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# Farmer's Stance on Antibiotic Resistance to *E. coli* and Extended Spectrum - β -lactamase Producing (ESBL) *E. coli* Isolated from Poultry Droppings

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## ABSTRACT

**Background:** This study was conducted to explore the contribution of poultry farms to the contamination of the environment with ESBL-producing *Escherichia coli* and there with, potentially to the spread of these bacteria to humans and other animals. Hence, the present work is a poultry farm based study aimed to detect prevalence of ESBL producing *E.coli* among poultry of small scale farmers.

**Methods:** ESBL-producing *E. coli* were detected at poultry farm (n=40). The *E. coli* was isolated from poultry droppings in irrespective of diseases. The required data were collected through well-structured interview schedule in farm premises. *E.coli* isolates were more susceptible to Gentamicin, Aztreonarm, Cefrtrazindime and Cefotoxime.

**Result:** Detection of ESBL isolates was performed by Combined Disc Diffusion Methods. Out of 40 *E.coli* isolates 12 were phenotypically identified as ESBL producers. The prevalence of CTX-m gene is 50% and Bla (TEM) gene is 50%.


**Key words:** Antibiotics, *E. coli*, ESBL, Poultry droppings, Resistance, Susceptibility.

## INTRODUCTION

Extended spectrum - β-Lactamase producing (ESBL) bacteria is a major threat to public health across the globe. The occurrence of ESBLs is due to improper usage of antibiotics both in animal husbandry practices and in human health care (Alisadi *et al*., 2015; Nalband *et al*., 2020). Thereby increasing the risk of emergence of resistance bacteria that can cause infections in animals and humans (Who 2014; Ngwai *et al*., 2012). Most of the bacterial pathogens associated with human illness originated from animals and indirectly through egg and chicken meat, contaminated water *etc*., (Kamini *et al.,*2012; Boamah *et al*., 2016; Newel *et al.,* 2010; Kanj *et al.,* 2011). Currently the appropriate methods for ESBL detections are seriously concerned because of failure of treatment of 3rd generation cephalosporins and Aztreonam (Nordman *et al*., 2011). Poultry industry also using different antibiotics for different purposes but reliable data about the quantity and pattern of usage such as dose and frequency of use is not available (Samrah *et al*., 2006 and Corriquen *et al.,* 2013). Due to scanty report to poultry and on the association of ESBL producing enteric bacteria in humans and foods of poultry origin as in the state of Tamilnadu. Therefore the present work is a poultry farm based study aimed to detect prevalence of ESBL producing *E. coli* among poultry of small scale farmers.

## MATERIALS AND METHODS

A total of 48 poultry farms with the age groups of 1-5 months were included in the study in and around Melmaruvathur, Tamilnadu. The study was conducted from July 2019 to March 2020 in the Veterinary University Training and Research centre, Melmaruvathur, Tamil Nadu-603 3, India. Random sampling techniques were adopted to choose 48 poultry farms. The required data were collected through well structured interview schedule in farm premises. Faecal samples (n=60) was collected in sterilized McConkey broth for enrichment at 37°C for 18-24 h. Further inoculate the growth of McConkey Lactose Agar (MLA) plates and incubate overnight at 37°C for 24 hrs. The typical lactose fermented colonies are picked and inoculated in Eosine methylene blue agar (EMBA) by streaking and incubated at 37°C for 24 hrs. All the samples were subjected to biochemical charectrization as Catalase, Indole and Motility test to confirm as *E.coli.*

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**Antimicrobial susceptibility test and multiple antibiotic resistances (MAR) index**

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 Clavunic acid (10 µg) and ceftazidime (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. The MAR Index was also calculated for all *E. coli* isolates, by applying formula a/b where "a" is the number of antibiotics to which an isolate was resistant and "b" is the number of antibiotics to which the isolates exposed (Krumperman. 1983).

