ISSN 2989-0705 (Online)|Vol 3|Issue 2|July 2025

Isolation, identification, antimicrobial susceptibility testing and public awareness of *Escherichia coli* on raw beef, pork and chicken meat in Western and Southern provinces of Sri Lanka

Eirene Ecclesia Harold[1], Malith Shehan Keraminiyage[1], S.M.D. Wijesinghe[1] and Lihini Ranesha Weerakkody[1*]

[1]Faculty of Life and Medical Sciences, Business Management School (BMS), Sri Lanka

[*]vetlihini@gmail.com

Abstract

Escherichia coli (*E. coli*) is a major foodborne pathogen with significant health and economic implications due to its virulence and antibiotic resistance. This cross-sectional study assessed the prevalence, antimicrobial resistance, and public awareness of *E. coli* in raw beef and chicken from the Western province and pork from the Southern province of Sri Lanka. Meat samples were collected under aseptic conditions and cultured on MacConkey agar. Presumptive *E. coli* colonies were identified using Gram staining and biochemical tests (indole and citrate utilization). Antimicrobial susceptibility was tested against Gentamicin, Chloramphenicol, and Erythromycin. Additionally, a questionnaire-based survey evaluated public knowledge and practices regarding *E. coli* and meat safety. The prevalence of *E. coli* was 33.3% in beef, 10% in pork, and 50% in chicken samples. All *E. coli* isolates from meat samples exhibited 100% resistance to Erythromycin. Susceptibility to Gentamicin was 76.9% in beef, 83.4% in pork, and 50% in chicken isolates. For Chloramphenicol, susceptibility was 100% in beef, 50% in pork, and 83.4% in chicken isolates. Survey results from 100 beef, 123 pork, and 429 chicken consumers revealed that 56%, 27%, and 47.1% respectively were unaware that *E. coli* is a foodborne pathogen, while only about half recognized undercooked meat as a source of infection. This study highlights the need for improved household hygiene, increased public awareness, and better handling practices by meat retailers. The observed antibiotic resistance underscores the necessity for stricter antibiotic usage guidelines. These findings provide baseline data for future interventions to enhance meat safety in Sri Lanka.

Keywords: *Escherichia coli,* foodborne pathogen, beef, pork, chicken, antimicrobial susceptibility testing

1. Introduction

Foodborne diseases are a global health issue due to the rise in demand for foods of animal origin with the increase of the world population. The risk of foodborne infections has grown tremendously over the past two decades because of the emerging foodborne pathogens hence, food safety and prevention of foodborne outbreaks is a public health concern.[1] Some of the major foodborne bacterial pathogens related to meat are *Salmonella* spp., *Escherichia coli (E. coli), Campylobacter jejuni* and *Listeria monocytogenes*.[2] Among them, *E. coli* infection is considered one of the important health issues[3].

Beef and pork are considered to be the key sources of foodborne transmission of *E. coli* and pork[4-8]. Chicken meat also poses a significant risk *E.coli* through food borne transmission.[9,10] In 2019, the most consumed meat in the world was poultry (14.7 kilogram/capita/year) followed by pork (11.1 kilogram/capita/year) and beef (6.4 kilogram/capita/year).[11] In a study conducted in Sri Lanka, the most preferred types of meat were chicken (84 %) followed by mutton (44 %), beef (33 %) and pork (24 %). Since chicken is not restricted by ethno-religious

beliefs and is regarded as a nutritious white meat, its consumption may be higher.[12] Sri Lanka is a nation with multi-ethnicity and religion, therefore the growth of the meat industry and beef and pork consumption is highly influenced by ethnoreligious views.[13]

E. coli is a rod-shaped, gram-negative and facultative anaerobic bacterium that belongs to the family of Enterobacteriaceae in the class of Gammaproteobacteria. It was first discovered and isolated in 1885 by T. Escherich during his study of intestinal microbes in infants and it was initially named Bacterium coli commune.[14] Majority of the *E.coli* strains are part of the intestinal microbiota, harmlessly colonizing the gastrointestinal tract. However, some *E. coli* strains have developed pathogenicity due to much phylogenetic diversity with certain lineages acquiring diverse combinations of virulence genes.[15] Several highly adapted *E. coli* clones have gained unique virulence properties, allowing them to adapt to new habitats and causing a wide range of diseases.[16] The virulence factors are acquired through transposons, bacteriophages and pathogenicity islands.[17] The first time *E. coli* was associated with human outbreaks was in 1982 due to the consumption of undercooked meat and beef patty was one of the common ingredients.[18] More than 40 non-O157 Shiga toxin-producing *E. coli* (STEC) outbreaks were discovered between 2000 and 2010. Almost half of them were caused by food poisoning, but many more were spread from person to person, particularly in child day care centre, waterborne transmission or contact with animals in public were responsible for a few epidemics.[19]

The five main foodborne diarrheagenic *E. coli* pathotypes based on virulence factors, invasiveness, toxin production, patterns and effect of bacterial attachment to host cells are Enteropathogenic *E. coli* (EPEC), *STEC*/Enterohemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and Enterotoxigenic *E. coli* (ETEC).[20] The most widely recognized pathotype associated with foodborne illnesses is the STEC, representing the predominant serotype *E. coli* O157:H7.[21] STECs are estimated to be responsible for 2.8 million acute illnesses worldwide.[22] The global prevalence (Figure 1) in cattle is 5.68%, with a higher prevalence in African (31.2%) and Northern American regions (7.35%).[23]

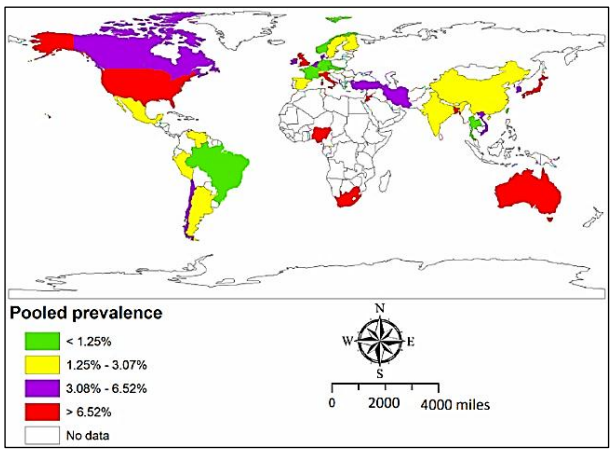

Figure 1. Estimated prevalence of *E. coli* O157 in cattle in different countries.[23]

Pathogenic *E. coli* ingested by the animal is excreted in feces leading to faecal contamination of the environment (Figure 2). Musca domestica (housefly) is a significant insect vector for STEC infection in farms and other vectors include birds, rodents and ruminants.[24]

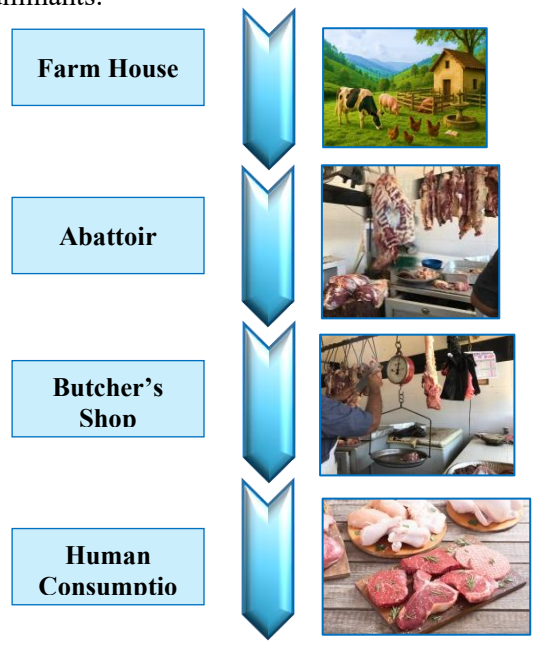

Figure 2. Mode of transmission of *E. coli*

Cross contamination can occur in the abattoir during evisceration. Handling money with bare hands, unwashed cutting board, knives and unhygienic practices can be reason for contamination at butcher's shop. The contamination of the carcass at the slaughterhouse can also occur by faecal shedding or by hides.[25, 26] Human exposures to *E. coli* is mainly through consumption of contaminated food or via direct contact.[27]

