Full Length Article

ISOLATION AND CHARACTERIZATION OF BIOFILM FORMING *ESCHERICHIA COLI* FROM CHICKEN MEAT SAMPLES

I. Manikkavasagan[1], K. Vijayarani[2] *, B. Murugan[1], S. Meignanalakshmi[3] and S. Eswari[4]

Department of Food Safety and Quality Assurance
College of Food and Dairy Technology
Tamil Nadu Veterinary and Animal Sciences University
Koduveli, Chennai – 600 052

ABSTRACT

The present study was aimed to investigate the presence of Escherichia coli (E.coli) in raw chicken meat samples collected from retail shops, as well as the biofilm-forming ability of field isolates, and to characterize different adhesion genes. Out of 20 chicken meat samples, 17 (85%) were positive for E. coli. Fifteen E. coli strains were characterized by PCR using the 16S rDNA primers and all the isolates were positive which confirmed that all the isolates were E.coli. Out of the 15 confirmed E.coli field isolates which were subjected to biofilm-forming assay, 46% of them were found to be strong biofilm producers. While all the isolates were screened for the presence of adhesion genes viz. luxS, csgA, fimH, fimA, and papC, the adhesion gene luxS was detected in all the strains (100%). The other adhesion genes csgA, fimH and fimA were detected in 93%, 93%, and 73% of the isolates, respectively. The E. coli field isolates were screened blaTEM gene was detected in only four strains, which was categorized under strong biofilm producers. This study demonstrated the presence of biofilm forming E. coli in the raw chicken meat samples as contaminants, causing spoilage and potentially posing risk to consumer's health and safety.

Keywords: *E. coli*, Biofilm, Crystal violet assay, PCR, adhesion genes

Received : 13.05.2024 Revised : 08.07.2024 Accepted : 11.07.2024

[1] Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

[2] Director of Research, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 51, Corresponding author

Email id:vijayaranikumanan@gmail.com

[3] Professor, Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

[4] Professor and Head, Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Chennai – 600 007, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

INTRODUCTION

Foodborne diseases affect over 200 million people globally each year and have a significant impact on public health, the economy, and society. These diseases arise from contaminated food at any point in the production, delivery, or consumption process, caused by various forms of environmental pollution, unsafe storage, and processing. According to the World Health Organization,

approximately 1 in 10 people worldwide is affected by contaminated food, leading to more than 420,000 deaths annually. Children under the age of 5 are particularly vulnerable, accounting for 125,000 deaths each year.

Escherichia coli (*E. coli*) is a bacterium that is commonly found in the gastrointestinal system of humans and warm-blooded animals. Majority of strains are harmless, a few are pathogenic causing intestinal or extra intestinal infections of varying severity, primarily transmitted via consumption of contaminated food. Many strains of *E. coli* are known to produce biofilm.

A biofilm is a structured colony of microorganisms that stick to a surface and form their own matrix, which is mostly made up of proteins, polysaccharides, and DNA. This matrix offers various advantages to the bacterial community, including immune cell protection and improved adherence, which is assisted by bacterial adhesions (Sharma *et al.,* 2023; Galié *et al.,* 2018).

Biofilms emerge in reaction to stress and function as a defense mechanism to help microorganisms survival. Biofilms play animportant role in microbial pathogenicity and genetic exchange, and can operate as a barrier to shield the microbial community from standard antimicrobial drugs used in the food business, making them more resilient and harder to eradicate (Vysakh *et al.,* 2018; Katongole *et al.,* 2020; Bhardwaj *et al.,* 2021). The development of acute and chronic infections caused by gram negative bacteria can be attributed, in part, to biofilm formation and quorum sensing (QS) controlled virulence factors, which are another interesting factor to consider (Vysakh *et al.,* 2018).

