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Antibiotic resistance pattern of *Escherichia coli* NM O157 strains isolated from ostrich feces in Lorestan province

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Abstract

Antibiotic resistance is a significant challenge in human and veterinary medicine. It has become a serious issue for the livestock industry and public health. Foodborne illnesses are a concern, and antibiotic-resistant pathogens can cause difficult-to-treat infections. *Escherichia coli* O157:H7 is a culprit in foodborne illnesses. Poultry meat, due to antibiotic use, can harbor resistant bacteria. This study investigated antibiotic resistance in *E. coli* O157:H7 strains from ostrich feces in Iran. In a cross-sectional study conducted in Lorestan province, Iran, antibiotic resistance in *E. coli* O157:H7 strains isolated from ostrich feces was investigated. A total of 100 samples were collected from healthy ostriches on farms. Enrichment and isolation methods were used, and antibiotic susceptibility profiles were determined using the Kirby-Bauer disk diffusion method. The study aimed to assess risk factors and the occurrence of *E. coli* O157:H7 in chicken cloacae. Statistical analysis revealed associations between different risk factors and pathogen occurrence. In this study, 43 of 100 samples were found to be infected with *E. coli*, and of these, 15 samples (15% of all samples or 34.88% of all isolates) were identified as serotype O157:H7. The isolates exhibited high resistance to tetracycline and clindamycin, while showing sensitivity to nalidixic acid, norfloxacin, ceftriaxone, and imipenem. A national monitoring program for Shiga toxin-producing *E. coli* strains in food systems is crucial due to antibiotic resistance in *E. coli* O157:H7, necessitating updated antibiotic practices and further epidemiological studies to understand regional variations and the role of ostriches in pathogen distribution.

Introduction

Antibiotic resistance, as a significant challenge in the fields of human and veterinary medicine, has become a serious and concerning issue for the livestock industry and public health (Levy, 1998). In recent decades, due to the increased use of antibiotics in the treatment and prevention of livestock diseases, it has evolved into one of the most significant challenges in the domains of animal and human healthcare extending its ominous shadow to the realm of food safety (Sun *et al*., 2022). Foodborne illnesses have become a significant public health concern today and are considered one of the key indicators of community health (Capita and Alonso-Calleja, 2013; Singh, 2017; Ge *et al*., 2022). Among these, one of the primary challenges in the treatment of foodborne infections is the emergence of antibiotic resistance against a wide range of antibiotics (Giraffa, 2002; Newell *et al*., 2010). The consequences of antibiotic-resistant foodborne pathogens are profound. They can cause difficult-to-treat infections in humans and animals, raising concerns about the efficacy of antibiotics in treating such illnesses (Nataro and Kaper, 1998). Furthermore, these resistant pathogens can be transmitted through contaminated food products, potentially resulting in foodborne disease outbreaks. *Escherichia coli* O157:H7 is one of the potential culprits in the occurrence of foodborne illnesses (Schoeni and Doyle, 1994). *E. coli* is a bacterial species that resides in both humans and animals and is able to infect a variety of hosts. It is frequently found in the surroundings and is used as a sign of fecal pollution in food and water. It has the ability to obtain, retain, and pass on resistance genes from different organisms in the surrounding area. *E. coli* is a gram-negative, rod-shaped bacterium without spores and is facultative anaerobic. It is a member of the Enterobacteriaceae family and is known to cause devastating foodborne infections in humans. *E. coli* O157:H7 is a type of Shiga toxin-producing *E. coli* (STEC) and is responsible for causing hemorrhagic colitis (HC), hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Doyle *et al*., 1984; Boyce *et al*., 1995; Rasko *et al*., 2011; Allocati *et al*., 2013; Shenkman *et al*., 2014).

