D-5400
[1-4]

RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Phenotypic Characterization and *bla* Gene Detection in Antibiotic Resistant *Escherichia coli* Obtained from Poultry Fecal Samples

R. Durairajan, M. Murugan

10.18805/ag.D-5400

ABSTRACT

Background: The current situation of multidrug resistant bacteria has become a worrisome issue in occurrence of disease outbreak. Multidrugresistant *E. coli* can be readily encountered in farms during daily clinical practice and veterinarian should act timely. Over past many reported increased incidences of Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli*. The introduction of ESBL-producing *E. coli* from poultry farms to the environment may pose a health risk if these bacteria reach places where people may become exposed and we report that different methods of identifications.

Methods: 60 fecal samples were collected from apparently healthy and infected poultry in the mixed age group. Bacterial isolates were identified on colony morphology and biochemical properties. ESBL producers were identified by using a combination disk diffusion method. ESBL positive isolates were further assessed using conventional polymerase chain reaction (PCR) to detect the TEM, SHV and CTX-M genes.

Result: In the present study, 40 isolates of *E.coli* were isolated from 60 faecal samples. Out of 40 isolates, 12 isolates were resistant to two or more than two antibioticsand were positive for ESBL by combination disc test. CTX-M genes were detected in 6 (50%) isolates. TEM and SHV were not detected in any of the isolates. This study showed high resistance of *E. coli* to antibiotics, particularly in the third generation cephalosporins but were more susceptible to Gentamicin, Aztreonarm, Cefrtrazindime and Cefotoxime. Laboratory monitoring and detection of *E. coli* of ESBL producing bacteria important steps in the appropriate treatment for farm based poultry industry and infection control efforts.

Key words: Antibiotic resistance, *bla* genes, *E. coli,* Poultry droppings.

INTRODUCTION

Extended spectrum-β-Lactamase (ESBL) producing bacteriaare a major threat to public health threat worldwide. The emergence of ESBL is due to improper use of anti biotics both in veterinary and human medicine (Geidam *et al*., 2014; Alisadi *et al*., 2015.). Antibiotics are widely used inpoultry and livestock breeding to improve growth and feed efficiency and reduce the disease outbreaks. Most of the bacterial pathogens associated with human diseases derived from animals and occurs indirectly through eggs, chicken meat and contaminated water, *etc*., (Donoghue, 2003; Kamini *et al.,* 2012; Boamah *et al*., 2016; Newel *et al.,* 2010.). β-Lactamase encoded by plasmids and transmitted between different bacterial species, which promotess increase emergence of ESBL strains. The proper methods for ESBL detection raise serious concern due to treatment failure of 3rd generation cephalosporins and Aztreonam Nordman *et al*. (2011). Currently, there is paucity of information on ESBL producing *E. coli* from poultry droppings and the possible contribution of these resistant species to the ever growing antimicrobial resistance observed in humans. Therefore, the present study demonstrates to detect ESBL producing *E.coli* from healthy poultry of small-scale farmers.

Veterinary University Training and Research Centre, Tamil Nadu Veterinary and Animal Sciences University, Melmaruvathur-603 319, Tamil Nadu, India.

Corresponding Author: Durairajan, R., Veterinary University Training and Research Centre, Tamil Nadu Veterinary and Animal Sciences University, Melmaruvathur-603 319, Tamil Nadu, India. Email: duraivet2006@gmail.com

How to cite this article: Durairajan, R. and Murugan, M. (2022). Phenotypic Characterization and *bla* Gene Detection in Antibiotic Resistant *Escherichia coli* Obtained from Poultry Fecal Samples. Agricultural Science Digest. DOI: 10.18805/ag.D-5400.

Submitted: 02-06-2021 **Accepted:** 08-08-2022 **Online:** 19-08-2022

MATERIALS AND METHODS

Study materials

A total of 60 poultry farms with the age groups of 1-5 months was included in the study. The study was conducted from July 2019 to March 2020 by the Veterinary University Training and Research Centre, Melmaruvathur. Random sampling techniques were adopted to choose 60 poultry farms.