Antibiotic resistance is one of the factors that determine biofilm production in bacteria. Especially β-lactamases enzyme production by certain bacteria that inhibits the activity and mechanisms of β-lactam antibiotics. Several studies have focused on the prevalence of β-lactam antibiotic resistance genes in *E. coli*, particularly in organisms capable of transferring resistance genes (Khalifa *et al.,* 2021; Bhardwaj *et al.,* 2021).

Biofilm formation occurs in three main stages which includes bacterial adhesion, quorum sensing, and biofilm biomass formation (Thakur *et al.,* 2016; Alshammari *et al.,* 2023). Several studies have reported a series of genes involved in biofilm formation by bacteria. The *E. coli* requires a set of genes to facilitate initiation, attachment, and maturation. The major genes involved encode for flagella, type I fimbriae (*fimH*) (Barilli *et al.,* 2020) and P fimbriae gene (Katongole *et al.,* 2020).

The *fimH* gene is required to produce fimbriae, which mediates the adhesion mechanism and stimulates biofilm formation (Alshammari *et al.,* 2023). Curli fimbriae are crucial components of the biofilm matrix of *E. coli* and other Enterobacteriaceae species. These fibrous surface proteins primarily contribute to bacterial attachment, cell accumulation, and biofilm formation (Özdemi and Arslan, 2021).

The *luxS* gene is a crucial component in *E. coli* for the production of auto inducer - 2, which plays a significant role in the quorum sensing mechanism by promoting biofilm formation and controlling biofilm architecture (Sharma *et al.,* 2016).

The present study was aimed to investigate the prevalence of *E. coli* in chicken meat samples collected from retail market. Among the isolates, the ability of the strains to form biofilm was tested. Further, the genes that encode for biofilm formation and antibiotic resistance (gene β-lactamase) were assessed in *E. coli.*

MATERIALS AND METHODS

Chemicals and media

MacConkey agar, Nutrient broth, Brain heart infusion (BHI) broth, biochemical test kit for *E.coli* (Himedia: KB010), crystal violet, PCR Master mix (Amplicon) were used for isolation and detection of *E. coli.*

Collection of food samples

A total of 20 chicken meat samples were collected aseptically in separate containers from the retail outlet in and around Chennai, Tamil Nadu. The samples were transported to Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Koduveli, Chennai – 600 052 for further investigation.

Isolation and biochemical identification

About 25 g of sample was mixed with 225 ml of nutrient broth for enrichment at 37°C for 18–24 h. After enrichment, 100 µL of the inoculum was placed on MacConkey agar. The plates were incubated at 37°C for 18-24 h (IS5887). Bacterial colonies with pink colour were subjected to biochemical tests (Himedia: KB010). After biochemical confirmation, each *E. coli* isolate was inoculated and incubated in microtubes containing BHI broth medium at 37°C for 24 hrs and subsequently stored at -20°C in glycerol for further analysis.

Quantification of biofilm

Biochemically positive isolate was grown in 10 ml of Brain Heart Infusion broth and the turbidity of the bacterial cultures was adjusted to 0.08 optical density (OD) at 600 nm. In each well, 180 µL of BHI broth and 20 µL of inoculum were transferred to microtitre plate in triplicate, with wells containing only BHI broth serving as a negative control. A 96-well plate was incubated at 37°C for 48 h in order to promote biofilm formation. After 48 hrs, the contents of each well were removed and the wells were washed three times with sterile distilled water to remove planktonic cells. The plate was air dried for 15 min and added with 200 µL of 0.1% crystal violet solution, which was used to stain the cells that were attached to the wells. The plate was incubated at room temperature for 30 min before the dye was removed by washing the wells five times with sterile distilled water. The plate was air dried for 15 min before 200 µL of a mixture consisting of 75% ethanol and 25% acetone was added to each well to resolve the stained cells. The ability of each strain to form biofilms was evaluated by measuring the

Table 1. Primers used to study the adhesion-associated genes for biofilm formation in *E. coli*