**Detection of ESBL isolates by combined disc diffusion methods**

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. Placed the disks containing cefotaxime (30 µg) or ceftazidime (30 µg) alone and with clavulnic acid (10 µg) diagonally with a distance of 25 mm, center to center. An increase of 5 mm (50%) or more in the zone of inhibition around the combined disk containing clavulnic acid than the corresponding disk with cefotaxime or ceftazidime is considered positive for ESBL production. All the isolates of ESBL producing *E. coli* isolates were screened for the detection of *bla*TEM, *bla*SHV and *bla*CTX-M genes as described by Monstein *et al* (2007).

## RESULTS AND DISCUSSION

About 50 questionnaires were added, all questionnaires filled at the farmers premises. Farmer's perception towards antimicrobial usage pattern and knowledge on antimicrobial resistance were summarized in Table 1-4. The majority of farmers reported that antimicrobials are used fairly in their farms, with the recommendation of veterinarians (75% of farmers). Almost 90% of respondents reported that antimicrobial usage in their farms solved their problems. The results of the drug usage pattern in the study area are depicted in Table 5. Results shows that majority of farmers rearing chicken are in the age of 30-39 (16) and 40-49 (16) and most of them are male and they all are educated up to tertiary (44). Majority of farmers are married (33) and have other occupations (36) also. Most of them using medicine for treatment purpose only and they have prescribed by Veterinary Doctors (30) and by self (5). The finding of this study in accordance to the previous report (Kabir *et al*., 2011; Sridhar *et al.*,2012).

Farmer's views on antimicrobials are summarized in Table 1-4. Majority of farmers with regard to encourage of farms by AMU in farms reported that this is light (33) and few farms reported such found is high (15). 45 farmers is telling that AMU could be decreased and how much it could be promise to reduce AMU in their farms and reduction may be 20-30% possible was assured by farmers. Drug was administered through drinking water, similarly Ameichi (2014) and Kamini *et al.* (2016) also reported that most of the drug was administered through drinking water. The farmers following prescription from veterinarian were likely to be higher than the self-medication and they were purchase from drug store only. Similar results were observed by Krishnasamy *et al*. (2015) who observed that 50% farmers purchase medicine prescribed by farmers. In contrast, Bashhun and Odochi (2015) reported that 63.3% following paravet prescription only.

The main parameter may concentrate to veterinary AMU reduction according farmers opinion are feed quality improvement (30) and animal genetic improvement (7). Most of the farmers (45) reported that quality of meat and meat product not affected by the AMU and AMU in farms may

**Table 1:** Demographic characteristics of the poultry farmers.

| Characteristics | Particulars | Result |
|---|---|---|
| Age of respondents | 20-29 | 8 |
| | 30-39 | 16 |
| | 40-49 | 4 |
| | 50-59 | 16 |
| | 60 and above | 4 |
| Gender | Male | 44 |
| | Female | 4 |
| Farmer's level of education | Primary School and below | - |
| | Secondary | 4 |
| | Tertiary | 44 |
| Marital status | Single | 15 |
| | Married | 33 |
| Religion | Islam | - |
| | Christianity | - |
| | Others | 48 |
| Other occupations | Yes | 36 |
| | No | 12 |

**Table 2:** Farming characteristics in the poultry farms.

| Characteristics | Particulars | Result |
|---|---|---|
| Breed /type of poultry | Broilers | 4 |
| | Layers | 4 |
| | Others | 40 |
| Number of birds | < 50 | - |
| | 50-99 | - |
| | 100-199 | 8 |
| | 200 and above | 40 |
| Types of rearing | Intensive system | 9 |
| | Extensive system | 39 |
| Means of poultry | Use as manure | 48 |
| Waste disposal | Around the poultry farm | - |
| | Inside the stream | - |
| | Others | - |

