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Prevalence and Molecular Characterization of Multidrug-resistant ESBL-producing *E. coli* in Commercial Poultry

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ABSTRACT

Background: Rural poultry farmers are gradually changing from the backyard poultry production to intensive production, which made poultry more vulnerable to diseases and lead to indiscriminate use of antimicrobials in poultry production. As per OIE, FAO and WHO, multiple spread antimicrobial resistant pathogenic bacteria are a serious global human and animal health problem. Hence the study has been carried out to know the antimicrobial resistance status of poultry in Tirunelveli, Tamil Nadu, India.

Methods: The study was aimed to find the occurrence of extended spectrum beta lactamases (ESBLs) producing *E. coli* from poultry origin. Caecum samples from the broiler (n=180), desi chicken (n=180) and Japanese quail (n=180) were processed for the isolation and phenotypic identification of ESBL *E. coli*. The isolates were tested for antimicrobial susceptibility test and characterization of ESBL and virulence genes done by PCR.

Result: On phenotypic confirmation of ESBL producing *E. coli* by combined disk diffusion test (CDDT), a total of 9.63% samples were positive for extended spectrum beta lactamase producer (ESBL). Multi drug resistance (MDR) was observed in 38.23% of the broiler isolates, 27.27% and 57.14% in desi chicken and Japanese quail isolates, respectively. All the phenotypically confirmed ESBL producing *E. coli* isolates (n=52) were positive for *uspA* gene by PCR confirming the isolates as *E. coli*. The overall presence of the *blaTEM* gene was 57.69%, with broiler isolates having the highest prevalence of 67.47%, followed by desi chicken 36.36% and Japanese quail 42.85%. In contrast, the *blaSHV* gene was found in only 17.64% of the broiler isolates, but none were positive for the *blaCTXM* and *blaOXA-1* gene.

Key words: Antimicrobial resistance, *E. coli*, ESBL, PCR, Poultry.

INTRODUCTION

Poultry meat is preferred more by peoples all over the world because of its low market price, nutritional value and less space requirement for production (Kalakuntla *et al*., 2017). Antimicrobials are injudiciously used in poultry production for the purpose of prevention of chick mortality, treatment and as growth promoters in feed. The WHO has classified extended spectrum β-lactamase (ESBL) producing *E. coli* as one of the important AMR pathogens to human health and a major public health concern. The prevalence of ESBL-producing *E. coli* in hospitals, livestock and poultry has increased globally (Hu *et al*., 2019). ESBL producing organisms are more often noticed in the gram-negative bacteria of Enterobacteriaceae family. The bacteria commonly involved in production of extended spectrum β-lactamase particularly *E. coli* and *K. pneumoniae* were reported in recent year all over the world including in India (Lalzampuia *et al*., 2013). ESBL organism may act as a potential source for transfer of resistance to other organism, intern these organisms are transmitted to susceptible host through contaminated water, environment and food chain *etc*. Several investigators have reported the occurrence of ESBL *E. coli* in different samples of chicken meat (Sivakumar *et al*., 2021); chevon meat (Bhoomika *et al*., 2016); raw milk (Chauhan *et al*., 2013); cloacal swabs of broilers (Shrivastav *et al*., 2016) and poultry fecal samples (Lalzampuia *et al*., 2013) from various states of India but not many reports are available on the occurrence of ESBL *E. coli* in poultry in Tamil Nadu, India. Hence, the study was undertaken to find out the occurrence, antimicrobial resistance pattern and their characterization of ESBL *E. coli* in different poultry species (Broiler, desi chicken and Japanese quail).

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MATERIALS AND METHODS

Sample collection

The present study was carried out during the year 2021-2023, at Department of Veterinary Pharmacology and Toxicology, VCRI, Tirunelveli, Tamil Nadu. A total of 540 caecum samples, 180 each from broilers, desi chicken and Japanese quail were collected randomly in aseptic polythene bag at different poultry meat retail outlets of Tirunelveli. Samples were processed at the laboratory or maintained at 4°C until further processing.