The production of Shiga toxins (Stx) by the stx1 and stx2 genes carried by lysogenic phages is the major virulence factor of STEC.[28] Intimin is essential for bacterial adherence to epithelial cells, resulting in a histopathological lesion called "attaching and effacing" (A/E lesion) controlled by locus of enterocyte effacement (LEE) which is a huge pathogenicity island leading to type III secretion system, Tir, and other secreted proteins.[29] STEC strains interact with the gut through long polar fimbriae (LPF), forming A/E lesions (Figure 3-A); Stx is produced in the intestine and transported in blood causing inhibition of protein synthesis and host inflammatory response leading to clinical manifestations.[30]

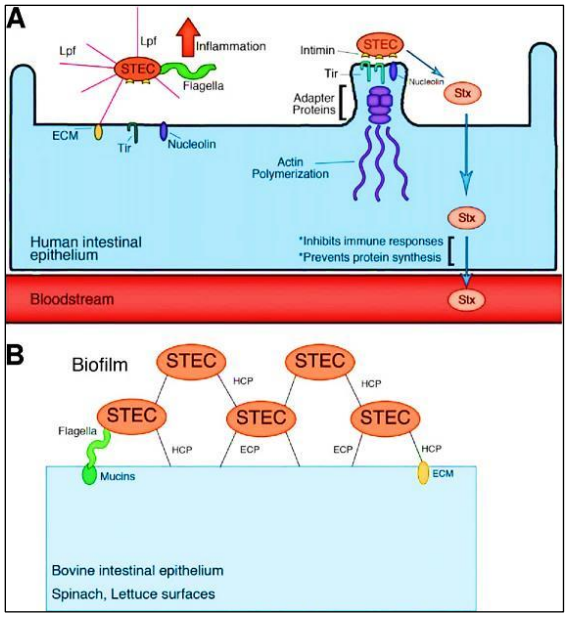

Figure 3. A- Colonization of STEC in intestine, B- Biofilm formation.[30]

Some infected people may be asymptomatic, while others may experience symptoms including fever, abdominal cramps, bloody diarrhea or even life-threatening conditions such as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura.[31] STEC strains may also adhere and colonize other surfaces, like the bovine intestine forming biofilm using adhesins such as haemorrhagic coli pilus (HCP), *E. coli* common pilus (ECP) and flagella (Figure 3-B). In swine, ETEC strains with fimbriae F5, F6, and F41 mostly colonise the posterior jejunum and ileum while ETEC with fimbriae F4 colonises the jejunum and ileum.[32]

In a study conducted contemporaneously across Great Britain, *E. coli* O157 was common in British beef cattle; the estimated herd-level prevalence was high in Scotland (23.6%) than in England and Wales (21.4%).[33] Similar studies conducted in other countries such as in India, the occurrence of *E. coli* O157:H7 in beef was 25.46% 25.8% in exported Malaysian beef and 11.1% in Thailand beef and 29.70% in Iran therefore contamination of beef meat with pathogenic *E. coli* continues to be a health concern worldwide.[34-36]

In Sri Lanka, the prevalence of STEC was found to be 53% in cattle calves.[37] In recent years, studies have been conducted on the prevalence of *E. coli* in chicken meat (20%), small fish (70%) and large fish (5%), but not on raw beef and pork.[38,39] However in a study conducted on ready-to-eat meat-based food products in Colombo, Sri Lanka including beef the prevalence of *E. coli* was 59%.[40]

Preventive and control strategies to minimise the risk of food/cross-contamination of *E. coli* include using safe water, hygienic conditions, vaccination for cattle and public awareness. Targeting super-shedders can also have a significant advantage.[26] Further methods to reduce contamination include pre-harvest interventions such as probiotics, vaccines (Epitopix SRP®), bacteriophages (Finalyse®) and post-harvest interventions such as physical interventions (knife trimming,

steam), using acid antimicrobials and oxidizer antimicrobials.[41] Preventative strategies for pigs include immunoprophylaxis (live attenuated and live wild type avirulent *E. coli*, subunit vaccines (purified F4 fimbriae), breeding of resistant pigs and diet management.[42]

Sri Lanka has implemented different measures in food safety such as the HACCP system, Food Act no.26 of 1980, including its amendments in 1991[43-45]. Understanding the potential factors for microbial meat contamination along the whole meat supply chain is required to identify targets for interventions and to minimise the number of meat-borne *E. coli* outbreaks.

This study focuses on the isolation and identification of *E. coli* from raw beef, pork and chicken samples from different regions of Sri Lanka, perform antimicrobial susceptibility test and determine the level of public awareness, as well as assess the knowledge, attitude and practice of beef, pork and chicken consumers via a questionnaire-based survey.

2. Methodology

2.1 Sample collection and preparation. The cross-sectional study was conducted between November 2021 and January 2022. A total of 21 beef and 20 chicken samples were collected from the Western Province, while 21 pork samples were obtained from the Southern Province of Sri Lanka. The study populations were all beef, chicken and pork carcasses which were fresh, unprocessed and slaughtered in the abattoir. Processed and spoiled samples were excluded. The samples were collected in sterile zip lock bags from the butcher's shop and were transported at 4°C in an icebox. The samples were then finely chopped using a mortar and pestle. All the procedures were done under aseptic conditions.

2.2 Pre-enrichment. Approximately 5 g of the meat was distributed into 50 mL falcon tube containing 30 mL of buffered peptone water using a sterile spoon spatula. The tubes were vortexed until the samples were homogenised and were incubated at 37°C for 4 hours.

2.3 Initial Culture. Isolation and identification of *E. coli* were performed following the flow chart in the Bergey's Manual of Determinative Bacteriology (Figure 4).[46] The inoculated peptone water was taken from the incubator and vortexed. It was used to streak plate using a sterile inoculation loop between 2 Bunsen burners and spread plate using a cotton swab inside a biosafety cabinet onto MacConkey agar Petri plate. The Petri plates were sealed with parafilm and were kept inside the incubator at 37°C for 24 hours.

2.4 Sub-culturing of presumptive E. coli colonies. After 24 hours of incubation, the MacConkey agar petri plates were observed for colony morphology. According to the colony morphology of *E. coli* on MacConkey agar: circular, moist, flat bright pink/red smooth colonies of entire margins were selected and were sub-cultured onto another new MacConkey agar Petri plate which was divided into 4 sections. The plates were sealed with parafilm and were incubated at 37°C for 24 hours.

2.5 Gram's staining. Colonies were picked using a sterile inoculation loop from sub-cultured MacConkey agar petri plates. Thin smears were prepared on a clean, dry glass slide using the inoculation loop and were air-dried and heat-fixed. The glass microscope slides were placed onto the staining tray and were stained according to the order and duration in Table 1. After the addition of each reagent, the slides were washed with distilled water. The slides were then placed on a clean surface and were left to air dry. Finally, the Gram's stained microscope slides were observed under different magnifications using a compound light microscope. A drop of immersion oil was added onto the smear and a coverslip was placed for observation at 100X magnification.