In today's world, protein sources, including poultry, are considered a fundamental and vital source of nutrition for humans. With the global population increasing and changes in dietary habits, the demand for poultry consumption is on the rise, and poultry meat has gained significant attention in recent years as one of the primary sources of protein, iron, phosphorus, and more. In animal production, antimicrobials are widely used as growth promoters and for treating infectious diseases. Their use in poultry production contributes to high resistance among normal poultry flora and pathogenic microorganisms(Romanus *et al*., 2012). Antimicrobial use in feed can alter intestinal flora, favoring resistant bacteria like *E. coli*, which may enter the environment and food chain (Furtula *et al*., 2010). Because of its widespread presence in both humans and animals and its dual role as both a pathogenic and commensal organism, *E. coli* has emerged as a frequently resistant microorganism to antimicrobials (Zhao *et al*., 2012). Due to frequent antimicrobial usage, bacteria develop resistance to both single and multiple antimicrobials, leading to challenges in treating certain diseases (Sellah *et al*., 2015). Unfortunately, due to the excessive use of antibiotics, the contamination of food products with antibiotic-resistant bacteria has increased and also, poultry meat can serve as a suitable breeding and growth medium for various bacteria (Manges, 2016). According to conducted studies, *E. coli* bacteria can easily contaminate poultry meat. The indiscriminate and unprincipled prescription of antibiotics, the disregard for the results of antibiotic sensitivity testing, and excessive use of disinfectants are the primary reasons for the emergence of resistance among *E. coli* O157 strains. Antibiotic administration in poultry farms, in some countries, goes beyond disease control and treatment, as it is also used to stimulate the growth of chicks, which could be another potential factor contributing to the increased prevalence of antibiotic resistance (Marangoni *et al*., 2015, İnana and Mustafa, 2018). Hence, based on the information provided, the study was carried out to examine the antibiotic resistance of *E. coli* O157:H7 strains obtained from ostrich feces in Lorestan province, Iran.

Materials and Methods

Study design

From March to May 2021, a targeted cross-sectional study was carried out to isolate, identify, and analyze the antimicrobial susceptibility of *E. coli* O157:H7 in the designated region. A total of 100 samples were gathered randomly from healthy ostriches on farms in Lorestan province, Iran (Fig. 1). In the research, potential risk factors such as the age, breed, and farm location of the birds were taken into account and documented.

Fig. 1. Map showing the study area. Lorestan province, Iran.

Sampling

In spring 2021, a descriptive-sectional study was conducted on 100 samples of ostrich feces randomly collected from the surfaces of breeding farms. The information related to ostriches (antibiotic consumption, age, etc.) was recorded in the designed questionnaire. The samples were collected in sterile containers and promptly transported, along with ice packs, to the microbiology laboratory of the Faculty of Veterinary Medicine at Lorestan University for further processing and were taken into account and recorded.

Enrichment

To enrich 10 g of fecal samples, 225 ml of Tryptic Soy Broth (TSB) (Merck Co., Germany) supplemented with Novobiocin (20 mg/l) (Merck Co., Germany) was added, and the mixture was incubated for 24 hours at 37°C (Stampi *et al*., 2004).

Isolation of bacteria

The enriched samples were cultured on Sorbitol MacConkey Agar (SMAC) (Merck Co., Germany) containing 0.05 mg/l cefixime (Oxoid Co., UK) and 2.5 mg/l of potassium tellurite (Oxoid Co., UK), and after 24 hours of incubation at 37°C, sorbitol-negative colonies were purified (Lee and Choi, 2006). For the confirmation of suspected *E. coli* strains, culture on Triple Sugar Iron (TSI) medium and the Indole-Methyl Red-Voges-Proskauer-Citrate (IMViC) test were used (Stampi *et al*., 2004).