Isolation and phenotypic identification of ESBL *E. coli*

All the collected samples were processed for isolation of *E. coli* on MacConkey agar, EMB agar (HiMedia, India) followed by biochemical characterization (IMViC test) as per the standard methods. Biochemically characterized isolates of *E. coli* were cultured on MacConkey agar, which was supplemented with cefotaxime at 2 mg/L. Any growth on the plates was considered as ESBL producer and/or AmpC resistant *E. coli* (Costa *et al*., 2009). Further, isolates shown growth on these plates was also subjected to a disk diffusion assay with commercially available antimicrobial disc (HiMedia, India): cefotaxime (CTX, 30 µg), ceftriaxone (CTR, 30 µg), ceftazidime (CAZ, 30 µg) and cefepime (CPM, 30 µg). The isolates showing resistant to any of the above antimicrobials were further subjected for combination disc method for detection of ESBL production, which is based on the principle that the ESBL producing isolate will exhibit an expanded zone of inhibition against third or fourth generation cephalosporin in the presence of beta-lactamase inhibitor like clavulanic acid (CLSI, 2015).

Antimicrobial susceptibility testing

Phenotypically confirmed ESBL producing *E. coli* isolates were tested for antimicrobial susceptibility using six classes of twelve different commercially available antimicrobial discs such as Cefpodoxime (CPD-10 µg), Cefepime (CPM-30 µg), Ceftazidime (CAZ-30 µg), Cefperazone (CPZ-75 µg), Cefotaxime (CTX-30 µg), Ceftriaxone (CTR-30 µg), Amoxycillin+Clavulanic acid (AMC-20/10 µg), Co-trimoxazole (COT-1.25/23.75 µg), Oxytetracycline (O-30 µg), Gentamicin (GEN-10 µg), Enrofloxacin (EX-5 µg) and Chloramphenicol (C-30 µg) by Kirby and Bauer disk diffusion method (Hudzicki, 2009). The Clinical Laboratory Standards Institute (CLSI) guidelines were followed to measure the zone of inhibition and isolates were categorized accordingly as sensitive, intermediate and resistant.

Characterization of ESBL genes and virulence genes by PCR

All the phenotypically confirmed ESBL producing *E. coli* isolates were further confirmed by PCR amplification of *uspA* gene specific primers for *E. coli*. The *E.coli* isolates confirmed by PCR were further subjected to PCR amplification of important ESBL genes *viz*., bla_{TEM}, bla_{CTXM} and bla_{SHV} and *blaOXA-1*. Shiga toxin virulence genes *stx1* and *stx2* by using gene specific primers (Table 1). In brief, the bacterial DNA extraction from the isolates was done by Snap chill method (Mandal *et al*., 2017). The uniplex PCR assays were performed with reaction mixture comprised of 12.5 µL of 2X Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark), 1 µL (10 pM) of each primer, 2 µL of extracted DNA template and the final volume of 25 µL adjusted by addition of NFW and subjected to amplification in thermocycler (Eppendorf Mastercycler® nexus X2, Germany). The PCR cyclical conditions of *uspA*, bla_{TEM}, bla_{SHV}, bla_{CTXM} and bla_{OXA-1} were set at an initial denaturation of 94°C for 5 min followed by 35 cycles each of denaturation at 94°C for 30 sec, annealing at 60°C for 15 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min whereas for *stx1* and *stx2* initial denaturation at 94°C for 5 min followed by 35 cycles each of denaturation at 94°C for 1.5 min, annealing at 62°C for 1.5 min, extension at 72°C for 1.5 min and final extension at 72°C for 7 min. Electrophoresis was carried out in 1.5% agarose gel to visualize the PCR products and the images were captured by gel documentation system (Gelstan-1312).

DNA sequencing and phylogenetic analysis

Among the ESBL genes, only the bla_{TEM} gene from broiler, desi chicken and Japanese quail was subjected for gene sequencing and phylogenetic analysis. GeneJET gel extraction kit (Thermo Fisher scientific, USA) was used for purification of the amplified products from the excised gel as per manufacturer's directions. The purified DNA was sequenced using the same set of PCR primers at Eurofins Genomics India Pvt Ltd., Bangalore. bla_{TEM} gene sequences were submitted to NCBI database and obtained accession numbers. The present study sequences were compared with 11 distinct isolates of the *E. coli* bla_{TEM} gene from GenBank database. Multiple alignment and comparison of the study sequences with GenBank references were performed using clustal W. Further, phylogenetic tree construction and molecular evolutionary analysis were conducted using MEGA (Molecular Evolutionary Genetics Analysis) version 11.0 by neighbour joining method with maximum likelihood substitution model at 1000 boot straps replicates (Tamura *et al*., 2021).