Gene	Nucleotide sequences (5′→3′)	Amplicon size (bp)	Annealing temperature	Reference
rrsH/16s rDNA	CGGACGGGTGAGTAATGTCT CTCAGACCAGCTAGGGATCG	200	57°C	(Bhardwaj *et al.,* 2021)
Type 1 fimbriae (*FimH*)	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	55°C	(Wang *et al.,* 2016)
Curli major subunit (*csgA*)	GGTAATGGTGCAGATGTTG GTCACGTTGACGGAGGAGTT	188	55°C	(Bhardwaj *et al.,* 2021)
luxS Regulation of AI-2, cell signaling	TTCTTCGTTGCTGTTGATGC TGGAAGACGTGCTGAAAGTG	152	57°C	(Bhardwaj *et al.,* 2021)
fimA Type 1 fimbriae major subunit	ATCGTTGTTCTGTCGGCTCT GCGGTACGAACCTGTCCTAA	167	57°C	(Bhardwaj *et al.,* 2021)
papC P fimbriae	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAAC	482	55°C	(Wang *et al.,* 2016)
blaTEM beta lactamase gene	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	54°C	(Eid and Samir, 2019)

optical density at 597 nm using a microplate reader (Obe *et al.,* 2021). The biofilm forming capacity of each isolate was then classified into three categories based on their final OD values: (1) weak biofilm producers with values less than 0.3, (2) moderate biofilm producers with values between 0.3 and 0.6, and (3) strong biofilm producers with values greater than 0.6 (Obe *et al.,* 2021).

Molecular identification and screening of adhesion genes

Bacterial genomic DNA was extracted from *E.coli* isolates using the QIAamp DNA Mini kit (QIAGEN) according to the manufacturer's instructions. All positive colonies were confirmed with PCR using *rrsH/16S rDNA* primers specific to *E.coli*. Adhesion-associated genes for biofilm

formation in *E.coli* were identified using primers targeting *luxS, csgA, fimH, fimA, and papC. E.coli* isolates were subjected for PCR to detect the presence of *blaTEM* gene. Gene primers used in this study are listed in Table 1. PCR was carried out using a thermal cycler (QIAGEN). The PCR reaction mix (25 µL) contained 2X PCR master mix 12.5µL, forward and reverse primer each 0.5 µL and 1µLof extracted DNA and 10.5µL nuclease free water. PCR was performed with the following conditions 95°C-3 min; 95°C-30 s; 72°C-1.5 min; 72°C-7 min and the annealing temperature of different genes as mentioned in the Table 1. The products were analyzed using 1.8% agarose gel electrophoresis with a molecular weight marker of 100bp and documented with a UV light transilluminator.

RESULTS AND DISCUSSION

In the present study, out of 20 chicken meat samples used for isolation and characterization of *E. coli*, 17 samples were positive for *E. coli,* characterized by typical pink colonies in MacConkeyagar, which is a selective medium for isolation and identification of *E. coli* from foods. Out of 20 isolates, 15 isolates were found to be positive for *E. coli* through biochemical characterization. Hussain *et al.*(2017) reported that 78% of broiler chicken meat specimens from retail shops of India were contaminated with *E. coli.*

Biofilm forming ability of the 15 isolates was carried out using crystal violet assay and were categorized into three groups based on the optical density value at 597 nm. Seven isolates were strong biofilm producers, four isolates were moderate, and four isolates were weak biofilm producers. (Table 2 and Fig. 1). Hussain *et al.,* (2019) found that 100% of *E. coli* isolates produced biofilms, and Senapati *et al.* (2020) reported that 12.5% of *E. coli* isolates produced strong biofilms isolated from raw chicken meat samples.

