

ESBL producing *Escherichia coli* prevalence in chicken stools in selected broiler farms in Galle district

RMCS Karunarathna¹, WMDGB Wijyaratne², HA Ubeysekara³

¹Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka.

²Department of Microbiology, Faculty of Medicine, University of Ruhuna, Sri Lanka.

³Provincial Director of Health Office Southern Province, Ministry of Health, Sri Lanka.

Correspondence: Dr. W.M.D. Gaya Bandara Wijyaratne
e-mail: gayabw@yahoo.co.uk
 <https://orcid.org/0000-0002-8330-3597>

Dr. H.A. Ubeysekara
e-mail: hubysekara@yahoo.co.uk
 <https://orcid.org/0000-0002-1917-6845>
Submitted on 18.07.2021 and accepted for publication on 15.03.2022

ABSTRACT

Introduction: Most of the animals reared for food are reservoirs for Extended Spectrum Beta-lactamase (ESBL) producing coliforms. ESBL producing *Escherichia coli* (*E. coli*) in chicken meat are increasing worldwide. Since the consumption of chicken in meals is higher compared to other livestock in Sri Lanka, there is a threat to transmit these organisms to humans via food. The aim of this study was to determine the ESBL producing *E. coli* prevalence in chicken stools and the presence of antibacterial substances in chicken feed and water in selected broiler farms in Galle district

Methods: Eighty stool samples were collected from selected broiler farms in Galle district. Isolates were confirmed as *E. coli* on the lactose fermentation, colony morphology, Gram staining and biochemical tests. ESBL production was detected according to the CLSI double disc diffusion method. Ready to serve chicken feed and water samples were collected and tested against two types of *E. coli* control cultures using agar well diffusion method to detect the effects of any antibacterial substances.

Results: Eighty-six different types of *E. coli* were isolated from 80 chicken stool samples. There were no ESBL producers among all isolated *E. coli*. There was no antibacterial activity demonstrated in food or water served for chicken except for in one farm. The percentage distribution of fecal microbial flora as well as their density was altered in the farm where antibiotics were used in chicken feed.

Conclusions: No ESBL producing *E. coli* were detected in chicken stool in the selected broiler farms of Galle district indicating the minimum risk of transmission of ESBLs to the human via consumption of chicken meat.

Keywords: Broiler farms, chicken stools, ESBL producing *E. coli*, Sri Lanka.

Introduction

Escherichia coli (*E. coli*) is a large and diverse group of bacteria that belong to the family Enterobacteriaceae. It is found in the environment, food and intestines of humans and animals.

Most of the animals reared for food are reservoirs for extended spectrum beta-lactamase (ESBL) - producing coliforms out of which poultry farms are the prominent (1). It is assumed that meat can be potentially contaminated by the own fecal flora of animals. This happens when consuming ground meat without proper cooking adequate enough to kill the bacteria or when the meat is tainted by the intestinal bacteria during the course of processing. Using untreated milk or any milk product, which has not been heated sufficiently to kill bacteria could also be a cause of the contamination. Fresh vegetable and fruit harvests can be polluted with soil or water contaminated with animal excreta in the farm itself. Using water containing *E. coli* can also contaminate food. In the kitchen, food can get contaminated by cross contamination of uncooked meat with food that will be eaten raw (2).

Prevalence of ESBL-producing *E. coli* among livestock population is increasing day by day. The study done in Austria tested for multi resistant bacteria observed in chicken meat found that the ESBL producing coliforms were the predominant (42%) type followed by vancomycin resistant Enterococci (6%) without any methicillin resistant *Staphylococcus aureus* (0%) (3).

Recent data suggest that the transmission of ESBL producing *E. coli* from poultry to humans is most likely through the food chain. Cindy *et al.*, have found that 1/3 of chicken farmers were contaminated with ESBL positive *E. coli* (4).

A study done in the Netherlands tested raw meat samples and the faecal carriage by human rectal swabs in four hospitals in the same area. It revealed a higher prevalence of ESBL genes in chicken meat (79.8%) with identical predominant ESBL genes in chicken meat and human rectal swab specimens (5).

CTX-M-1 gene is the most prominent ESBL gene in poultry as well as in humans. In human *E. coli*, 35% are poultry associated ESBLs types (6).

E. coli can cause many intestinal and extra-intestinal diseases. ESBL positive *E. coli* make them multidrug resistant and cause severe infection in humans. Treatment of such infections is increasingly challenging leaving only a few sensitive antibiotics (7). Therefore, ESBL producing organism containing meat can be a threat to the human lives.