Table 1. Reagents used for Gram's staining

Gram's Reagents	**Duration (s)**
Crystal Violet	60
Gram's iodine	60
Gram's decolourizer	20
Safranin	60

2.6 Biochemical tests. The positive control used for the indole and citrate utilization test was the reference strain, *E. coli* (ATCC 25922) and the negative control was the uninoculated indole/citrate tube. Indole and citrate utilization tests were performed according to standard procedures.[47]

2.6.1 Indole Test. 5 mL test tubes containing 4 mL of Trytophan broth was inoculated by stirring with presumptive *E. coli* colonies which were picked using an inoculation loop from the sub-cultured MacConkey agar. The inoculated tryptophan broth was incubated at 37°C for 24 hours. After incubation, 0.5 mL of Kovacs reagent was added to the broth to observe cherry-red ring formation at the meniscus.

2.6.2 Citrate utilization test. Citrate utilization test was only carried out when the indole test was positive. A presumptive *E. coli* colony was picked using an inoculation loop from the sub-cultured MacConkey agar Petri plate and the citrate agar was streaked according to the conventional tube method. The tubes were incubated at 37°C for 48 hours. Results were observed to confirm the presence or absence of *E. coli* in the beef, pork and chicken samples.

2.7 Identification of E. coli. Result interpretation was guided using the taxonomic characteristics from Bergey's Manual of Determinative Bacteriology (Figure 4*)*.[46]

2.8 Antimicrobial Susceptibility Testing. Antibiotic susceptibility testing was performed using the disk diffusion method according to CLSI guidelines.[48] Luria Bertani (LB) broth was prepared according to the manufacturer's instructions (Himedia, India). An *E. coli* colony was picked using an inoculation loop from the sub-cultured MacConkey agar petri plate and was dipped into LB broth. The inoculated broth was incubated at 37°C for 24 hours. After incubation, 1 mL of the cultured LB broth was poured into an empty falcon tube. The turbidity of the LB broth was compared to the prepared 0.5M McFarland standard using a Wickerham card. If the turbidity of the inoculated LB broth was higher, fresh LB broth was poured into the falcon tube containing inoculated LB broth, until the turbidity of the inoculated LB broth was similar to that of 0.5M McFarland standard. Mueller-Hinton agar (MHA) was prepared according to the manufacturer's instructions (Himedia, India). It was cultured by spread plating using a cotton swab that was dipped into inoculated LB broth which had similar turbidity to that of 0.5M McFarland standard. The Petri plates were divided into 4 sections. Gentamicin 10µg/disc (Himedia, India), chloramphenicol 30µg/disc (Himedia, India), erythromycin 15µg/disc (Himedia, India) and a negative control filter paper dipped in autoclaved distilled water were placed in the MHA Petri plate using sterile forceps. The plates were sealed with parafilm and were kept in the incubator at 37°C for 24 hours. After incubation, the diameters of the zones of inhibition were measured and were compared to the zone size interpretative chart to determine whether the samples are sensitive, intermediate or resistant.

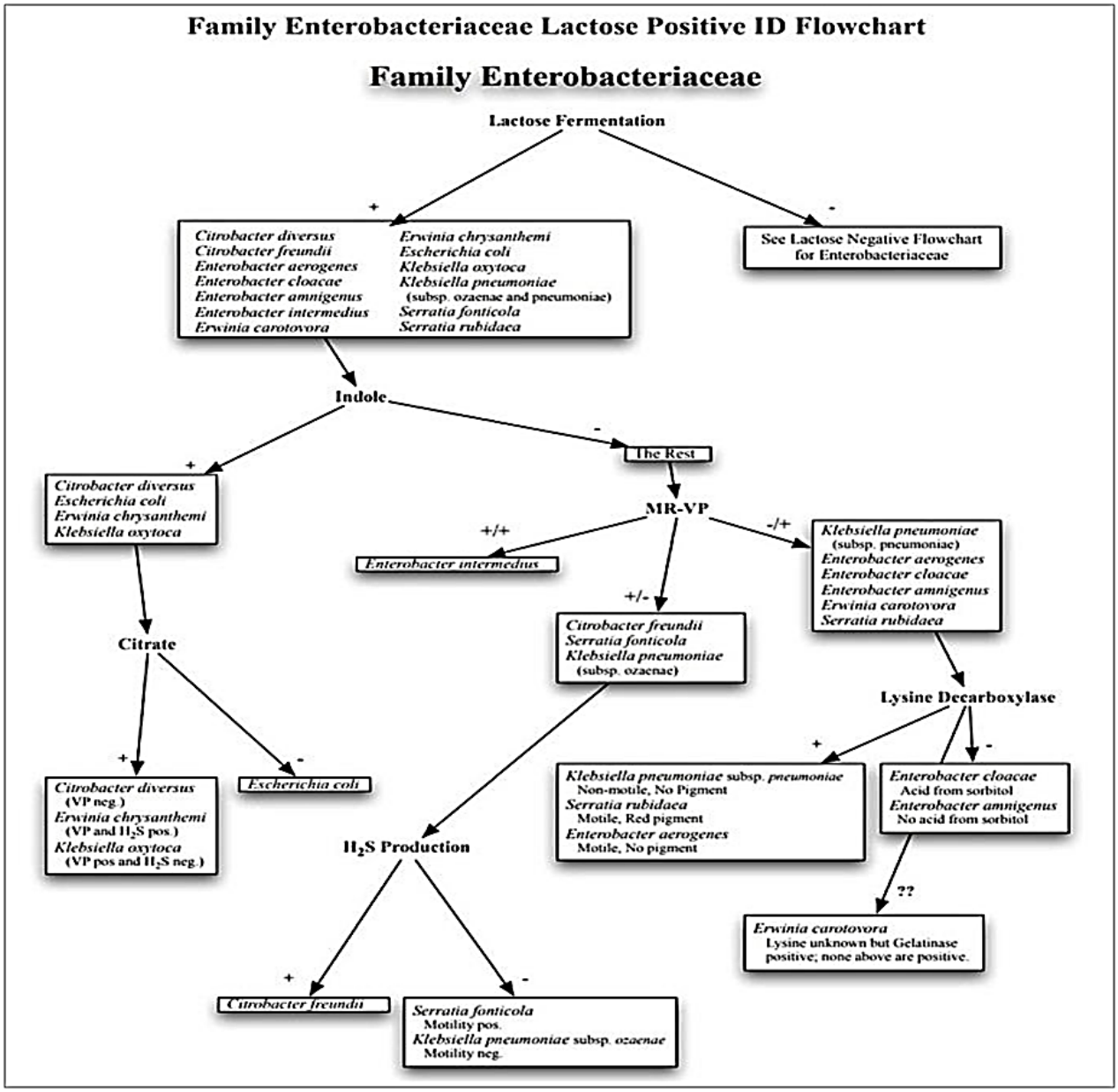

Figure 4. Flowchart for the Identification of Lactose-Positive Enterobacteriaceae based on Biochemical Tests.[46]

2.9 Data Analysis. A self-reported questionnaire-based survey was employed to assess the knowledge, attitude and practice of the beef, pork and chicken consumers. The participation was entirely voluntary, and no personal or identifying information was collected. As the study posed minimal risk and was conducted for academic purposes without involving sensitive topics, formal ethical approval was not sought. Consent was implied through completion of the form. Participants were regular consumers of meat, aged ≥18, and residents of Sri Lanka. Participants who were vegan, <18 years and incomplete responses were excluded. The questionnaire was created using Google forms and was shared via social media. The survey data were analyzed using Microsoft Excel 2010 and SPSS version 20. Descriptive statistics, including frequencies and percentages, were generated to summarize participant responses.