Sample collection

The faecal samples were collected by inserting a swab into the vent and gently swabbing the mucosal wall than the swab

was removed gently from the cloaca and placed the swab into sterile tubes containing enough enrichment medium (Mueller Hinton broth) to moisten and cover the end of the swabs and incubated at 37°C for 18-24 h.

Identification of pathogens

For identification of *E. coli* a loop-full culture from enrichment broth was taken and streaked onto MacConkey's lactose agar (HiMedia, India) and incubated at 37°C for 24 h. The suspected *E. coli* colonies, pink to red were picked up and further streaked on eosin methylene blue agar (HiMedia, India) and incubated at 37°C for 24 h. Dark-centered and flat colonies with metallic sheen were considered as *E. coli*. On microscopic examination motile long slender Gram negative bacilli on the gram staining were suggestive of *E. coli*. All the samples were subjected to biochemical charectrization as Catalase, Indole and Motility test to confirm as *E. coli.*

Antimicrobial susceptibility test

All the confirmed *E. coli* isolates were tested for their antimicrobial drug susceptibility test on Mueller-Hinton agar (MHA) (HiMedia, India) by the disc diffusion method (CLSI. 2012). The antibiotics used were oxytetracycline (30 µg), cefpodoxime (30 µg), enrofloxacin (30 µg), gentamicin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg) with Clavulanicacid (10 µg) and ceftazidime (30 µg) with Clavulanicacid (10 µg) (HiMedia, India). The diameter of the zones of complete inhibition was measured and compared with the zone size interpretation chart and was graded as sensitive, intermediate and resistant.

Detection of ESBL isolates by combined disc diffusion methods

All confirmed *E. coli* isolates were tested for their antibiotic sensitivity pattern on Mueller-Hinton agar (MHA) (HiMedia, India) by the disc diffusion method. Prepared the inoculums of the suspected test isolate and streak in the MHA plates and kept the plates for not more than 15 min for evaporation of excess media. The antibiotics used were oxytetracycline (30 µg), cefpodoxime (30 µg), Enrofloxacin (30 µg), gentamicin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg) with Clavunic acid (10 µg) andceftazidime (30 µg) with Clavunic acid (10 µg) (HiMedia, India). The diameter of the zones of complete inhibition was measured and compared with the zone size interpretation chart and was graded as sensitive, intermediate and resistant.

For detection of ESBL producing islolatesthe disks containing cefotaxime (30 µg) or ceftazidime (30 µg) alone and with clavulanic acid (10 µg) were placed diagonally with a distance of 25 mm, center to center. Incubate at 35°C for 16-18 hour. An increase of 5 mm (50%) or more in the zone of inhibition around the combined disk containing clavulanic acid than the corresponding disk with cefotaxime or ceftazidime is considered positive for ESBL production. Phenotypically confirmed ESBL-producing *E. coli* isolates were analyzed for the presence of genes encoding *bla* TEM, *bla* SHV and *bla* CTX-M by multiplex polymerase chain reaction (m-PCR) described by Monstein *et al* (2007). Primers used in this study for ESBL resistance and cyclic conditions for the polymerase reaction to detect ESBL resistance genesis given in Table 1 and 2.

RESULTS AND DISCUSSION

In the present study, 40 isloates of *E. coli* were isolated from 60 faecal samples. Among 40 isolates, 12 isolates were resistant to two or more than two antibiotics. The resistance in isolates was as follow (Table 3): Oxytetracycline (80%), Gentamicin (37.5%), Enrofloxacin (75%), Cefotoxime (62.5%), Ceftazidime (50%), Cefpodoxime (47.5%), Aztreonam (45%), Cefotoxime +Clavulanicacid (CEC) (25%)

Table 1: Primer details and amplicon size for detection of ESBL.