Consumption of chicken meat is higher when compared to other livestock in Sri Lanka. But there is no published data on the prevalence and burden of ESBL producing coliforms in livestock in Sri Lanka.

Hence, this study aims to detect the presence of ESBL producing *E. coli* in poultry by determining the prevalence of ESBL producing *E. coli* in chicken stools and to determine the presence of antibacterial substances in chicken feed and water in selected broiler farms in Galle district.

Methods

A descriptive cross-sectional study conducted among selected broiler chicken farms in Galle district. A. Sample size of 80 was calculated by the formula given by Charan and Biswas (8) using the prevalence (30.1%) of ESBL coliforms among gulls (*Leucophaeus pipixcan*) found in a study done in Chile (9).

Stool samples were collected from broiler chicken of four randomly selected farms in Galle District. Each farm was visited once and a representative sample of stools was obtained from all cages with broiler chicken who has grown for more than 40 days. These samples were collected and transported under recommended conditions and were processed on the same day.

Identification of *Escherichia coli* isolates

All stool samples were cultured on MacConkey agar and all types of lactose fermenting coliforms were sub cultured on MacConkey agar and kept minimum of 7 days at 4-8°C until it is processed at the Faculty of Allied Health Sciences. Isolates were confirmed as *E. coli* by the colony morphology, Gram's stain (Gram negative), motility test (motile) and standard biochemical tests such as urease test (negative), indole test (positive), growth on triple sugar iron agar (A/A, Gas (+), H₂S (0)).

Detection of ESBL production

Mueller-Hinton agar (MHA) plates were inoculated with confirmed *E. coli* isolates having a turbidity equal to the McFarland 0.5 standard. ESBL production detected by using antibiotics selected from the disc diffusion test methods described in the latest edition of the Clinical and Laboratory Standard Institute (CLSI) antimicrobial susceptibility testing standards at the time of conducting the study (10). All isolates were screened for ESBL production by using cutoff zone diameters for Ceftazidime 30µg (CAZ) (≤ 22 mm) and cefotaxime 30µg (CTX) (≤ 27 mm). In parallel, all *E. coli* isolates were tested using the confirmatory ESBL detection test by placing ceftazidime (30µg) (CAZ) and ceftazidime (30µg)/clavulanic acid (10µg) discs. Following 16 - 18 hours of incubation at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$, detection of smaller zone diameters than the cutoff levels in one or more of the above antibiotics were considered as possible ESBL production. Further detection of a ≥ 5 mm difference between the diameters of zones of inhibition of CAZ and CAZ/ clavulanic acid was considered as the confirmatory test method for ESBL detection.

Further, all isolates were tested using a double disc synergy test (DDST) to support the findings of the previous test method. DDST checks the synergy between a third generation cephalosporin and clavulanate by placing a disc of amoxicillin (20 µg)/ clavulanate (10 µg) and a disc of cefotaxime (30 µg), 30 mm apart (center to center) on an inoculated MHA plate (11). A clear extension of the edge of the cefotaxime inhibition zone towards the disc containing clavulanate (keyhole like) was interpreted as synergy, indicating the presence of an ESBL.

Detection of antibacterial activity of chicken feed and water

Two samples each from ready to serve chicken feed and water were collected from the selected farms. Five grams of chicken feed was dissolved in 10 ml of sterile distilled water. Dissolved food and water samples were cultured on MacConkey agar for the detection of coliforms. The concentrated fraction of remaining sample was tested against two types of *E. coli* control cultures (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218) using agar well diffusion method to detect the evidence of the presence of any antibacterial substances in chicken feed and water.

Ethical approval was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna and data collected after obtaining permission from the owners of the selected farms in Galle District. The results of the study were presented as group data and all the specimens and culture isolates were discarded according to standard guidelines.

Results

A total of 80 chicken stool samples were collected from 4 broiler farms in Galle District. Out of the 102 coliform isolates 86 (84.3%) were *E. coli*, 14 (13.7%) were *Klebsiella* spp. and 2 (2%) were other coliforms. There were no ESBL producers detected among all coliform isolates confirming a zero prevalence of ESBL producing coliforms in chicken stools in these selected broiler farms (Table 1). Figure 1 and Figure 2 demonstrate the percentage of isolated organisms and their colony density in stool culture isolates of each farm.