3. Results

3.1 Culture plates. Pink/red, flat, bright, circular, moist smooth colonies of entire margins were observed in the culture plates as shown in Figure 5 and 6. Colourless colonies were observed too.

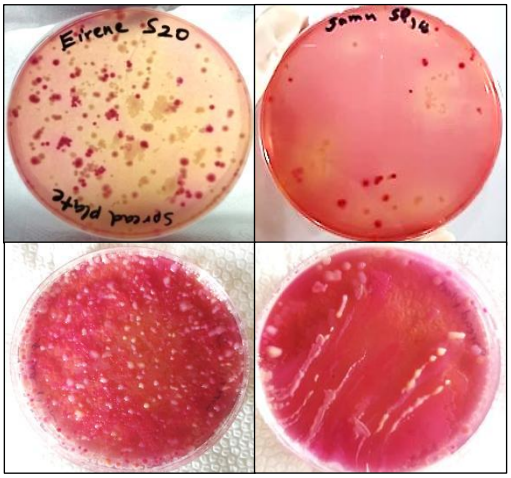

Figure 5. Initial culture plates

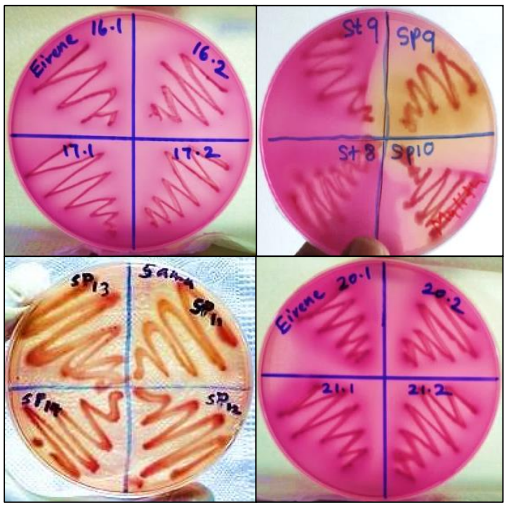

Figure 6. Sub-cultured Plates

3.2 Microscopic Observation. Pink, rod shaped, gram-negative bacteria arranged singly or in pairs (Figure 7) were observed in all samples under 100X magnification using compound light microscope.

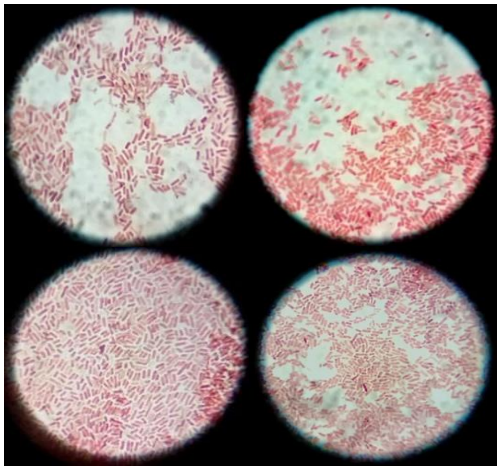

Figure 7. Gram-negative bacteria (100X)

3.3 Biochemical tests. A cherry red ring formation (Figure 8) was observed soon after adding Kovacs reagent indicating a positive result. Absence of a cherry red ring formation with the solution being yellow indicated a negative indole test result.

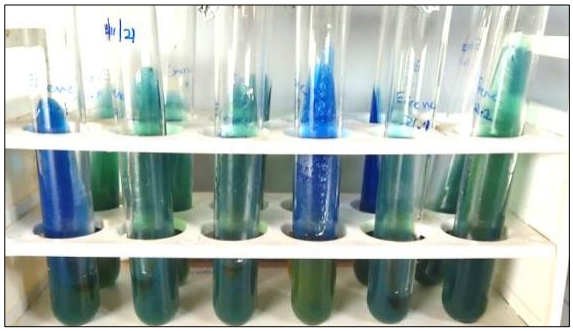

Figure 8. Indole Test

Citrate utilization test (Figure 9) was done only for the samples which had positive indole result. A negative citrate tested resulted in no colour change while a colour change from green to blue of the citrate agar indicated a positive test.

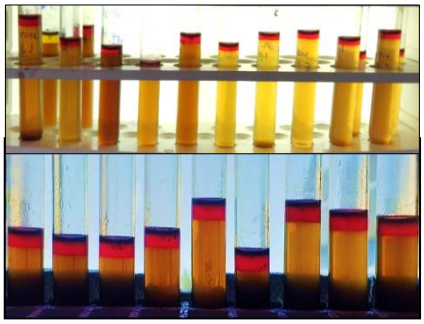

Figure 9. Citrate Utilization Test

3.4 Antimicrobial susceptibility test (AST). AST was done for all meat samples with a positive citrate utilization test as shown in Figure 10. The zone of inhibition was recorded and categorised as sensitive (S), intermediate (I), and resistant (R) as illustrated in Figure 11. Zone of inhibition was observed for gentamicin and chloramphenicol. Erythromycin and negative control (filter paper soaked in autoclaved distilled water) had no zone of inhibition.

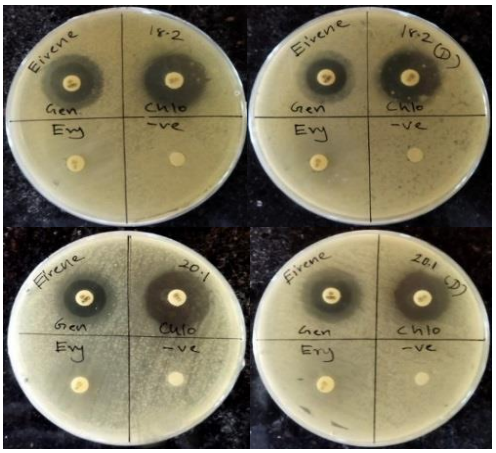

Figure 10. AST Plates

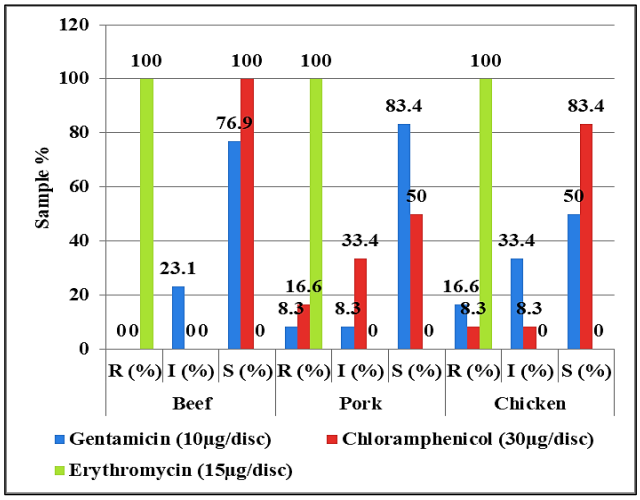

Figure 11. Zone of Inhibition for each meat sample

3.5 Data Analysis. Table 2 shows the knowledge, attitude and practice of the meat consumers. X is the specific meat sample which could be beef, pork or chicken.