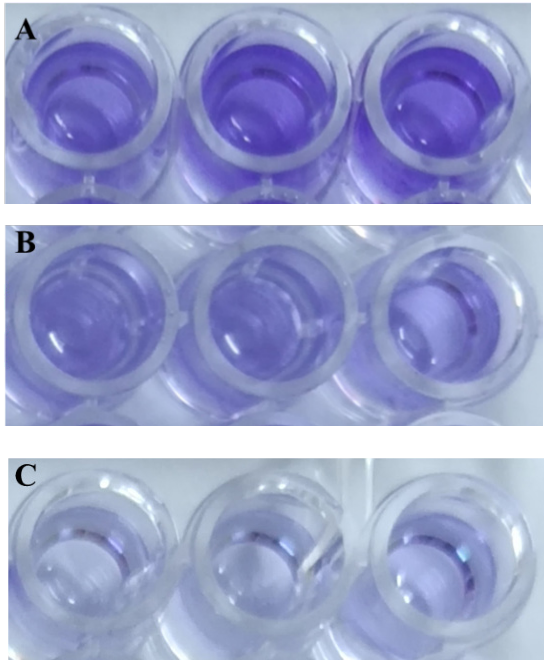

Fig.1. Microtitre plate assay for biofilm production.

A - Strong biofilm producer;

B - Moderate biofilm producer;

C - Weak or none biofilm producer

In the present study, 15 isolates were used for screening of 16S rDNA specific to *E. coli.* All the 15 isolates tested positive for

rrSH/16SrDNA gene. Further investigation was carried out for five genetic markers targeting biofilm associated genes in *E. coli viz. luxS* (100%), *csgA* (93%), *fimH* (93%) and *fimA* (73%).All the isolates were negative for *papC* gene. The results of the agarose gel electrophoresis were presented in Fig.2, 3, 4, 5 and 6.

All biofilm producers harbored *luxS* gene which is responsible for quorum sensing properties of microorganisms. In this study, strong biofilm producers were not positive for all the genes but positive for two genes (RCE1) or three genes (RCE6). All the isolates were positive and possessed biofilm producing genes, such as *luxS, csgA, fimH*, and *fimA*. Similar results were reported in a study conducted on retail meat products (Barilli *et al.,* 2020). The primary elements to produce biofilm are csgA and fimH, which respectively produce curli fimbriae and type I fimbriae are crucial for the initiation of biofilm development.

The *luxS gene* was present in all the isolates and this *luxS*gene could also influence biofilm development by producing AI-2 signaling molecule (Niu *et al.,* 2013). The *luxS* gene plays a vital role to initiate cell to cell communication at an early stage of biofilm formation (Bhardwaj *et al.,* 2021). All the isolates were negative for *papC* gene, and less frequency of *papC* was recorded by other researchers(Stromberg *et al.,* 2017; Crecencio *et al.,* 2020). *E. coli* strains potentially able to form structured biofilm due to the presence of curli fimbriae, cellulose, and type I fimbriae–associated genes were isolated from meat purchased at supermarkets, and a large number of these were MDR (Barilli *et al.,* 2020).

Out of a total of 15*E.coli* isolates that were screened for antibiotic resistance gene *blaTEM*, four isolates tested positive for target gene. These four isolates that contained the gene *blaTEM,* had the potential to form strong biofilm. The result of the agarose gel electrophoresis was presented in Fig.7.The *blaTEM* gene was the most commonly detected gene responsible for the antibiotic resistance(Mohammedkheir *et al.,* 2024).

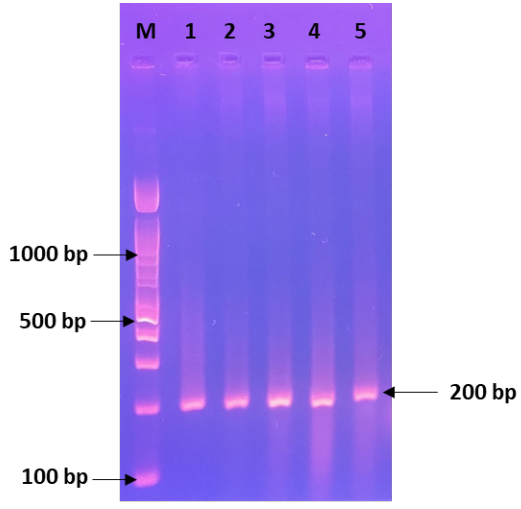

Fig. 2. 1.8% agarose gel electrophoresis for identification and confirmation of *E.coli* field isolates by PCR (16S rDNA). M- Marker 100bp, Lane 1 – 5 *E. coli* field isolates