**Table 3:** Medicine usage pattern in the selected farms.

| Variables | Particulars | Result |
|---|---|---|
| Use of antimicrobial agent on farm | Yes | 45 |
| Source of information about antimicrobial agent used | Veterinary doctor | 30 |
| | By self | 05 |
| | Animal health workers | 01 |
| | Through the seller | 02 |
| | Through a friend | 20 |
| Reason for antimicrobial usage | To prevent and treat disease | 41 |
| | To prevent diseases | - |
| | To treat diseases | 06 |
| | Promote growth of birds | - |
| | To prevent, treat and promote growth | 01 |
| Routes of administration to poultry | Through water | - |
| | Through water and injection | 01 |
| | Through water and food | - |
| | Through injection | - |
| | Through water, food and injection | 47 |
| Frequency of use of antimicrobial agent | Daily-once a week | 2 |
| | Once in two weeks- once a month | 2 |
| | When they are sick | 44 |
| | Others | - |

**Table 4:** Data on perception of farmers of antibiotic usage and AMR in farms.

| Variables | Particulars | Result |
|---|---|---|
| The quantity of antibiotics used in the farm is | Limited | 44 |
| | Fair, | - |
| | in line with veterinarians' recommendations | 03 |
| | Sometimes excessive | 01 |
| | Unknown | - |
| The outcome of antibiotic treatment is: | Always resolutive | 43 |
| | Often resolutive | 03 |
| | Sometimes resolutive | - |
| | Seldom resolutive | - |
| | Never resolutive | - |
| Antibiotic treatment affects the economy of the farm | Very heavily | - |
| | Heavily | 15 |
| | Only lightly | 33 |
| | Not at all | - |
| Antibiotic usage in the farms could be decreased: | Yes | 45 |
| | No | 03 |
| Antibiotic usage in the farm could be decreased by | 5-10% | 42 |
| | 20-30% | 06 |
| | 50% 5 | - |
| | >50% | - |
| | Unknown | - |
| The main factors that can contribute to decrease antibiotic use are: (possible more answers) | Animal genetic improvement | 07 |
| | Feed quality improvement | 30 |
| | Housing microclimate improvement | 02 |
| | Marketing of more effective drugs | 09 |
| | Other | - |

**Table 4: Continue...**

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| | | |
|---|---|---|
| Antibiotics may affect the quality of meat products: | Very heavily | - |
| | Heavily | - |
| | Only lightly | 03 |
| | Not at all | 45 |
| Antibiotic use in farms may influence human health: | Yes | 40 |
| | No | 08 |
| Factors that influence the development of antibiotic resistance in humans (possible more answers) | Use of antibiotics administered to humans when a health issue occurs | 04 |
| | Use of antibiotics administered in farms | 20 |
| | Ingestion of resistant bacteria present in contaminated fresh products | 24 |
| | Others | - |

**Table 5:** Antimicrobial susceptibility test for *E.coli* isolates from poultry are shown in.

| Antibiotic | Disc contents (µg) | No. (%) resistance in *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 + Clavunic acid (CEC) | 10 µg | 10 (25%) |
| Ceftazidime + Clavunic acid (CAC) | 10 µg | 10 (25%) |

**Table 6:** Primer details and amplicon size for detection of ESBL.

| Genes | Primers | Amplicon size (bp) |
|---|---|---|
| CTXMGp9_for | TCAAGCCTGCCGATCTGGT | 561 |
| CTXMGp9_rev | TGATTCTCGCCGCTGAAG | |
| TEM variants_for | CATTTCCGTGTCGCCCTTATTC | 800 |
| TEM variants_rev | CGTTCATCCATAGTTGCCTGAC | |
| SHV variants_for | AGCCGCTTGAGCAAATTAAAC | 713 |
| SHV variants_rev | ATCCCGCAGATAAATCACCAC | |