Table 1: Number of organisms isolated from chicken stools

Farm No	Number of chicken in the herd	Number of stool samples obtained	Number of isolated <i>E. coli</i>	Number of isolated <i>Klebsiella</i>	Number of other coliform isolates	Total number of coliforms isolated	Number of ESBL producing isolates
1	500	29	29	0	0	29	0
2	1200	30	41	0	1	42	0
3	100	07	08	0	0	08	0
4	200	14	08	14	1	23	0
Total	2000	80	86	14	2	102	0

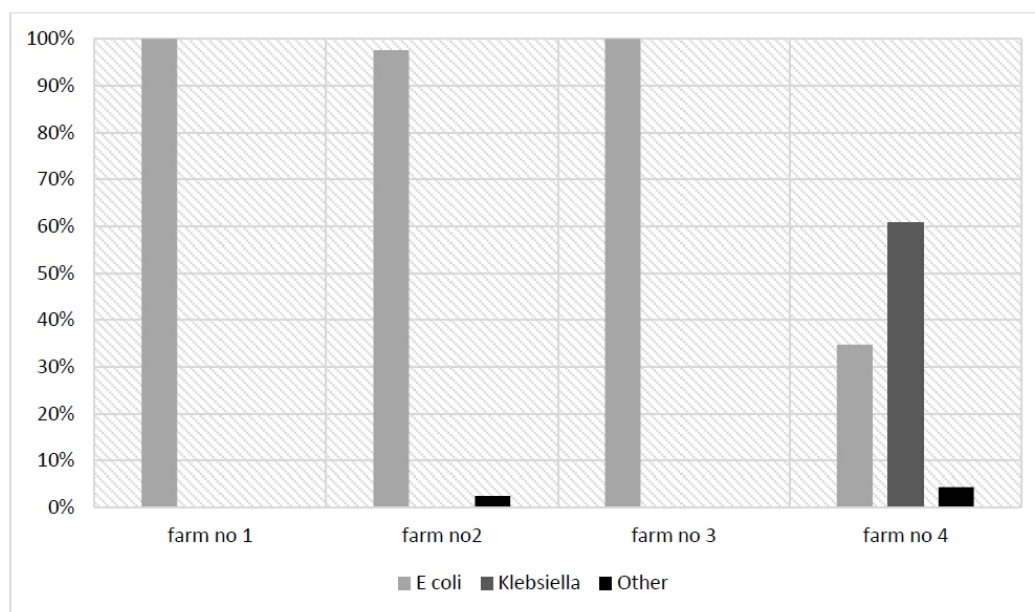


Figure 1: Percentage of isolated organism from chicken stools

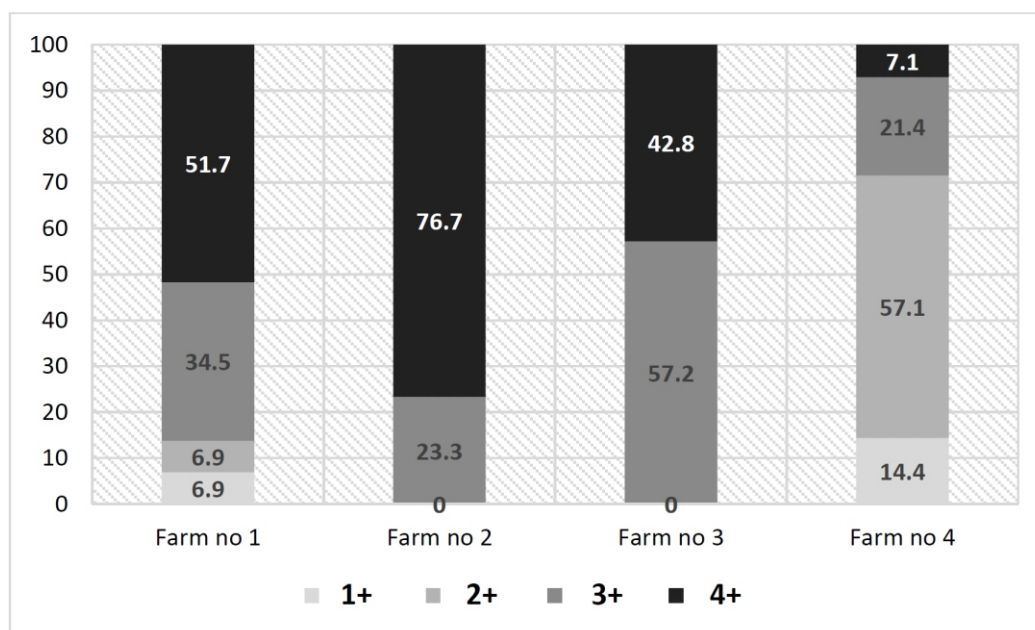


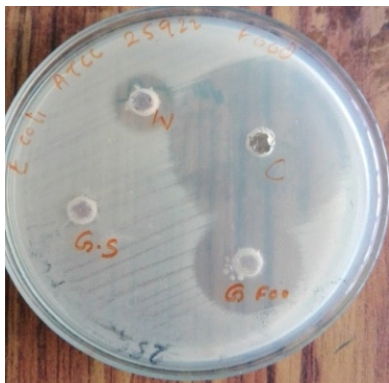
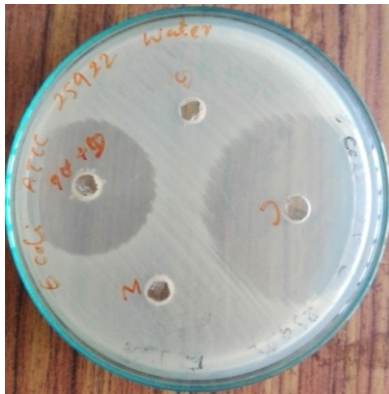
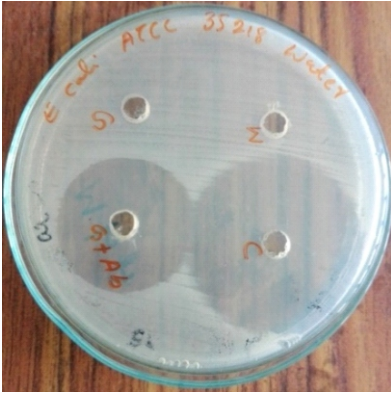
Figure 2: Colony density of bacterial isolates in the stool culture isolates

All food and water samples were cultured on MacConkey agar and there were no coliform detected. No antibacterial activity was detected in the food and water samples collected from the farm numbers 1, 2 and 3.

Farm number 4 declared that they add 1 ml of Co-trimoxazole to 1000ml of water served to

animals. The antibacterial activity of the collected food and water samples are demonstrated in Table 3. Further, colony density of bacterial isolates in the stool culture isolates of farm number 4 was proportionately less when compared to other three farms who did not use any additives to chicken feed or water. Cefotaxime sodium (10 mg/mL) was used as the positive control (Table 3).

Table 3: Antibacterial activity (diameter of the zone of clearance) of food and water samples collected from farm number 4

	Label on culture plate	<i>E. Coli</i> ATCC 25922	<i>E. Coli</i> ATCC 35218
Food samples			
Food sample 1	W	15 mm	18 mm
Food sample 2	G	25 mm	27 mm
Food sample 3 (starter food)	GS	0	0
Control - Cefotaxime	C	44 mm	45 mm
Water samples			
Water sample 1	W	0	0
Water sample 2	G	0	0
Water sample 3 (with antibiotics)	G Ab	34 mm	33 mm
Control - Cefotaxime	C	45 mm	45 mm

Discussion