Statistical analysis

All the statistical analyses were performed using SPSS computer software version 22. Chi-square analysis was performed to compare the isolation of *E. coli* and phenotypic confirmation of ESBL *E. coli* by CDDT for broiler, desi chicken and Japanese quail.

RESULTS AND DISCUSSION

Isolation and phenotypic identification of ESBL *E. coli*

In the present study, a total of 89.44% (483/540) *E. coli* isolates were isolated from caecum samples. Among them highest isolation rate was obtained in broiler 91.6% (165/180), followed by Japanese quail 89.4% (161/180) and

Table 1: Details of primers used in this study.

Target genes	Primer sequence (5′ to 3′)	Amplicon size (bp)	Reference
uspA	F: AATGCAGGCTACCCAATCAC	162	Kanokudom *et al*., 2021
	R: GGTGTTGATCAGCTGACGTG		
bla_{TEM}	F: TCCGCTCATGAGACAATAACC	931	Sturenburg *et al*., 2004
	R: TTGGTCTGACAGTTACCAATGC		
bla_{CTXM}	F: ATGTGCAGYACCAGTAARGTKATGGC	593	Hasman *et al*., 2005
	R: TGGGTRAARTARGTSACCAGAAYCAGCGG		
bla_{SHV}	F: TGGTTATGCGTTATATTCGCCC	868	Kim *et al*., 1998
	R: GGTTAGCGTTGCCAGTGCT		
bla_{OXA-1}	F- GCAGCGCCAGTGCATCAAC	198	Maynard *et al*., 2004
	R- CCGCATCAAATGCCATAAGTG		
stx1	F- CAGTTAATGTGGTGGCGAAGG	348	Cebula *et al*., 1995
	R- CACCAGACAATGTAACCGCTG		
stx2	F- ATCCTATTCCCGGGAGTTTACG	584	Cebula *et al*., 1995
	R- GCGTCATCGTATACACAGGAGC		

Table 2: Sample-wise isolation rate of *E. coli.*

Type of sample	Isolation rate of *E. coli*		No. of. samples collected
	Present	Absent	
Broiler ceacum	165 (91.67)	15 (8.33)	180 (100.00)
Desi chicken ceacum	157 (87.22)	23 (12.78)	180 (100.00)
Japanese quail ceacum	161 (89.44)	19 (10.56)	180 (100.00)

Chi-square test value = 1.88^{NS} (P value=0.39), ^{NS}- Non-significant (P≥0.05). Figures in parenthesis indicate percentage to the row total.

Table 3: Phenotypic characterization of ESBL *E. coli* by combined disc diffusion test (CDDT).

Type of sample	CDDT		No. of. samples Collected
	Positive	Negative	
Broiler ceacum	34 (18.89)	146 (81.11)	180 (100.00)
Desi chicken ceacum	11 (6.12)	169 (93.88)	180 (100.00)
Japanese quail ceacum	7 (3.89)	173 (96.11)	180 (100.00)

Chi-square test value = 27.11 (P value = 0.00), Significant (P≤0.01). Figures in parenthesis indicate percentage to the row total.

desi birds 87.2% (157/180). Chi-square analysis showed that no significant association (P≥0.05) between broiler, Desi chicken and Japanese quail for the isolation of *E. coli* (Table 2). The prevalence of *E. coli* in this study also indicates that poultry meat is significant sources of *E. coli* and potential risk factors for *E. coli* infection. Further, phenotypic confirmation of ESBL producing *E. coli* done by CDDT. A total of 9.63% (52/540) of isolates were ESBL producer in which broiler, desi chicken and Japanese quail were positive for 18.89% (34/180), 6.12% (11/180) and 3.88% (07/180), respectively. Chi-square analysis showed that the highly significant association (P<0.01) between broiler, desi chicken and Japanese quail for the phenotypic confirmation of ESBL *E. coli* by CDDT method (Table 3).