affects human health (40). The resistance in isolates were as follow: Oxytetracycline (80%), Gentamicin (37.5%), Enrofloxacin (75%), Cefotoxime (62.5%), Ceftazidime (50%), Cefpodoxime (47.5%), Aztreonam (45%), Cefotoxime +Clavunic acid (CEC) (25%) and Ceftazidime + Clavunic acid (CAC) (25%). Among 40 isolates, 12 isolates were resistant to two or more than two antibiotics and the highest MAR=0.7 and lowest by 2 isolates and the MAR index is 0.2. The prevalence of MAR in *E.coli* isolates was also reported by Jaulkar *et al*. (2011). The indiscriminate use of antibiotics in mass production of poultry has promoted the emergence of MAR *E.coli* in poultry. Out of 40 *E.coli* isolates 12 were phenotypically identified as ESBL producers. Tame *et al.* (2019) 46.9% of ESBL producers from faecal dropping of poultry. Out of 12 *E.coli* presumptive ESBL producers; 6 isolates either one or two genes in PCR. Tewari *et al*. (2019) were found same percentage of ESBL producing organism. The prevalence of CTX-m gene is 50% and Bla (TEM) gene is 50%. The interesting finding was the percentage of occurrence of Bla CTX-m. 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 (Schink *et al.,* 2013; WHO 2014). Prevalence of extended spectrum beta lactamase (ESBL) 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 (2014) and Dewangan *et al.* (2017) reported plasmid mediated gene transfer is responsible for AMR and ESBL. It was observed that *E. coli* isolates were more susceptible to Gentamicin, Aztreonarm, Cefrtrazindime and Cefotoxime. This finding is agreement with finding of Unal *et al*. (2017) and Tame *et al*. (2019). The high susceptibility of antibiotics 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 Kabir *et al*. (2014) or substandard antibiotics in animal husbandry especially in poultry. Some feed formulation may contain antibiotics and thus can change the microflora of the gut and these can transfer. However, farmers also replied that the use of antimicrobials would be decrease particularly in desichicken and broiler farms (Martino *et al*., 2018). Moreover feed and feed supplement and animal genetic improvement as the main factor can contribute to decrease AMU in poultry sector. However, integrated companies might also provide more efficient support and education campaigns to farmers in order to achieve specific targets on drug use reduction to satisfy consumer's demands. This hypothesis agrees with the results of Wei and Aengwanich (2012), which suggested that biosecurity levels of company-owned poultry farms were better than those of individual farms due to a harmonized policy of investments in farmers' education. The role farm veterinarian will be crucial in the years to come in

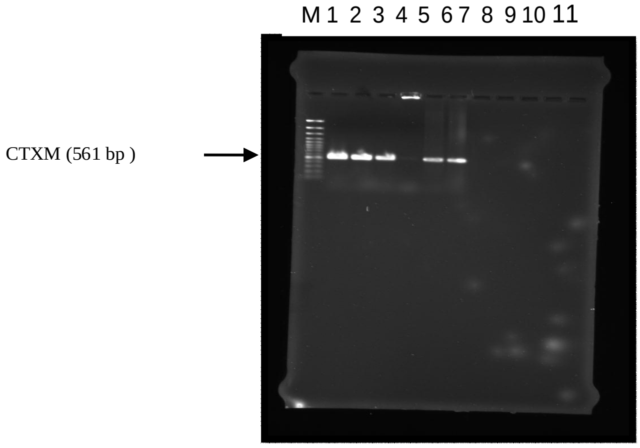


**Fig 1:** Multiplex PCR For detection of CTXM, TEM and SHV. Agarose gel image of PCR for detection of Extended spectrum - β-Lactamase producing (ESBL) bacteria. Lanes 2, 3, 4, 5, 6 and 7-positive amplification for CTXm gene (561 bp). Lane M-100 bp marker.

order to support farmer's education and expected transition to lower AMU, while maintaining high animal health and welfare standard. The molecular findings are similar to Olowe *et al*. (2015) and Apka *et al.* (2010) who reported that none of the isolates were expressed Bla (SHV) genes for resistance to antibiotics.

## CONCLUSION

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 in single gene is 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 farm based poultry industry and infection control efforts.

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