ESBL-producing coliforms including *E. coli* in poultry farms are increasing day by day. It can be a threat to the human via food cycle. *E. coli* is an organism which lives in normal gut flora of human and animals. Antibiotic sensitivity pattern of isolated *E. coli* in chicken stool indicate antibiotic pressure on the normal flora. The frequent administration of antibiotics to the chicken is the main reason for developing ESBL-producing coliforms in poultry. In this study, 86 *E. coli* colony types were observed among the 80 samples but there were no ESBL-producing *E. coli* detected among these isolates. None of the other coliform isolates found in the stool cultures were ESBL producers.

Although there are no studies conducted for *E. coli* prevalence in chicken stool worldwide, a study done in Chile on *E. coli* in fecal flora in gulls found 30.1% of ESBL-producing bacteria (9). Further studies done on other countries in chicken meat have detected ESBL-producing coliforms with a higher prevalence ranging from 42% in Austria (3) to 66.32% in India (12).

Antibacterial activity of food and water served for poultry were tested in the selected farms. Antibacterial activity was observed only in one of the farms, in which the farm owner declared the use of co-trimoxazole containing water to feed chicken. The percentage of *E. coli* in stool samples collected from this farm has been reduced to 34.7% when compared to nearly 100% in rest of the farms. This change resulted in an overgrowth of *Klebsiella* species and other coliforms in the stools samples of this farm. Further, the total colony density of the culture isolates of this farm is low when compared with the rest of the farms that do not use any antibiotics. Therefore, incorporation of this antibiotic must have imposed pressure on changing the faecal bacterial flora of poultry in this farm.

Antibiotics and antibacterial compounds containing food were less commonly used in the poultry farms in Galle District which is reflected by not detecting any ESBL-producing *E. coli* in chicken stool.