4. Discussion

This is the first study conducted in Western Province and Southern Province, Sri Lanka to isolate, identify, perform antimicrobial susceptibility test and determine the level of public awareness of *E. coli* in raw beef, pork and chicken meat. The prevalence of *E. coli* in the beef (B) and chicken (C) meat bought from butcher's shop in the Western province, Sri Lanka is 33.3%, 50% and for pork (P) meat is 10% from Southern Province, Sri Lanka, respectively in our study. In a previous study conducted in South China, beef had the highest prevalence (13.32%) of contamination by *E. coli*, followed by pork (6.90 %) and chicken (3.28%).[49] In Ghana, the prevalence of *E. coli* in beef and chicken was 86.67% and 80%, in Northern Egypt, it was 6.7%, 16.7% in South Korea, 42.3%, 75.9%, and for pork 39.2% respectively[50-52]. In a study conducted in Italy, STEC isolates were obtained in pork samples indicating a stx-positive rate of 7.1%, and 2.8%.[53] According to reported prevalence rates of stx-positive *E. coli* isolates in live swine, slaughtered swine, and retail pork samples around the world varied from 4.4%-68.3%, 22%-86.3%, and 0.10%-80%, respectively, which depended on the sample classifications, detection methods, and the sanitation of the slaughterhouses and retail markets.[54]

The differences in prevalence and contamination rates of meat isolated from various countries could be due to sample types, seasonal influences, and detection methods utilized or due to the hygienic measures used in the whole beef, pork and chicken meat supply chain which varies among countries and also from the farm to abattoir to butcher's shop to the consumer.

Cross-contamination is an important source of carcass contamination in abattoir which could happen by faeces during evisceration, cattle transport or in the lairage, handlers' hands, and knives.[55] As a result of simultaneous handling of money and meat, *E. coli* was detected in meat and in money samples with a 100% contamination of

Table 2. Data Analysis of the questionnaire based survey

Variables	**Values**	**Beef**		**Pork**		**Chicken**	
		Frequency	**%**	**Frequency**	**%**	**Frequency**	**%**
Place of buying *X*	Butcher's shop	65	65	75	61	175	40.7
	Supermarket	39	39	75	61	249	58.0
	Local farms	1	1	24	19.5	24	5.5
	Small retail shops	3	3	20	16.3	66	15.3
	Other	4	4	14	11.4	7	1.6
Priority criterion when purchasing *X*	Quality (purity, taste and nutritive value)	38	38	66	53.7	236	55.0
	Price	10	10	42	34.1	120	27.9
	Freshness	45	45	92	74.8	290	67.5
	Other	8	8	7	5.7	7	1.6
Methods of consumption	Boiled	20	20	13	10.6	80	18.6
	Fried/deep fried	30	30	52	57.7	194	45.2
	Cooked	82	82	98	79.7	359	83.6
	Roasted	12	12	25	20.3	92	21.4
	Baked	11	11	33	26.8	68	15.8
	Smoked	9	9	9	7.3	31	7.2
	Raw	2	2	0	0	5	1.1
	Other	4	4	5	4.1	0	0
Knows that undercooked meat is a source of *E.coli*	Yes	52	52	52	42.3	173	40.3
	No	48	48	71	57.7	256	59.7
Thinks that cooked meat is always safe to eat	Yes	38	38	67	54.5	218	50.8
	No	62	62	56	45.5	211	49.2
History of meat *X* poisoning	Yes	11	11	9	7.3	25	5.8
	No	89	89	114	92.7	404	94.2
Symptoms	Headache	13	13	35	28.5	48	11.1
	Fever/chills	19	19	26	21.1	56	13.0
	Diarrhea	65	65	81	65.9	193	44.0

	Abdominal cramps	48	48	70	43.1	241	56.1
	Nausea and vomiting	63	63	74	60.2	262	61.0
	Other	11	11	14	11.4	0	0
Thinks that *X* slaughtered in abattoir is always safe to eat	Yes	37	37	10	8.1	51	11.9
	No	63	63	33	26.8	378	88.1
Heard of *E.coli* as a foodborne pathogen	Yes	44	44	10	8.1	227	52.9
	No	56	56	33	26.8	202	47.1
Knows that *E.coli* can be transmitted through the consumption of contaminated *X* meat	Yes	34	34	43	35	119	27.7
	No	66	66	79	64.2	310	72.3

currency from the same butcher.[56] Similar results were reported in another study with 100% prevalence of *E. coli* in money samples from meat sellers.[57]

Culturing in MacConkey agar helped to differentiate between lactose and non-lactose fermenting bacteria, since *E. coli* is lactose fermenting bacteria with its colonial morphological features which include pink, bright, flat, deeper central depression, dome-shaped appearance.[58] Gram's staining was done to determine the shape, size and arrangement of the bacteria. All the samples were gram-negative because the microscope slides were inoculated from the sub-cultured MacConkey agar plates (Figure 6), which were selective for the growth of gram-negative bacteria. Gram-negative bacilli were arranged singly or in pairs under microscopic observations and these were suspected to be *E. coli* as observed in Figure 7.

According to Bergey's manual of determinative bacteriology (Figure 4), if the Indole test was positive, it was presumed that the isolated organism could be *E. coli,* *Klebsiella oxytoca, Erwinia chrysanthemi*, or *Citrobacter diversus*.[46] However, a negative citrate utilization test further narrows down the identification, confirming the presence of *E. coli*. This biochemical distinction is important given that the indole-positive microorganisms have also been isolated from raw meat in previous studies. For instance, in a study conducted in Egypt, the prevalence of *E. coli* was 54.0% and *Klebsiella* spp. was 6.0% in beef meat.[59] In another study to assess the presence of Enterobacteriaceae in raw meat, the prevalence of *K. oxytoca* was 27.4% and *E. coli* was 12.1%.[60] In a study in Ghana the prevalence in beef meat was 8.2% for *C. diversus* and 17.3% for *K.oxytoca.*[61] In a study conducted among 500 pork samples, 68% were positive for coliforms including *Citrobacter* spp. and *Klebsiella* spp.[62] *E. chrysanthemi* is a phytopathogenic bacterium which is not typically found in meat samples but the presence of it could indicate cross-contamination. These findings highlight the need for confirmatory biochemical tests to accurately distinguish *E. coli* from other closely related species.

All *E. coli* isolates (100%) were resistant to Erythromycin, as shown in Figure 10, where no zone of inhibition was observed around the Erythromycin discs on the ABST plates. Similar results were obtained in another study.[60] B - 76.9%, P - 83.4%, C - 50% of the *E. coli* isolates were susceptible to Gentamicin. Figure 11 reveals that Chloramphenicol had the highest (100%) antimicrobial activity in *E.coli* isolates from beef, followed by C - 83.4% and P - 50% The findings were similar to a study done in Bangladesh, where the *E.coli* isolates were susceptible to Chloramphenicol (83%), Gentamicin (73%) and resistant (83%) to erythromycin.[63] However, colonies were observed inside the zone of inhibition for Chloramphenicol. This could indicate the presence of different strains of *E. coli* which are resistant to the antibiotic. Contrastingly, without the bacteria having developed a resistance phenotype, it could also indicate the presence of tolerant and persistent bacteria.[64] B - 23.1%, P - 8.3%, C - 33.4% were intermediately resistant to Gentamicin, whereas for Chloramphenicol, it was B - 0%, P - 33.4% and C - 8.3% for *E. coli* isolates In a previous study, B - 2.2%, P - 0.4%, C - 30.1% of the *E. coli* isolates were intermediately resistant to Gentamicin, Chloramphenicol and Erythromycin.[65] A study from 2002-2011 conducted among Italian swine herds showed an increase in resistance to Erythromycin (92.4–100%) and Gentamicin (63.6–85.7%).[66]

