study revealed that, 61.8 per cent samples were confirmed for *E. coli* by PCR. Since *E. coli* is one among the common foodborne pathogens, there is a need for surveillance and control of this organism. A multifaceted One Health approach can combat foodborne diseases. This includes collaborative approach by various disciplines including human medicine, veterinary medicine, epidemiology, environmental specialist, public health institutes and epidemiological surveillance agencies. Upgradation and proper implementation of biosecurity measures are mandatory to control the spread of foodborne zoonotic pathogens.

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Conflicts of interest

There were no conflicts of interest reported by the authors.

References

Abdelrahman, I.O., Elbagir, N.M., Osman, A.M.A., Sharfi, S.A., Saeed, A.M.A., Musa, H.A., Ashmaig, A.A. and Aradaib, I.E. 2008. PCR Detection of *E. coli* in Chicken Fecal Samples. *Int. J. Mol. Med. Adv. Sci.* **4**: 82-85.

Akond, M.A., Alam, S., Hassan, S.M.R. and Shirin, M. 2009. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. *Int. J. Food Safety*. **11**: 19-23.

Alqahtani, J.M., Asaad, A.M., Ahmed, E.M. and Qureshi, M.A. 2015. Drinking water quality and public health in Southwestern Saudi Arabia: The need for a national monitoring program. *J. Family Community Med.* **22**: 19.

Andrews, H.P. and Aswathy, S. 2019. Occurrence of *Escherichia coli* in Broilers and their Farm Settings. *J. Foodborne Zoonotic Dis.* **7**: 1-4.

Bej, A.K., Dicesare, J.L., Haff, L. and Atlas, R.M. 1991. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for *uid*. *Appl. Environ. Microbiol.* **57**: 1013-1017.

Ejeh, F.E., Lawan, F.A., Abdulsalam, H., Mamman, P.H. and Kwanashie, C.N. 2017. Multiple antimicrobial resistance of *Escherichia coli* and Salmonella species isolated from broilers and local chickens retailed along the roadside in Zaria, Nigeria. *Sokoto J. Vet. Sci*. **15**: 45-53.

Eze, E., Nwakeze, E., Oji, A., Ejikeugwu, C. and Iroha, I. 2013. Microbiological investigation of *Escherichia coli* isolates from cloacal and feacal swabs of broiler chickens for extended-spectrum beta-lactamase (ESBL) enzymes. *J. Pharmacy Biological Sci.* **7**: 96-99.

Fratamico, P.M. 2003. Comparison of culture, polymerase chain reaction (PCR), TaqMan Salmonella, and Transia Card Salmonella assays for detection of *Salmonella* spp. in naturally-contaminated ground chicken, ground turkey, and ground beef. *Mol. Cell. Probes*. **1**: 215-221.

Hossain, M.T., Siddique M.P., Hossain, F.M.A., Zinnah, M.A., Hossain, M.M., Alam, M.K., Rahman, M.T., and Choudhury, K.A. 2008. Isolation, identification, toxin profile and antibiogram of *E. coli* isolated from broilers and layers in Mymensingh district of Bangladesh. *Bangladesh J. Vet. Med.* **6**: 1-5.

Kwoji, I.D., Musa, J.A., Daniel, N., Mohzo, D.L., Bitrus, A.A., Ojo, A.A. and Ezema, K.U. 2019. Extended-spectrum beta-lactamase-producing *Escherichia coli* in chickens from small-scale (backyard) poultry farms in Maiduguri, Nigeria. *Int. J. One Hlth*. **5**: 26-30.

Lee, S. H., Jung, B. Y., Rayamahji, N., Lee, H. S., Jeon, W.J., Choi, K. S., Kweon, C. H. and Yoo, H. S. 2009. A multiplex real-time PCR for differential detection and quantification of *Salmonella* spp., *Salmonella enterica* serovar Typhimurium and Enteritidis in meats. *J. Vet. Sci.* **10**: 43-51.

Meguenni, N., Chanteloup, N., Tourtereau, A., Ahmed, C.A., Bounar-Kechih, S. and Schouler, C. 2019. Virulence and antibiotic resistance profile of avian *Escherichia coli* strains isolated from colibacillosis lesions in central of Algeria. *Vet. Wld*. **12**: 1840.

Nazir, K.H.M.N.H. 2004. Molecular base of diversified E. coli potentiating antibiotic resistant pattern and compromising epidemiology. *MS Thesis*, Department of Microbiology and Hygiene, BAU, Mymensingh-2202.

Saidani, M., Tabib, I., Chaouechi, A., Zouaoui,

S., Soudani, A., Haenni, M., Daaloul, M., Ben, F., Chehida, A., Chakroun, C. and Madec, J. 2017. Surveillance of antimicrobial resistance in *Escherichia coli* strains isolated from cattle and broiler chickens in Tunisia. *J. New Sci.* **25**: 1-6.

Samanta, I., Joardar, S.N., Das, P.K., Sar, T.K., Bandyopadhyay, S., Dutta, T.K. and Sarkar, U. 2014. Prevalence and antibiotic resistance profiles of Salmonella serotypes isolated from backyard poultry flocks in West Bengal, India. *J. Appl. Poult. Res*. **23**: 536-545.

Stella, A.E., Oliveira, M.C.D., Fontana, V.L.D.D.S., Maluta, R.P., Borges, C.A. and Ávila, F.A.D. 2016. Characterisation and antimicrobial resistance patterns of *Escherichia coli* isolated from faeces of healthy broiler chickens. *Arq. Inst. Biol*. **83**: 1-5.

WHO (World Health Organisation). 2015. *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015*. World Health Organisation, Geneva, 265p.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. CTX Gene of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* on Broilers in Blitar, Indonesia. *Sys. Rev. Pharm.* 2020. **11**: 396-403.

Yulistiani, R. and Praseptiangga, D. 2019. Occurrences of *Salmonella* spp. and *Escherichia coli* in chicken meat, intestinal contents and rinse water at slaughtering place from traditional market in Surabaya, Indonesia. *International Conference on Food Science and Engineering*, 25–26 September 2018, Central Java, Indonesia. 7p.

Zinnah, M.A., Bari, M.R., Islam, M.T., Hossain, M.T., Rahman, M.T., Haque, M.H., Babu, S.A.M., Ruma, R.P. and Islam, M.A. 2007. Characterisation of *Escherichia coli* isolated from samples of different biological and environmental sources. *Bangladesh J. Vet. Med*. **1**: 25-32.

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