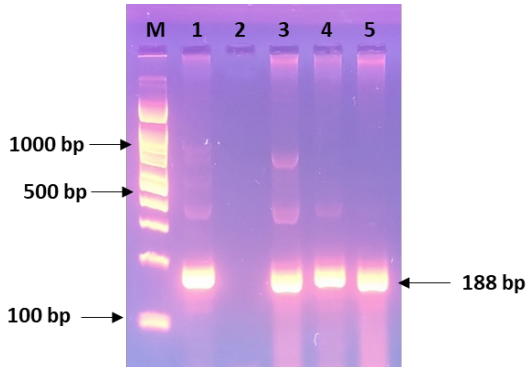

Fig. 3. 1.8 % Agarose gel electrophoresis for PCR products for detection of biofilm forming *E. coli* field isolates with *csgA* gene. M- Marker 100bp, Lane 1 – 5 *E. coli* field isolates

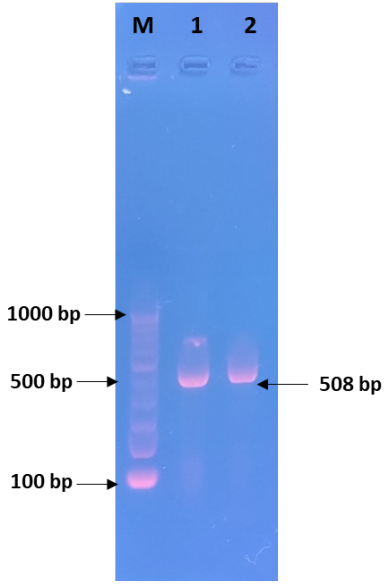

Fig. 4. 1.8 % Agarose gel electrophoresis for PCR products for detection of biofilm forming *E. coli* field isolates with *fimH* gene. M- Marker 100bp, Lane 1 – 2 *E. coli* field isolates

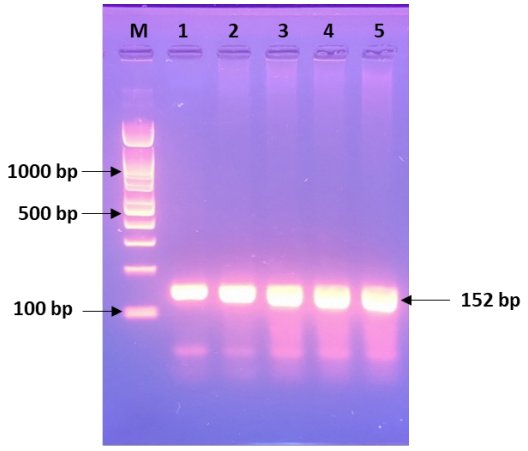

Fig. 5. 1.8 % Agarose gel electrophoresis showing PCR products *luxS* gene of *E. coli.*

M- Marker 100bp, Lane 1 – 5 *E. coli* field isolates

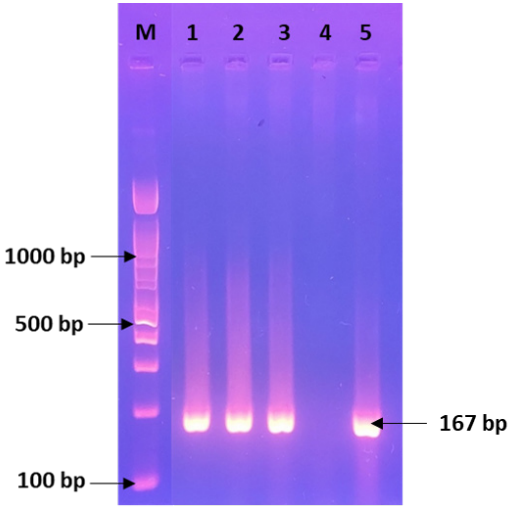

Fig. 6. 1.8 % Agarose gel electrophoresis *fimA* gene of *E. coli* M- Marker 100bp, Lane 1 – 5