100, 123, 429 respondents in beef, pork and chicken participated in the self-reported questionnaire-based survey respectively, which was used to determine the public awareness including knowledge, attitude and practice of beef and pork consumers comprising hygiene and food safety as seen in table 2, 65% of the beef consumers and 40.7% of chicken consumers buy from butcher's shop and 39% and 58.7% buy from supermarkets. 61% of pork consumers buy from supermarket and butcher shop. Similar to our results, in a study conducted in Hungary, butcher shops (45.36%) are where most pork meat is purchased, followed by hypermarkets and supermarkets (28.56%).[67] A study reported that beef samples from wet markets had high contamination rate (89.50%) than in hyper markets (35.35% and 20%).[68]

When purchasing beef, 45% of the participants considered freshness as the priority criterion compared to quality (38%) as seen in Table 2. For pork and chicken, 74.8% and 67.5% of respondents prioritized freshness, while 53.7% and 55% prioritized quality, respectively. Similarly in a study conducted in Venezuela, freshness of the meat was an important attribute when buying/consuming meat.[69] In a research conducted in four European nations (France, UK, Germany and Spain), participants did not consider packed beef products as fresh, and it is likely that the perceived healthiness of branded and labelled beef is linked to its perceived quality; contrastingly, fresh meat is typically unbranded and unlabeled, so consumers assess its healthiness mostly based on its appearance and labelled, branded, fresh. Lean beef was seen as healthy compared to further processed packed beef.[70] Pork consumers' primary quality criterion is cleanliness, followed by moderate fat layer, freshness, colour, texture and smell when buying pork.[71] In another recent study conducted in Spain and Brazil, participants defined the traits for purchasing beef based on the intrinsic (colour, freshness, fat distribution) and extrinsic aspects (price and expiration date); freshness provided the buyer the impression of a good hygienic quality product; in Brazil, frozen packaged beef meat was seen as a lower-quality product and was purchased poorly, with customers assuming that freezing reduces freshness resulting in loss of quality but in comparison, Spanish consumers consider packaged beef as convenient and safe.[72]

Table 2 shows that majority of the consumers' preferred method of consumption for beef, pork and chicken is cooked 82%, 79.7%, 83.6%; boiled 20%, 10.6%, 18.6%; fried/deep fried 30%, 57.7%, 45.2%; roasted 12%, 20.3, 21.4%. Grilling or broiling beef

patty samples at 65°C resulted in higher reduction of overall bacterial and *E. coli* O157:H7 populations than at 60°C, however this reduction was not seen in pan-fried samples, moreover, broiling and grilling when combined, may have significant cooking temperature effect on *E. coli* O157:H7 reduction in comparison to panfrying.[73] This suggests that although frying is a commonly preferred method, it may be less effective in eliminating *E. coli* compared to grilling or broiling at higher temperatures. The pan-cooking method for pork meat was associated with an appetizing and nutritive product that is tasty/salty, juicy and soft/tender, with pepper and toasted flavours, and with a spicy aroma. In contrast, ohmic cooked pork meat was associated with golden and green colour, intense, spicy, and beer aroma, toasted flavour, and beer and cumin flavour, related to the brine solution used prior to cooking. Toasted flavours and aromas could be attributed to the formation of poly-cyclic aromatic hydrocarbons or polycyclic aromatic hydrocarbons, which are produced in greater quantity in cooking methods such as smoking, grilling and roasting. Interestingly, it was found that in ohmic cooking, the formation of these compounds could occur despite the meat not being exposed to temperatures above 100°C during cooking, suggesting that ohmic cooking is indeed a promising alternative for processing meat products with attributes such as toasted aroma, toasted flavour, and golden appearance. Moreover, pan-cooked pork meat was significantly more preferred than the other cooking methods.[74]

In the current study as stated in Table 2, 62%, 50.8% thought that cooked beef, chicken is not always safe to eat and 63%, 88.1% think that beef, chicken slaughtered in the abattoir is not always safe to eat whereas 54.5% of the pork consumers think that cooked pork is always safe to eat and 76.7% think that pork slaughtered in the abattoir is always safe to eat. More than half of the respondents (52%) were aware that undercooked beef meat can be a source of *E. coli*, while 59.7% of chicken consumers and 57.7% of pork consumers were unaware of this risk. A previous study revealed that *E. coli* isolates of various serotypes were present even in cooked meat, with 26.67% of chicken kofta and 20% of beef kofta samples testing positive. Among the bacterial isolates, *Enterobacteriaceae* was found to be the most prevalent group in chicken.[75] From the research survey, it was observed that B - 66%, P - 64.2%, C - 72.3% of the participants did not know that *E. coli* can be transmitted through contaminated meat. Majority of the participants had no history of B - 89%, P - 92.7% and C - 94.2% meat poisoning. Diarrhea (65%, 65.9%, 44%) and nausea/vomiting (63%, 60.2%, 61%) were selected as the common symptoms associated with contaminated beef, pork and chicken consumption and other symptoms included abdominal cramps (48%, 43.1%, 56.1%), headache (13%, 28.5%, 11.1%) and fever/chills (19%, 21.1%, 13%), respectively. The results were similar to a study conducted in Grampian and North Wales where they frequently selected vomiting, and cramps were the secondly placed symptom; 54% of the respondents also had heard about *E. coli*.[76] In a research done in Uganda, 50% of the participants reported that they have heard of foodborne illnesses; all participants agreed that they can contract disease from consuming pork; symptoms included worms (26%) and stomach ache (20%), diarrhea (16%), and fever (13%).[71]

More than half (52.9%) of the chicken respondents knew *E.coli* as a foodborne pathogen but majority of the beef (56%) and chicken (26.8%) participants were unaware and stated that only because of this questionnaire that they learned about *E.coli* and undercooked meat as a source.

Livestock-derived food demand is expected to surge globally by 14% per person and by a total 38% between 2020 and 2050; this demand growth is predicted to be greatest in South Asia (49%) and also in sub-Saharan Africa (55%) with the fastest growth in beef and pork.[77] Ground-breaking researches are being conducted to find safe alternatives to the

conventional beef and pork meat production due to the demand in meat and rising global population. Globally, there are around 32 cultured meat companies, with an emphasis on cultured beef (25%) and pork (19%) while 31% of these businesses are present in Asia.[78] In 2018, 2 companies developed cell-cultured pork utilizing stem cell technology and a successful prototype of pork sausage was produced using fat and muscle cell culture from live pig samples.[79] 3D bio-printed beef was recently made using bovine satellite cells and adipose-derived stem cells.[80] Lab-grown 'clean meat' or the cultured meat is also progressively making its way from academic laboratories towards the factory production line. Further researches conducted on these novel and 'non-traditional' beef, pork and chicken products, may have the potential to minimize the ethical concerns involving animal slaughter and diminish the environmental and health hazards related to conventional meat production, such as antibiotic resistance, food-borne and zoonotic infections.

The objectives of this study were successfully achieved. The isolation and identification of *E. coli* from raw beef, pork, and chicken meat confirmed its presence in all three types of meat, with varying prevalence. Antimicrobial susceptibility testing revealed high resistance to Erythromycin among all *E. coli* isolates. Furthermore, the public awareness survey highlighted significant gaps in knowledge regarding food safety, particularly among meat consumers. These findings collectively emphasize the need for improved hygiene practices and consumer education in meat handling and preparation.