Resistance gene	Sequence(5′ to 3′)	Size (bp)
CTX-M	F: CGC TTT GCG ATG TGC AG	590
	R: ACC GCG ATA TCG TTGGT	
SHV	F: GAT GAA CGC TTT CCC ATG ATG	214
	R: CGC TGT TAT CGC TCA TGG TAA	
TEM	F: ATG AGT ATT CAA CAT TTC CG	847
	R: GTC ACA GTT ACC AAT GCT TA	

Table 2: Cyclic conditions for the ESBL resistance genes.

	Cycling conditions				
Primers	Initial denaturation	Denaturation	Annealing	Extension	Final extension
CTX-M	94°C for 5 min	94°C for 25 s	52°C for 40s	72°C for 50s	72°C for 6 min
		Repeat for 30 cycles			
SHV	94°C for 5 min	94°C for 60 s	61°C for 60s	72°C for 60s	72°C for 5 min
		Repeat for 35 cycles			
TEM	96°C for 5 min	96°C for 1 min	58°C for 1 min	72°C for 1min	72°C for 10 min
		Repeat for 35 cycles			

and Ceftazidime+Clavulanicacid (CAC) (25%). Among 40 isolates, 12 isolates were resistant to two or more than two antibiotics. The *E. coli* isolates were also reported by Jaulkar *et al*. (2011) and Durairajan *et al*. (2021). The indiscriminate use of antibiotics in mass production of poultry has promoted the emergence of antimicrobial resistance (AMR) *E. coli* in poultry. Tame *et al.*(2019) reported 46.9% of ESBL producers from fecal dropping of poultry. The molecular methods helped to confirm the presence of genes in *E. coli* which is regard to ESBL production. Out of 12 *E.coli* presumptive ESBL producers; 6 isolates have CTX-m genes in PCR (Fig 1) and the prevalence of CTX-m gene is 50%. There was no presence of Bla-SHV and Bla-TEM in the confirmed *E. coli* isolates. Though AMR is hot title for concerning in human and animal health (Schwarz *et al.,* 2001; Schink *et al.,* 2013; WHO 2014). The failure is in accordance with WHO survey (WHO, 2015B) where the majority of responsibilities indicated the need for a more rationale antimicrobial use (AMU) in veterinary medicines as the first priorities of actions. Incidence of beta lactamase producing *E. coli* is due to frequent administration of drug such as penicillin, cephalosporin, monobactum and carbapenam (Cheaito and Matar, 2014), which is associated with resistance to other type of antibiotics leading to multidrug resistance. Haldorsen (2011) reported plasmid mediated gene transfer is responsible for AMR and ESBL. The contamination of several resistant genes causes bacteria to be resistant most classes of antibiotics in both farm and hospital set up Allocati *et al.* (2013). From the study it was observed that *E.coli* isolates were more susceptible to Gentamicin, Aztreonarm, Cefrtrazindime and Cefotoxime. This report are similar with finding of Unal *et al*. (2017) and Tame *et al*. (2019). The high susceptibility of antibiotics mentioned above may be due to the fact that the drugs not like abused and not affordable by farmers. Also, Gentamicin and cefotoxime are available in injectable form only and because of pain and laborious to administration such antibiotics not likely to be used indiscriminately or substandard antibiotics in animal husbandry especially in poultry. Parent stocks used combination of antibiotics which simultaneously transmitted to progeny also. The genotypic charecterization are same as to Olowe *et al*. (2015) and Apka *et al.* (2010) who reported that none of the isolates were expressed Bla (TEM) genes for resistance to antibiotics. The percentage of ESBL genes observed in this study suggestive of the gene may be responsible for the production of ESBL enzymes that is resistant to most Beta lactam antibiotics. Sometimes multiples genes are responsible for production of ESBL enzymes than single gene alone. This implies that the antibiotics are useful in the treatment of infection caused by *E. coli* in particular area. Laboratory monitoring and detection of *E. coli* of ESBL producing bacteria important steps in the appropriate treatment for a farm based poultry industry and infection control efforts.