E. coli field isolates

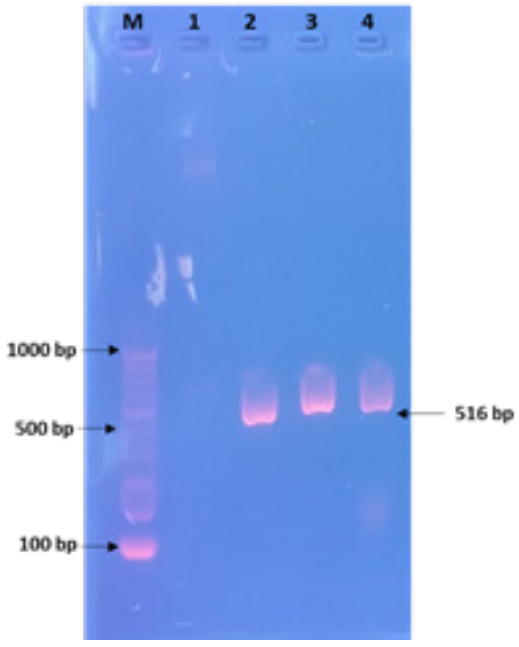

Fig. 7. 1.8 % Agarose gel electrophoresis *blaTEM* gene of *E. coli* M- Marker 100bp, Lane 1 – 4

E. coli field isolates

CONCLUSION

A high prevalence of *E. coli* in chicken meat samples was observed, indicating poor hygienic practices prevalent during processing. Majority of the isolates were strong biofilm producers. Our study confirmed that *luxS*, *csgA*, and *fimH* were responsible for biofilm formation in *E. coli* isolates. Further studies are required to investigate these genes for better understanding and prevent the biofilm formation in food industry. The strong biofilm forming bacteria have more resistance to antibiotics (β-lactamase).

REFERENCES

Alshammari, M., Ahmad, A., AlKhulaifi, M., Al Farraj, D., Alsudir, S., Alarawi, M., Takashi, G. and Alyamani, E. (2023). Reduction of biofilm formation of *Escherichia coli* by targeting quorum sensing and adhesion genes using the CRISPR/Cas9-HDR approach,

Table 2. Results of different adhesion genes detected in *E.coli* strains from chicken samples and biofilm formation characteristics

Sample No.	Different adherent genes for *E. coli*						*blaTEM* gene	Biofilm formation
	rrsH/16s rDNA	*luxS*	*csgA*	*fimH*	*fimA*	*papC*		
RCE1	+	+	+	+	-	-	-	Strong
RCE2	+	+	+	+	+	-	+	Strong
RCE3	+	+	+	+	+	-	-	Weak
RCE4	+	+	-	+	+	-	-	Weak
RCE5	+	+	+	+	+	-	-	Moderate
RCE6	+	+	+	-	-	-	-	Strong
RCE5	+	+	+	+	+	-	+	Strong

Sample No.	Different adherent genes for *E. coli*						*blaTEM* gene	Biofilm formation
	rrsH/16s rDNA	*luxS*	*csgA*	*fimH*	*fimA*	*papC*		
RCE6	+	+	+	+	+	-	+	Strong
RCE7	+	+	+	+	+	-	-	Moderate
RCE8	+	+	+	+	+	-	-	Weak
RCE9	+	+	+	+	+	-	-	Weak
RCE10	+	+	+	+	+	-	+	Strong
RCE11	+	+	+	+	+	-	-	Strong
RCE12	+	+	+	+	-	-	-	Moderate
RCE13	+	+	+	+	-	-	-	Moderate

and its clinical application on urinary catheter. *Journal of Infection and Public Health*, **16**(8): pp.1174 – 1183. [Online]. Available at: doi:10.1016/j.jiph.2023.05.026.