In agreement with this study, the phenotypic results of ESBL *E. coli* in poultry isolates were 5.22% (Lalzampuia *et al*., 2014); foods of animal origin and human clinical samples were 10.99% (Bhoomika *et al*., 2016). In contrast, existing investigations reported a higher prevalence rate of 30% in poultry droppings, (Durairajan *et al*., 2021) and 33.35% in healthy broilers (Shrivastav *et al*., 2016).

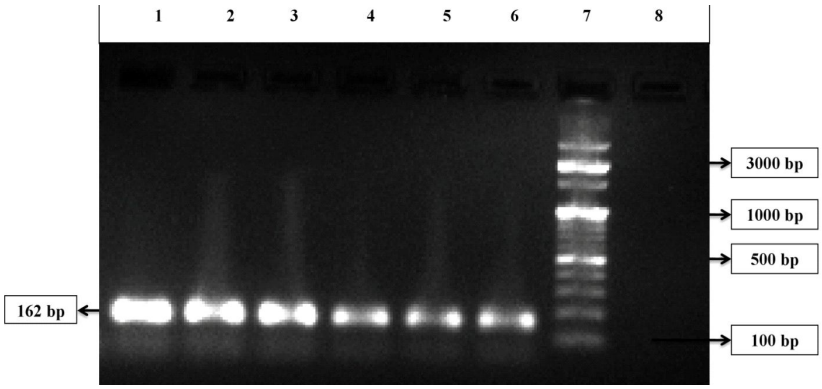
Antimicrobial susceptibility testing

All the ESBL isolates further subjected for antimicrobial testing of which highest resistance was observed to oxytetracycline, cefotaxime and cefperazone (100%) followed by ceftazidime (94.23%), cefpodoxime (88.46%), ceftriaxone (82.69%), chloramphenicol (76.92%) and low resistance in gentamicin (11.53) and enrofloxacin (9.61%). However, no resistance was observed in cefepime and amoxycillin+clavulanic acid. Resistance of antimicrobials obtained in this study in field conditions were also are reported by the different researchers (Bhoomika *et al*., 2016; Durairajan *et al*., 2021). Of the 52 isolates 38.46 % (20/52) of them were MDR isolates, including 38.23% (13/34), 27.27% (3/11) and 57.14% (4/7) isolates from broilers, desi chicken and Japanese quail, respectively. Rahman *et al*. (2020) observed the prevalence of MDR *E. coli* in broiler 78.17% and layer is 73.71% with overall prevalence of 75.06% in Bangladesh. The transmission of MDR *E. coli* in healthcare and food chain influenced by factors such as the incorrect usage of antimicrobials and inadequate hygiene practices. Thereby, consuming meat and meat products that contain MDR *E. coli* increases the risk of human infection (Adzitey *et al*., 2021).

Characterization of ESBL genes and virulence genes by PCR

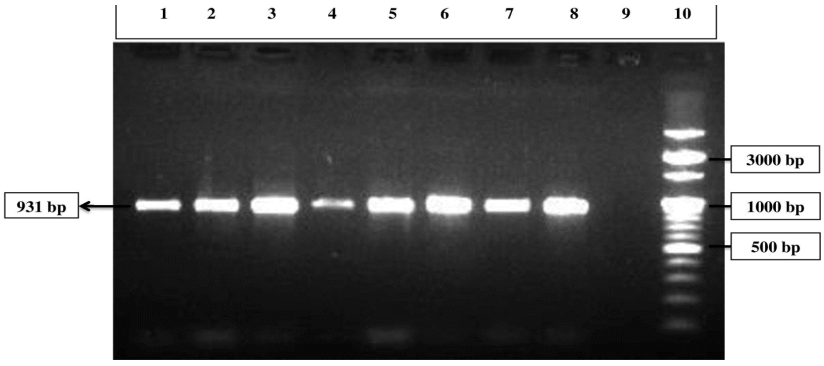
All the phenotypically confirmed 52 isolates of broiler (n=34), desi chicken (n=11) and Japanese quail (n=7) were subjected to PCR for genotypic detection of *uspA* and ESBL genes *viz*. bla_{TEM}, bla_{SHV}, bla_{CTXM}, $bla_{OXA-1,}$ *stx1* and *stx2*. The presence of *uspA* gene were 100% (52/52), of which 100%

in broiler (34/34), desi chicken (11/11) and Japanese quail (7/7) respectively (Fig 1). The overall presence of bla_{TEM} gene were 57.69% (30/52), of which predominant in broiler isolates 67.47% (23/34) followed by 42.85% (3/7) in Japanese quail and 36.36% (4/11) in desi chicken (Fig 2). Whereas bla_{SHV} gene detected in 17.64% (6/34) only from broiler isolates (Fig 3) and none of the broiler, desi chicken and Japanese quail isolates were positive for bla_{CTXM} gene