Table 3: Antimicrobial susceptibility test for *E.coli* isolates from poultry are shown.

Antibiotic	Disc contents (µg)	No. (%) inresistance *E.coli* (N=40)
Oxytetracycline (O)	30 µg	32 (80%)
Gentamicin (G)	30 µg	15 (37.5%)
Enrofloxacin (Ex)	30 µg	30 (75%)
Cefotoxime (CTX)	30 µg	25 (62.5%)
Ceftazidime (CTZ)	30 µg	20 (50%)
Cefpodoxime (CPD)	10 µg	19 (47.5)
Aztreonam (AT)	30 µg	18 (45%)
Ceftriaxone (CTR)	30 µg	11 (27.5%)
Cefotoxime + Clavulanicacid (CEC)	10 µg	10 (25%)
Ceftazidime+ Clavulanicacid (CAC)	10 µg	10 (25%)

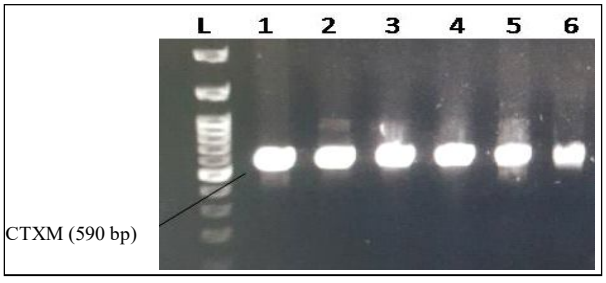

Fig 1: PCR assay detecting CTXM gene 590 bp.

CONCLUSION

The explosions of Indian population enhance the demand of poultry. The high human population needs higher polutry production for satisfying the animal nutritive nutritive protein requirements. The continuous use of antibiotics for treatment and antibiotics free feed and feed materials has also reduced the problem of multidrug resistence Indian soils and protect to poultry health. Screening and surveillance of *E. coli* for ESBL producing bacteria important steps in the appropriate treatment in farm based poultry industry and infection control efforts.

ACKNOWLEDGEMENT

The authors are grateful to the TANUVAS, Chennai, Tamil Nadu, India for providing necessary financial assistance to carry out this research programme.

Conflict of Interest: None.

REFERENCES

Aliasadi, S. and Dastmalchi Saei, H. (2015). Fecal carriage of *Escherichia coli* harboring extended-spectrum beta-lactamase (ESBL) genes by sheep and broilers in Urmia region, Iran. Iranian Journal of Veterinary Medicine. 9: 93-101.

Aloocati, N., Masulli, M., Alexe, V. and Illio, C.D. (2103). *Escherichia coli* in Europe: An overview. International Journal of Environment Public Health. 10: 6235-6254.

Apaka, P.E., Legall, B., Padman, J. (2010). Molecular detection and epidemiology of extended spectrum beta-lactamase genes prevalent in clinical isolates of *Kle bs iell apne umonae* and *E. coli* from Trinidad and Tobago. West Indian Medical Journal. 59(6): 591-596.

Boamah, V.E., Agyare, C., Odoi, H. and Dalsgaard, A. (2016). Practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. Journal of Antimicrobial Agent. 2(2): 1-8.

Cheaito, K. and Matar, G.M. (2014). The Mediterranean region: A reservoir for CTX-M-ESBL producing. Journal of Biological Sciences. 7(1): 1-6.

CLSI. (2012). Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement. M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Donoghue, D.J. (2003). Antibiotic residues in poultry tissues and eggs: Human health concerns. Poultry Science. 82(4): 618-621.