Determination of antibiotic resistance (Agar diffusion method)

The antibiotic sensitivity of bacterial isolates was assessed using the Kirby-Bauer disk diffusion method on Mueller Hinton Agar culture medium (Merck Co., Germany) and according to the CLSI (Clinical and Laboratory Standards Institute) guidelines. To do this, initially, the bacteria were cultured for 24 hours in a tryptic soy agar medium. Subsequently, a bacterial suspension was prepared from the bacterial colonies grown in sterile physiological saline, with a concentration equivalent to half of the McFarland standard. Then, using a sterile swab, lawn cultures were performed on the blood agar medium, and antibiotic discs (PadtanTeb Co., Iran) were placed on the medium at appropriate intervals. The culture plates were maintained at 37°C for 24 to 48 hours, and the diameter of the zones of growth inhibition was measured. Sensitivity, intermediate sensitivity, and resistance categories were determined for each bacterial isolate based on the standards provided by the customer company. The studied antibiotics included ciprofloxacin (5 µg), gentamicin (10 µg), streptomycin (10 µg), ampicillin (10 µg), erythromycin (15 µg), vancomycin (30 µg), sulfamethoxazole (75/23 µg), nalidixic acid (30 µg), norfloxacin (10 µg), clindamycin (20 µg), cefazolin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), and amoxicillin-clavulanic acid (20 µg). Furthermore, *E. coli* ATCC 25922 was utilized as a control for antibiotic resistance assessment.

Data analysis

The information was coded and transferred to SPSS version 27.0 for statistical analysis as needed. Descriptive statistics were utilized to identify the presence of the pathogen. Chi square (x^2) and odds ratio were employed to assess the relationship between various risk factors and the presence of *E. coli* O157:H7 in chicken cloacae. Results were considered statistically significant when the *p*-value was below 0.05 ($p < 0.05$).

Results

Based on bacteriological tests, 43 of the 100 study samples were found to be infected with *E. coli*, and of these, 15 samples (15% of all samples or 34.88% of all isolates) were identified as serotype O157:H7, based on the characteristic of not fermenting sorbitol. The results of the resistance and sensitivity of *E. coli* isolates to antibiotics were summarized in Table 1. As can be seen, the isolates showed the highest resistance to tetracycline (100%), clindamycin (100%), followed by vancomycin (66.86%), erythromycin (66.86%). On the other hand, the lowest resistance was related to the antibiotic's amoxicillin/clavulanic acid (66.6%), ampicillin (20%), cefazolin (66.26%), ciprofloxacin (66.26%), and streptomycin (40%). *E. coli* isolates showed complete sensitivity to nalidixic acid, norfloxacin, ceftriaxone, and imipenem.

Table1. Antimicrobial resistance profiles of isolated *Escherichia coli* O157:H7.

Antibiotics	Codes	Disks	Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%
Vancomycin	V	30 µg	14	93.33	1	6.66	0	0
Ampicillin	AM	10 µg	3	20	1	6.66	11	73.33
Gentamycin	GM	10 µg	0	0	0	0	15	100
Nalidixic Acid	NA	30 µg	0	0	0	0	15	100
Cephalexin	CN	30 µg	4	26.66	5	33.33	6	40
Co-trimoxazole	SXT	1.25+23.75 µg	4	26.66	0	0	11	73.33
Clindamycin	CC	20 µg	15	100	0	0	0	0
Ceftriaxone	CRO	30 µg	0	0	0	0	15	100
Tetracycline	TE	30 µg	15	100	0	0	0	0
Erythromycin	E	15 µg	14	93.33	1	6.66	0	0
Ciprofloxacin	CP	5 µg	4	26.66	0	0	11	73.33
Streptomycin	S	10 µg	6	40	4	26.66	5	23.33
Imipenem	IMP	10 µg	0	0	0	0	15	100
Norfloxacin	NOR	10 µg	0	0	0	0	15	100
Amoxicillin-Clavolanic acid	AMC	20 µg	1	6.66	0	0	14	93.33