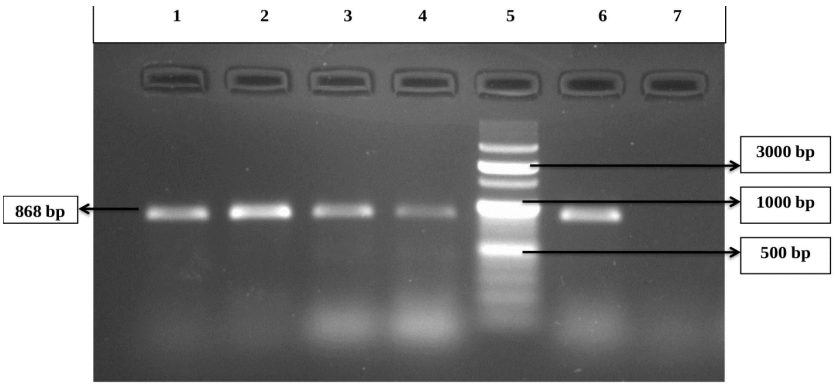
Lane-1 to 5 samples positive for *UspA* gene (162 bp); Lane-6: Positive control (ATC 2922); Lane-7: 100 bp DNA Ladder; Lane-8: Non template control.

Fig 1: PCR amplification of *Escherichia coli* specific *uspA* gene.



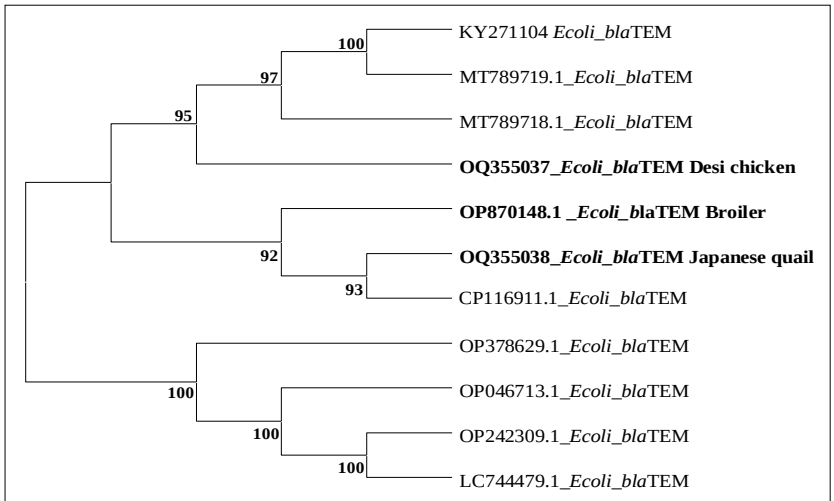
Lane-1 to 8: Positive for blaTEM gene (931 bp); Lane-9: Non template control; Lane-10: 100 bp DNA ladder.

Fig 2: PCR amplification of ESBL producing *Escherichia coli* bla_{TEM} gene.



Lane-1,2,3,4,6: Positive for blaSHV gene (868bp); Lane-5: 100 bp DNA Ladder; Lane-7: Non template control.

Fig 3: PCR amplification of ESBL producing *Escherichia coli* bla_{SHV} gene.



The tree was obtained by Neighbor-joining method with 1000 bootstrap replicates using MEGA 11. The representative sequences of the three isolates (OP870148, OQ355037 and OQ355038) from this study are highlighted bold.