5. Conclusion

Public awareness is low regarding *E. coli* and its potential risk as a foodborne pathogen despite meat being its major source. In this study, *E. coli* was isolated from raw beef, pork, and chicken samples, confirming its presence across all meat types. The isolates were fully resistant to Erythromycin and were susceptible to Gentamicin and Chloramphenicol. The questionnaire-based survey further revealed that a significant proportion of consumers were unaware of the risks associated with undercooked meat and poor handling practices. Awareness initiatives and a synchronized effort is required to mitigate or to effectively prevent the danger posed by *E. coli* at various levels in the entire beef, pork and chicken meat supply chain from farmers to consumers, as well as ensure that antimicrobials are used appropriately in both veterinary and human treatment regimes. Furthermore, public awareness should be raised about foodborne illnesses caused by *E. coli*, emphasizing the safe practices and consumption of beef, pork and chicken products, as well as the selection and safe use of antimicrobials.

References

1 A. Elbehiry, A. Abalkhail, E. Marzouk, A.E. Elmanssury, A.M. Almuzaini, H. Alfheeaid, M.T. Alshahrani, N. Huraysh, M. Ibrahem, F. Alzaben and F. Alanazi. *Vaccines*, 2023;**11**(4);725.

2 N. Heredia and S. García. *Animal nutrition*, 2018;**4**(3);250-5.

3 K. Bantawa, K. Rai, D. Subba Limbu and H. Khanal. *BMC research notes*, 2018;**11**;1-5.

4 R. Abdissa,W. Haile, A.T. Fite, A.F. Beyi, G.E. Agga, B.M. Edao, F. Tadesse, M.G. Korsa, T. Beyene, T.J. Beyene and L. De Zutter. *BMC infectious diseases*, 2017;**17**;1-6.

5 M.A. Croxen, R.J. Law, R. Scholz, K.M. Keeney, M. Wlodarska and B.B. Finlay. *Clinical microbiology reviews*, 2013;**26**(4);822-80.

6 R.A. Mir, T.A. Weppelmann, M. Elzo, S. Ahn, J.D. Driver and K.C. Jeong. *PLoS One*, 2016;**11**(2);e0148518.

7 I. Nastasijevic, J.W. Schmidt, M. Boskovic, M. Glisic, N. Kalchayanand, S.D. Shackelford, T.L. Wheeler, M. Koohmaraie and J.M. Bosilevac. *Applied and environmental microbiology*, 2020;**87**(1);e01711-20.

8 A. Pond, M.F. Miller, A. Echeverry, N, Huerta, A. Calle, M.S. Lozano, A. Chavez, T. Brashears and M.M. Brashears. *Food Protection Trends*, 2016;**36**(1).

9 R. Yulistiani, D Praseptiangga and S. Sudibya. *Materials Science and Engineering* 2019;**633**;012007.

10 G.T. Adeyanju and O. Ishola. *Springerplus*, 2014;**3**;1-9.

11 C. Whitton, D. Bogueva, D. Marinova and C.J. Phillips. *Animals*, 2021;**11**(12);3466.

12 P.H. De Silva, N.S. Atapattu and A.L. Sandika. *Journal of Agricultural Sciences–Sri Lanka*, 2011;**5**(2).

13 A.U. Alahakoon, C. Jo and D.D. Jayasena. *Korean Journal for Food Science of Animal Resources*, 2016;**36**(2);137.
14 T.H. Escherich. *Reviews of Infectious Diseases*, 1989;**11**(2);352–6.
15 H. Kittana, J.C. Gomes-Neto, K. Heck, A.L. Geis, R.R. Segura Muñoz, L.A. Cody, R.J. Schmaltz, L.B. Bindels, R. Sinha, J.M. Hostetter and A.K. Benson. *Frontiers in Immunology*, 2018;**9**;2318.
16 A.K. Kidsley, S. Abraham, J.M. Bell, M. O'Dea, T.J. Laird, D. Jordan, P. Mitchell, C.A. McDevitt and D.J. Trott. *Frontiers in Microbiology*, 2018;**9**;1207.
17 J.Y. Lim, J.W. Yoon and C.J. Hovde. *Journal of Microbiology and Biotechnology*, 2010;**20**(1);5.
18 L. W. Riley, R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Hebert, E.S. Olcott, L.M. Johnson, N.T. Hargret and P.A. Blake. *New England Journal of Medicine*, 1983;**308**(12);681–5.
19 T.H. Pennington. *Infection and Drug Resistance*, 2014;211–22.
20 S.C. Yang, C.H. Lin, I.A. Aljuffali and J.Y. Fang. *Archives of Microbiology*, 2017;**199**(6);811–25.
21 W. Beauvais, E.V. Gart, M. Bean, A. Blanco, J. Wilsey, K. McWhinney, L. Bryan, M. Krath, C.Y. Yang, D. Manriquez Alvarez and S. Paudyal. PLoS One, 2018;**13**(2);e0192149.
22 S.E. Majowicz, E. Scallan, A. Jones-Bitton, J.M. Sargeant, J. Stapleton, F.J. Angulo, D.H. Yeung and M.D. Kirk. *Foodborne Pathogens and Disease*, 2014;**11**(6);447–55.
23 M.Z. Islam, A. Musekiwa, K. Islam, S. Ahmed, S. Chowdhury, A. Ahad and P.K. Biswas. *PLoS One*, 2014;**9**(4);e93299.
24 P. Sapountzis, A. Segura, M. Desvaux and E. Forano. *Microorganisms*, 2020;**8**(6);877.
25 P. Sapountzis. A. Segura, M. Desvaux and E. Forano, *Microorganisms*, 2020;**8**(6);877.
26 J.M. Soon, S.A. Chadd and R.N. Baines. *Animal Health Research Reviews*, 2011;**12**(2);197–211.
27 R. Gautam, M. Kulow, D. Park, T.K. Gonzales, J. Dahm, M. Shiroda, A.J. Stasic, D. Döpfer, C.W. Kaspar and R. Ivanek. *Epidemiology & Infection*, 2015;**143**(2);274–87.
28 L. Rodríguez-Rubio, N. Haarmann, M. Schwidder, M. Muniesa and H. Schmidt. *Pathogens*, 2021;**10**(4);404.
29 A.I. Etcheverría and N.L. Padola. *Virulence*, 2013;**4**(5);366–72.
30 M.J. Farfan and A.G. Torres. *Infection and Immunity*, 2012;**80**(3);903–13.
31 R.A. Stein and D.E. Katz. *FEMS Microbiology Letters*, 2017;**364**(6);fnx050.
32 A. Luppi. *Porcine Health Management*, 2017;**3**;1–8.
33 M.K. Henry, S.C. Tongue, J. Evans, C. Webster, I.J. McKendrick, M. Morgan, A. Willett, A. Reeves, R.W. Humphry, D.L. Gally and G.J. Gunn. *Epidemiology & Infection*, 2017;**145**(15);3168–79.
34 C. Vijayan, V.J. Ajaykumar, A. Bhattacharya and V. Bhanurekka. *Journal of Entomology and Zoology Studies*, 2017;**5**(6);1395–403.
35 P. Sukhumungoon, Y. Nakaguchi, N. Ingviya, J. Pradutkanchana, Y. Iwade, K. Seto, R. Son, M. Nishibuchi and V. Vuddhakul. *International Food Research Journal*, 2011;**18**(1).
36 H. Momtaz, F.S. Dehkordi, E. Rahimi and H. Ezadi, R. Arab. *Meat Science*, 2013;**95**(2);381–8.
37 A.M. Tokhi, J.S. Peiris, S.M. Scotland, G.A. Willshaw, H.R. Smith and T. Cheasty. *Epidemiology & Infection*, 1993;**110**(2);197–208.
38 U.C. Gunasekera, M. Muralithas and M.P. Baumann. *Veterinary Journal*, 2016;**63**(2).
39 S. Ariyawansa, P. Ginigaddarage, K. Jinadasa, J. M. Chandrika, G.G. Arachchi, S. Ariyaratne. *Sri Lanka, Procedia Food Science*, 2016;**6**;246–52.
40 S.G.M.R.L. Wimalasekara and G.D. Gunasena. *International Journal of Agriculture Forestry and Plantation*, 2016;**4**;38–44.
41 T.L. Wheeler, N. Kalchayanand and J.M. Bosilevac. *Meat Science*, 2014;**98**(3);372–82.
42 M. Rhouma, J.M. Fairbrother, F. Beaudry and A. Letellier. *Acta Veterinaria Scandinavica*, 2017;**59**;1–9.
43 J. Munasinghe, A. De Silva, G. Weerasinghe, A. Gunaratne and H. Corke. *Quality Assurance and Safety of Crops & Foods*, 2015;37–44.
44 Food Act no. 26 of 1980, Chapter 544, Parliament of Sri Lanka, Sri Jayewardenepura Kotte, Sri Lanka.
45 Food Amendment Act no. 20 of 1991, The Gazette of the Democratic Socialist Republic of Sri Lanka, Colombo 8, Sri Lanka.
46 J.G. Holt. *Bergey's Manual of Determinative Bacteriology*, 9th Edition, Baltimore, Lippincott Williams and Wilkins; 1994.
47 J.G. Cappuccino and C. Welsh. *Microbiology: A Laboratory Manual*, 11th Edition, Pearson, Boston, 2017.
48 Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*, 30th Edition, CLSI supplement M100, CLSI, Wayne, PA, 2020.
49 S. Zhang, X. Zhu, Q. Wu, J. Zhang, X. Xu and H. Li. *Annals of Microbiology*, 2015;**65**;1993–9.
50 F. Adzitey, P. Assoah-Peprah, G.A. Teye, A.M. Somboro, H.M. Kumalo and D. G. Amoako. *International Journal of Food Science*, 2020;**2020**(1):8877196.
51 A.A. Moawad, H. Hotzel, O. Awad, H. Tomaso, H. Neubauer, H.M. Hafez and H. El-Adawy. *Gut Pathogens*, 2017;**9**;1–3.
52 H.J. Park, J.W. Yoon, E.J. Heo, E.K. Ko, K.Y. Kim, Y.J. Kim, H.J. Yoon, S.H. Wee, Y.H. Park and J.S. Moon. *Journal of Microbiology and Biotechnology*, 2015;**25**(9);1460–6.
53 L. Ercoli, S. Farneti, A. Zicavo, G. Mencaroni, G. Blasi, G. Striano, S. Scuota. *International Journal of Food Microbiology*, 2016;**232**;7–14.
54 M. Haque, J.M. Bosilevac and B.D. Chaves. *International Journal of Food Microbiology*, 2022;**377**;109832.
55 F.D. Gutema, G.E. Agga, R D. Abdi, A. Jufare, L. Duchateau, L. De Zutter and S. Gabriël. *International Journal of Environmental Research and Public Health*, 2021;**18**(5);2729.
56 M.U. Ukwuru and A. Gabriel. *Journal of Applied Sciences and Environmental*, 2012;**3**;42–8.
57 C.E. Ofoedu, J.O. Iwouno, I.M. Agunwah, P.Z. Obodoechi, C.O. Okpala and M. Korzeniowska. *PeerJ*, 2021;**9**;e10795.
58 L. Barcella, A.P. Barbaro, S.B. Rogolino. *Microbiologia Medica*, 2016;**31**(2).