Barilli, E., Vismarra, A., Frascolla, V., Rega, M. and Bacci, C. (2020). *Escherichia coli*strains isolated from retail meat products: evaluation of biofilm formation ability, antibiotic resistance, and phylogenetic group analysis. *Journal of Food Protection*, **83**(2): pp.233 –240. [Online]. Available at: doi:10.4315/0362-028X.JFP-19-330.

Bhardwaj, D. K., Taneja, N. K., Dp, S., Chakotiya, A., Patel, P., Taneja, P., Sachdev, D., Gupta, S. and Sanal, M. G. (2021). Phenotypic and genotypic characterization of biofilm forming, antimicrobial resistant, pathogenic *Escherichiacoli* isolated from Indian dairy and meat products. *International Journal of Food Microbiology*, **336:** p.108899. [Online]. Available at: doi:10.1016/j.ijfoodmicro.2020.108899.

Crecencio, R. B., Brisola, M. C., Bitner, D., Frigo, A., Rampazzo, L., Borges, K. A., Furian, T. Q., Salle, C. T. P., Moraes, H. L. S., Faria, G. A., et al. (2020). Antimicrobial susceptibility, biofilm formation and genetic profiles of *Escherichia coli* isolated from retail chicken meat. *Infection, Genetics and Evolution*, **84:** p.104355. [Online]. Available at: doi:10.1016/j.meegid.2020.104355.

Eid, S. and Samir, A.H. (2019). Extended-spectrum beta-lactamase and Class 1 integrons in multidrug-resistant *Escherichiacoli* isolated from turkeys. *Veterinary World*, **12**(7): pp.1167 – 1174. [Online]. Available at: doi:10.14202/vetworld.2019.1167-1174.

Galié, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J. and Lombó, F. (2018). Biofilms in the food industry:

health aspects and control methods. *Frontiers in Microbiology*, **9:** p.898. [Online]. Available at: doi:10.3389/fmicb.2018.00898.

Hussain, A., Shaik, S., Ranjan, A., Nandanwar, N., Tiwari, S. K., Majid, M., Baddam, R., Qureshi, I. A., Semmler, T., Wieler, L. H., et al. (2017). Risk of transmission of antimicrobial resistant *Escherichiacoli* from commercial broiler and free-range retail chicken in india. *Frontiers in Microbiology*, **8:** p.2120. [Online]. Available at: doi:10.3389/fmicb.2017.02120.

Hussain, A., Shaik, S., Ranjan, A., Suresh, A., Sarker, N., Semmler, T., Wieler, L. H., Alam, M., Watanabe, H., Chakravortty, D., et al. (2019). Genomic and functional characterization of poultry *Escherichiacoli* from India revealed diverse extended-spectrum β-lactamase-producing lineages with shared virulence profiles. *Frontiers in Microbiology*, **10:** Frontiers. [Online]. Available at: doi:10.3389/fmicb.2019.02766 [Accessed 7 May 2024].

Katongole, P., Nalubega, F., Florence, N. C., Asiimwe, B. and Andia, I. (2020). Biofilm formation, antimicrobial susceptibility and virulence genes of uropathogenic *Escherichiacoli* isolated from clinical isolates in Uganda. *BMC infectious diseases*, **20**(1): p.453. [Online]. Available at: doi:10.1186/s12879-020-05186-1.

Khalifa, S. M., El-Aziz, A. M. A., Hassan, R. and Abdelmegeed, E.S. (2021). β-lactam resistance associated with β-lactamase production and porin alteration in clinical isolates of *E. coli* and *K. pneumoniae*. *PLOS ONE*, **16**(5): Public Library of Science., p.e0251594. [Online]. Available at: doi:10.1371/journal.pone.0251594.