Fig 4: The phylogenetic tree is based on partial nucleotide sequences of *E. coli* bla_{TEM} gene.

and bla_{OXA-1} gene. Samanta *et al*. (2015) in poultry observed that bla_{TEM}, bla_{CTXM} and bla_{SHV} detected in 21.7%, 34.7% and 43.4% of the isolates respectively. In contrast, Durairajan *et al*. (2021) observed in poultry droppings that 50% of the isolates pose the bla_{CTXM} gene and bla_{TEM} genes, but bla_{SHV} genes were detected in none of the isolates. Screening of stx1 and stx2 gene specific PCR revealed none of the isolates were found positive for shiga toxin which was similar to the study conducted by (Sivakumar *et al*., 2021).

DNA Sequencing and phylogenetic analysis

In this study, bla_{TEM} genes were submitted to the NCBI database and received accession numbers OP870148, OQ355037and Q355038 respectively. Phylogenetic analysis revealed that the aforesaid accession numbered genes shared an evolutionary relationship with other bla_{TEM} genes from different *E. coli* serogroups reported. Even though all these isolates were descended from a common ancestor, isolates OP870148, OQ355038 are closely related and clustered in the same clade (Fig 4). Furthermore, Isolate OQ355038 was clustered with CP116911.1 sequence from an *E. coli* that showed resistance against third-generation cephalosporin (Pankok *et al*., 2022).

CONCLUSION

The overall isolation rate of ESBL *E. coli* in broiler, desi chicken and Japanese quail is 9.63%. The MDR- ESBL *E. coli* strains in poultry meat may transmit to humans by means of direct as well as indirect contact. This study highlighted the emergence of multidrug resistant ESBL *E. coli* in healthy poultry has potential threats to public. The continuous monitoring and surveillance of poultry, poultry products and their environment for the important food borne pathogens, antimicrobial resistance and virulence factors are necessary.

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Conflict of interest

On behalf of all authors there is no conflict of interest in publishing this research article.

REFERENCES

Adzitey, F., Huda, N. and Shariff, A.H.M. (2021). Phenotypic antimicrobial susceptibility of *Escherichia coli* from raw meats, ready-to-eat meats and their related samples in one health context. Microorganisms. 9(2): 326. doi: 10.3390/microorganisms9020326.

Bhoomika, S.S., Patyal, A., Gade, N.E. (2016). Occurrence and characteristics of extended-spectrum Beta-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. Vet. World. 9: 996-1000.

Cebula, T.A., Payne, W.L. and Feng, P. (1995). Simultaneous identification of strains of *Escherichia coli* serotype O157: H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. Journal of Clinical Microbiology. 33(1): 248-250.

Chauhan, S., Farooq, U., Singh, V. and Kumar, A.J.A.Y. (2013). Determination of prevalence and antibacterial activity of ESBL (Extended Spectrum Beta-lactamases) producing *Klebsiella* species isolated from raw milk of Doon Valley in India. Int. Pharma. Bio. Sci. 4(1): 417-23.

CLSI, (2015). Performance standards for antimicrobial susceptibility testing, Clinical and Laboratory Standard Institute. CLSI Document, Wayne, PA. pp. 108-111 and 132-134.

Costa, D., Vinué, L., Poeta, P., Coelho, A. C., Matos, M., Sáenz, Y. and Torres, C. (2009). Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers. Veterinary Microbiology. 138(3-4): 339-344.

Durairajan, R., Murugan, M., Karthik, K. and Porteen, K. (2021). Farmer's stance on antibiotic resistance to *E. coli* and extended spectrum-β-lactamase producing (ESBL) *E. coli* isolated from poultry droppings. Asian Journal of Dairy and Food Research. 40(1): 88-93. Doi: 10.18805/ajdfr. DR-1574.

Hasman, H., Mevius, D., Veldman, K., Olesen, I. and Aarestrup, F.M. (2005). β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. Journal of Antimicrobial Chemotherapy. 56(1): 115-121.

Hu, Y.J., Ogyu, A., Cowling, B.J., Fukuda, K. and Pang, H.H. (2019). Available evidence of antibiotic resistance from extended-spectrum β-lactamase-producing Enterobacteriaceae in paediatric patients in 20 countries: A systematic review and meta-analysis. Bulletin of the World Health Organization. 97(7): 486-501B.

Hudzicki, J. (2009). Kirby-bauer disk diffusion susceptibility test protocol. American Society for Microbiology. 15: 55-63.