However, an island-wide survey including different scales of broiler farms will demonstrate a better picture on burden of ESBL producing *E. coli* in chicken stool in Sri Lanka. The establishment

of antibiotic resistance surveillance in livestock in Sri Lanka will support to achieve goals in one health concept.

Limitations

ESBL detection in coliforms could have been increased by obtaining repeated samples from the same farms and by testing for the full panel of antibiotics listed in the screening and the confirmatory tests for ESBL detection in CLSI.

Conclusions & Recommendations

ESBL-producing *E. coli* prevalence in chicken stools in selected broiler farms in Galle district was found to be zero. However, these findings cannot be generalized to all broiler farms in the entire Galle District. An island-wide survey including different scales of broiler farms is recommended to reveal the true picture of the impact.

References

1. Gundogan N and Avci E. Prevalence and antibiotic resistance of extended-spectrum beta-lactamase (ESBL) producing *Escherichia Coli* and *Klebsiella* species isolated from foods of animal origin in Turkey. *African Journal of Microbiology Research*. 2013; **31**(7): 4059-4064.
2. Kimberly K. Consumers are buying contaminated meat, doctors' group says in lawsuit. 2019, April 17. The Washington Post. https://www.washingtonpost.com/national/consumers-are-buying-contaminated-meat-doctors-group-says/2019/04/16/978eed32-6073-11e9-9412-daf3d2e67c6d_story.html.
3. Gernot Z, Herbert G, Josefa L, Christian P, Franz FR, Doris H, Clemens K, Andrea JG, Peter P and Gebhard F. Multi-resistant bacteria isolated from chicken meat in Austria. *International Journal of Environmental Research and Public Health*. 2014; **11**: 12582-12593. DOI: 10.3390/ijerph111212582
4. Cindy D, Alieda van EZ, Kees V, Hilde S and Dik M. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Veterinary Microbiology*. 2010; **145**(3-4): 273-278. DOI: 10.1016/j.vetmic.2010.03.019

5. Overdeest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, Van der Zwaluw K, Huijsdens X and Kluytmans J. Extended-Spectrum β -Lactamase Genes of *Escherichia coli* in Chicken Meat and Humans, the Netherlands. *Emerging Infectious Diseases*. 2011; **17**(7): 1216-1222. DOI: 10.3201/eid1707.110209
6. Leverstein-van Hall MA, Dierikx CM, Stuart JC, Voets GM, Van den Munckhof MP, Van Essen-Zandbergen A, Platteel T, Fluit AC, Van de Sande-Bruinsma N, Scharinga J, Bonten MJM and Mevius DJ. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical Microbiology and Infection*. 2011; **17**(6): 873-80. DOI: 10.1111/j.1469-0691.2011.03497.x
7. Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, Giske CG, Naas T, Carattoli A, Martínez-Medina M, Bosch J, Retamar P, Rodríguez-Banõ J, Baquero F and Soto SM. *Escherichia coli*: An old friend with new tidings. *FEMS Microbiology Reviews*. 2016; **40**(4):437-463. DOI: 10.1093/femsre/fuw005
8. Charan J and Biswas T. How to Calculate Sample Size for Different Study Designs in Medical Research? *Indian Journal of Psychological Medicine*. 2013; **35**: 121-126. DOI: 10.4103/0253-7176.116232
9. Hernandez J, Johansson A, Stedt J, Bengtsson S, Porczak A, Granholm S, González-Acuña D, Olsen B, Bonnedahl J and Drobni M. Characterization and Comparison of Extended-Spectrum β -Lactamase (ESBL) Resistance Genotypes and Population Structure of *Escherichia coli* Isolated from Franklin's Gulls (*Leucophaeus pipixcan*) and Humans in Chile. *PLoS ONE*. 2013; **8**(9): e76150. DOI: 10.1371/journal.pone.0076150]
10. Clinical and laboratory standard institute. M100 - Performance standards for antimicrobial susceptibility testing. 28th edition. January 2018.
11. Drieux L, Brossier F, Sougakoff W and Jarlier V. Phenotypic detection of extended-spectrum β -lactamase production in Enterobacteriaceae: review and bench guide. *Clinical Microbiology and Infection*. 2008; **14** (1): 90-103. DOI: 10.1111/j.1469-0691.2007.01846.x
12. Bhoomika, Shakya S, Patyal A and Gade NE. Occurrence and characteristics of extended-spectrum β -lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. *Veterinary World*. 2016; **9**(9): 996-1000. DOI: 10.14202/vetworld.2016.996-1000