Mohammedkheir, M. I. A., Gaafar, E. M. and AbdAlla, E. G. E. (2024). Assessment of BlaTEM, BlaSHV, and BlaCTX-M genes of antibiotic resistance in Gram-negative bacilli causing urinary tract infections in Khartoum State: a cross-sectional study. *BMC Infectious Diseases*, **24**(1): BioMed Central., pp.1–7. [Online]. Available at: doi: 10.1186/s12879 -024-09023-7.

Niu, C., Robbins, C. M., Pittman, K. J., Osborn, joDi L., Stubblefield, B. A., Simmons, R. B. and Gilbert, E. S. (2013). LuxS influences *Escherichia coli* biofilm formation through autoinducer-2-dependent and autoinducer-2-independent modalities. *FEMS Microbiology Ecology*, **83**(3): pp.778 – 791. [Online]. Available at: doi:10.1111/1574-6941.12034.

Obe, T., Nannapaneni, R., Schilling, W., Zhang, L. and Kiess, A. (2021). Antimicrobial tolerance, biofilm formation, and molecular characterization of *Salmonella* isolates from poultry processing equipment. *Journal of Applied Poultry Research*,

30(4): p.100195. [Online]. Available at: doi:10.1016/j.japr.2021.100195.

ÖzdemiR, F. and Arslan, S. (2021). Molecular Characterization and Biofilm Formation of Escherichia coli from Vegetables. *Sakarya University Journal of Science*, **25**(1): pp.12 –21. [Online]. Available at: doi:10.16984/saufenbilder.793400.

Senapati, I. A., Mishra, R., Kundu, A. K., Mishra, B. P. and Rath, P. K. (2020). Prevalence and characterization of *Escherichiacoli* from poultry meat in Bhubaneswar. *International Journal of Current Microbiology and Applied Sciences*, **9**(9): pp.2047 – 2055. [Online]. Available at: doi:10.20546/ijcmas.2020.909.255.

Sharma, G., Sharma, S., Sharma, P., Chandola, D., Dang, S., Gupta, S. and Gabrani, R. (2016). Escherichia coli biofilm: development and therapeutic strategies. *Journal of Applied Microbiology*, **121**(2): pp.309 – 319. [Online]. Available at: doi:10.1111/jam.13078.

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L. and Aalinkeel, R. (2023). Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, **11**(6): p.1614. [Online]. Available at: doi:10.3390/microorganisms11061614.

Stromberg, Z. R., Johnson, J. R., Fairbrother, J. M., Kilbourne, J., Van Goor, A., Curtiss, R. and Mellata, M. (2017). Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. *PLoS ONE*, **12**(7): p.e0180599. [Online]. Available at: doi:10.1371/journal.pone.0180599.

Thakur, P., Chawla, R., Tanwar, A., Chakotiya, A. S., Narula, A., Goel, R., Arora, R. and Sharma, R. K. (2016). Attenuation of adhesion, quorum sensing and biofilm mediated virulence of carbapenem resistant *Escherichia coli* by selected natural plant products. *Microbial Pathogenesis*, **92:** pp.76 – 85. [Online]. Available at: doi:10.1016/j.micpath.2016.01.001.

Vysakh, A., Midhun, S. J., Jayesh, K., Jyothis, M. and Latha, M. S. (2018). Studies on biofilm formation and virulence factors associated with uropathogenic Escherichia coli isolated from patient with acute pyelonephritis. *Pathophysiology: The Official Journal of the International Society for Pathophysiology*, **25**(4): pp.381 – 387. [Online]. Available at: doi:10.1016/j.pathophys.2018.07.004.

Wang, Y., Yi, L., Wang, Y., Wang, Y., Cai, Y., Zhao, W. and Ding, C. (2016). Isolation, phylogenetic group, drug resistance, biofilm formation, and adherence genes of Escherichia coli from poultry in central China. *Poultry Science*, **95**(12): pp.2895 – 2901. [Online]. Available at: doi:10.3382/ps/pew252.