Kalakuntla, S., Nagireddy, N.K., Panda, A.K., Jatoth, N., Thirunahari, R. and Vangoor, R.R. (2017). Effect of dietary incorporation of n-3 polyunsaturated fatty acids rich oil sources on fatty acid profile, keeping quality and sensory attributes of broiler chicken meat. Animal Nutrition. 3(4): 386-391.

Kanokudom, S., Assawakongkarat, T., Akeda, Y., Ratthawongjirakul, P., Chuanchuen, R. and Chaichanawongsaroj, N. (2021). Rapid detection of extended spectrum β-lactamase producing *Escherichia coli* isolated from fresh pork meat and pig cecum samples using multiplex recombinase polymerase amplification and lateral flow strip analysis. PloS one. 16(3): e0248536. doi: 10.1371/Journal.pone. 0248536.

Kim, J., Kwon, Y., Pai, H., Kim, J.W. and Cho, D.T. (1998). Survey of *Klebsiella pneumoniae* strains producing extended-spectrum β-lactamases: Prevalence of SHV-12 and SHV-2a in Korea. Journal of Clinical Microbiology. 36(5): 1446-1449.

Lalzampuia, H., Dutta, T.K., Warjri, I. and Chandra, R. (2013). PCR-based detection of extended-spectrum β-lactamases (bla CTX-M-1 and bla TEM) in *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* isolated from pigs in North Eastern India (Mizoram). Indian Journal of Microbiology. 53: 291-296.

Lalzampuia, H., Dutta, T.K., Warjri, I. and Chandra, R. (2014). Detection of extended-spectrum? -lactamases (blaCTX-M-1 and blaTEM. Veterinary World. 7(11).

Mandal, A., Sengupta, A., Kumar, A., Singh, U.K., Jaiswal, A.K., Das, P. and Das, S. (2017). Molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* pathotypes in diarrheal children from low socioeconomic status communities in Bihar, India: Emergence of the CTX-M Type. Infectious Diseases: Research and Treatment. 10: 1178633617739018. doi: 10.1177/1178633617739018.

Maynard, C., Bekal, S., Sanschagrin, F., Levesque, R.C., Brousseau, R., Masson, L. and Harel, J. (2004). Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. Journal of Clinical Microbiology. 42(12): 5444-5452.

Pankok, F., Fuchs, F., Loderstädt, U., Kaase, M., Balczun, C., Scheithauer, S., Frickmann, H. and Hagen, R.M. (2022). Molecular epidemiology of *Escherichia coli* with resistance against third-generation cephalosporines isolated from deployed german soldiers-a retrospective assessment after deployments to the African sahel region and other sites between 2007 and 2016. Microorganisms. 10(12): 2448. doi: 10.3390/microorganisms10122448.

Rahman, M.M., Husna, A., Elshabrawy, H.A., Alam, J., Runa, N.Y., Badruzzaman, A.T.M. and Ashour, H.M. (2020). Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. Scientific Reports. 10(1): 21999. doi: 10.1038/s41598-020-78367-2.

Samanta, I., Joardar, S.N., Das, P.K. and Sar, T.K. (2015). Comparative possession of Shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in *Escherichia coli* isolated from backyard and farmed poultry. Iranian Journal of Veterinary Research. 16(1): 90-3.

Shrivastav, A., Sharma, R.K., Sahni, Y.P., Shrivastav, N., Gautam, V. and Jain, S. (2016). Study of antimicrobial resistance due to extended spectrum beta-lactamase-producing *Escherichia coli* in healthy broilers of Jabalpur. Veterinary World. 9(11): 1259-1263.

Sivakumar, M., Abass, G., Vivekanandhan, R., Singh, D.K., Bhilegaonkar, K., Kumar, S., Grace, M.R. and Dubal, Z. (2021). Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant *Escherichia coli* in street foods: A public health concern. Journal of Food Science and Technology. 58: 1247-1261.

Sturenburg, E., Lang, M., Horstkotte, M.A., Laufs, R. and Mack, D. (2004). Evaluation of the MicroScan ESBL plus confirmation panel for detection of extended-spectrum β-lactamases in clinical isolates of oxyimino-cephalosporin-resistant Gram-negative bacteria. Journal of Antimicrobial Chemotherapy. 54(5): 870-875.

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution. 38(7): 3022-7.