59 M. Gwida, H. Hotzel, L. Geue and H. Tomaso. *International Scholarly Research Notices*, 2014;**2014**;565671.
60 A. Kilonzo-Nthenge, E. Rotich and S.N. Nahashon. *Poultry Science*, 2013;**92**(4);1098–107.
61 M. Olu-Taiwo, P. Obeng, A.O. Forson. *Journal of Food Quality*, 2021**;2021**:6666683.
62 K. Schwaiger, S. Huther, C. Hölzel, P. Kämpf and J. Bauer. *International Journal of Food Microbiology*, 2012;**154**(3):206–11.
63 M.D. Gupta, M. Islam, A. Sen, M.S. Sarker and A. Das. *Microbes and Health*, 2017;**6**(1);1–4.
64 O. Gefen, B. Chekol, J. Strahilevitz and N.Q. Balaban. *Scientific Reports*, 2017;**7**(1);41284.
65 A. Medina, P. Horcajo, S. Jurado, R. De La Fuente, J.A. Ruiz-Santa-Quiteria, G. Domínguez-Bernal and J.A. Orden. *Journal of Veterinary Diagnostic Investigation*, 2011;**23**(1);91–5.
66 A. Luppi, P. Bonilauri, M. Dottori, Y. Gherpelli, G. Biasi, G. Merialdi, G. Maioli and P. Martelli. *Transboundary and Emerging Diseases*, 2015;**62**(1);67–71.
67 V. Vida. *APSTRACT: Applied Studies in Agribusiness and Commerce*, 2013;**7**:151–8.
68 J.M. Premarathne, C.Y. New, A. Ubong, Y. Nakaguchi, M. Nishibuchi and R. Son. *Food Research*, 2017;**1**(3);67–76.
69 L.A.D. Moreno, N.J. Jerez-Timaure, J.V. Hernández, N.H. Leidenz, A.R. González. *Foods*, 2020;**9**(2);202.
70 L. Van Wezemael, W. Verbeke, M.D. de Barcellos, J. Scholderer and F. Perez-Cueto. *BMC Public Health*, 2010;**10**;1–10.
71 K. Roesel, F. Ejobi, M. Dione, D. Pezo, E. Ouma, J. Kungu, P.H. Clausen and D. Grace. *Global Food Security*, 2019;**20**;26–36.
72 D.R. Magalhaes, M.T. Maza, I.N. Prado, G. Fiorentini, J.K. Kirinus, M.D. Campo. *Foods*, 2022;**11**(1);129.
73 Y. Yoon, I. Georgnaras, A. Mukherjee, K.E. Belk, J.A. Scanga, G.C. Smith and J.N. Sofos. *Meat Science*, 2013;**95**(2);317–22.
74 S.V. Ángel-Rendón, A. Filomena-Ambrosio, M. Hernández-Carrión, E. Llorca, I. Hernando, A. Quiles and I. Sotelo-Díaz. *Meat Science*, 2020;**163**;108089.
75 F.A. Shaltout, H.M. Lamada and E.A. Edris. *Journal of Protein Research and Bioinformatics*, 2020;**2**(005).
76 C.D. Jones, C. Hunter, A.P. Williams, N.J. Strachan and P. Cross. *Epidemiology & Infection*, 2011;**139**(10);1522–30.
77 A.M. Komarek, S. Dunston, D. Enahoro, H.C. Godfray, M. Herrero, D. Mason-D'Croz, K.M. Rich, P. Scarborough, M. Springmann, T.B. Sulser and K. Wiebe. 2021;**70**;102343.
78 D. Choudhury, T.W. Tseng and E. Swartz. *Trends in Biotechnology*, 2020;**38**(6);573–7.
79 T.K. Hong, D.M. Shin, J. Choi, J.T. Do and S.G. Han. *Food Science of Animal Resources*, 2021;**41**(3);355.
80 D.H. Kang, F. Louis, H. Liu, H. Shimoda, Y. Nishiyama, H. Nozawa, M. Kakitani, D. Takagi, D. Kasa, E. Nagamori and S. Irie. *Nature Communications*, 2021;**12**(1);5059.