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

Geidam, Y.A., Ibrahim, H.A., Grema, K.A., Sanda, A., Suleiman and Mohzo. (2014). Patterns of antibiotic sales by drug stores and usage in poultry farms: A questionnaire-based survey in Maiduguri, Northeastern Nigeria. Journal of Animal and Veterinary Advances. 11(16): 2852-2855.

Haldorsen, B.C. (2011). Aminoglycosides resistance in clinical gram negative isolates from Norway (thesis). North Norway (NO). University of Troms.

Jaulkar, A.D., Zade, N.N., Katre, D.D., Khan, D.D., Chaudhary, S.P. and Shinde, S.V. (2011). Plasmid characterization of *Salmonella* isolated from foods of animal origin. Journal of Veterinary Public Health. 9(1): 25-28.

Kamini, M.G., Keutchatang, F.T., Mafo, H.Y., Kansci, G. and Nama, G.M. (2012). Antimicrobial usage in the chicken farming in Yaounde, Cameroon: A cross-sectional study. International. Journal of Food Contamination. 3(10): 1-6.

Monstein, H.J., Ostholm-Balkhed, A., Nilsson. M.V, Dornbusch, K., Nilsso, L.E. (2007). Multiplex PCR amplification assay for rapid detection of bla $_{SHV}$ bla $_{TEM}$ and bla $_{CTX-M}$ genes in enterobacteriaceae. A.P.M.I.S. 115(1): 400-408.

Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threfall, J., Scheutz, F., Van Der Giessen, J. and Kruse, H. (2010). Food borne diseases the challenges of 20 years ago still persist while new ones continue to emerge. International Journal of Food Microbiology. 139: 3-15.

Ngwai, Y.B., Iliyasu, H., Young, E., Owuna, G. (2012). Bacteriuria and antimicrobial susceptibility of *Escherichia coli* isolated from urine of asymptomaticuniversity students in Keffi. Nigerian Journal of Microbiology. 5(1): 323-327.

Nordmann, P., Poirel, L. (2011). Emerging carbapenemases in gram negative aerobes. Clinical Microbiology Infecion. 8: 321-31.

Olowe, O.A., Adewumi, O., Odewale, G., Ojurongbe, O., Adefioye, O.J. (2015). Phenotypic and molecular characterization of extended-spectrum beta-lactamase producing *Escherichia coli* obtained from animal fecal samples in Ado Ekiti, Nigeria. Journal of Environment and Public Health. 40: 243-245.

Schink, A.K., Kadlec, K., Kaspar, H., Mankertz, J., Schwarz, S. (2013). Analysis of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates collected in the GERM-Vet monitoring programme. Journal of Antimicrobial Chemotherapy. 68: 1741-1749.

Schwarz., S., Kehrenberg, C., Walsh, T.R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agent. 17: 431-437.

Tame, S.C., Ngwai,Y.B., Nkenel, I.H and Abimiku, R.H. (2019). Molecular detection of extended spectrum betalact amase resistance in *Escherichia coli* from poultry droppings in keffi, Nigeria. Asian Journal of Medical and Health.15(4): 1-9

Unal, N., Karagoz, A., Askar, S., Dilik, Z., Yurteri, B. (2017). Extended-spectrum β-lactamases among cloacal *Escherichia coli* isolates in healthy broilers in Turkey. Turkish Journal of Veterinary and Animal Science. 41(1): 72-76.

World Health Organization (2014). Antimicrobial Resistance: Global Report on Surveillance. World Health Organization, Geneva, Switzerland. Accessed on August 6, 2018. http:/ apps.who.int/iris/bitstream/10665/112642/1/ 97 89 2415 64748-eng.pdf.

World Health Organization. (2015b). Antibiotic resistance: Multi country public awareness survey. World Health organization, Geneva, Switzerland. Accessed on August 6, 2018. http:/ apps.who.int/iris/bitstream/10665/194460/1/97892 415 09817-eng.pdf?ua¼1.