Discussion

E. coli is one of the most common causes of gastrointestinal infections, being a gram-negative bacterium naturally found in the human and animal intestines (Mushtaq *et al*., 2015). This bacterium is usually harmless but can, in some cases, lead to urinary tract infections, respiratory infections, and bloodstream infections (Shecho *et al*., 2017). One of the serious issues associated with *E. coli* is its resistance to antibiotics that are used to treat bacterial infections by eliminating or inhibiting bacterial growth. However, bacteria can develop resistance to antibiotics. Antibiotic resistance is a serious public health problem that can result in bacterial infections being difficult to treat and even fatal. The antibiotic resistance of *E. coli* can make infections caused by this bacterium more challenging to treat, leading to prolonged hospital stays, increased treatment costs, and an elevated risk of mortality. As a result, antimicrobial resistance is now a worldwide issue (Miles *et al*., 2006). The most significant factor in the development, selection, and spread of antimicrobial resistant microorganisms in human and veterinary medicine is the widespread use of antimicrobial agents in humans, veterinary, and agriculture (Simonsen *et al*., 2004).

Unfortunately, our investigation indicated that in the current situation, the treatment of cases infected with *E. coli* using common antibiotics such as tetracycline, vancomycin, and other widely used antibiotics is not effective. Horizontal transfer of resistance genes among different environments, foods, and hosts is quite likely. In this study, our *E. coli* isolates demonstrated the lowest resistance to ampicillin (20%), even though they showed complete sensitivity to nalidixic acid, norfloxacin, ceftriaxone, and imipenem. While our results align with previous studies regarding antibiotic groups, there are variations in the prevalence of antibiotic resistance. In fact, resistance to different antibiotics varies based on treatment patterns used against causative agents in different regions. Many studies have been conducted in various regions of Iran and the world has addressed this issue.

The research conducted by Gugsa and team in 2023 found that the prevalence of *E. coli* O157:H7 was 54.7% and 6.5% in cows from Dessie and Kombolcha, Ethiopia, respectively. Every *E. coli* O157:H7 isolate (100%) showed susceptibility to ampicillin, sulfamethoxazole-trimethoprim, and norfloxacin. In contrast, every single isolate (100%) exhibited resistance to penicillin G, vancomycin, and oxacillin (Abebe *et al*., 2023).

Kazemnia *et al*. (2014) investigated the antibiotic resistance pattern of various strains of *E. coli* isolated from poultry and humans in Iran. A total of 98% of the *E. coli* isolates were resistant to at least five antibiotics. The results indicated that out of the 50 tested *E. coli* strains, all of them (100%) were resistant to penicillin and erythromycin, 49 strains (98%) to nalidixic acid, 47 strains (94%) to cefalexin, 43 strains (86%) to amoxicillin, 42 strains (84%) to ampicillin, 37 strains (74%) to ciprofloxacin, 32 strains (64%) to tetracycline, 27 strains (54%) to cefixime, and 18 strains (36%) to gentamicin that the results obtained for some antibiotics were similar to the current study.

In a study by Rezaifar *et al*. (2013), the highest levels of *E. coli* resistance were observed against carbenicillin (100%), erythromycin (100%), co-amoxiclav (75%), ampicillin (62.5%), and tetracycline (53.1%). Conversely, no resistance was reported to cotrimoxazole, fluoroquinolones, or danofloxacin.

According to research by Carneiro *et al*. (2010) in Portugal, the isolation rate of *E. coli* from ostrich eggs was 16.66%. The resistance levels of these isolates were reported as follows: ampicillin (96.12%), tetracycline (96.12%), streptomycin (55.5%), amoxicillin-clavulanic acid, cefotaxime, and gentamicin (85.1%).

In a study by Ley *et al*. (2001) in Indiana and Ohio, the isolation rate of *E. coli* from ostrich carcasses was 91%. The highest resistance levels were observed against erythromycin (99%) and neomycin (65%). Additionally, resistance was reported to bacitracin, lincomycin, penicillin, and vancomycin.

Cabassi *et al*. (2001) showed that the isolation rate of *E. coli* from ostrich eggs was 19.3% (105 of 543). The highest antibiotic resistance in these isolates was reported against oxytetracycline, ampicillin, amoxicillin, and clavulanic acid.

A study in Thailand (Sarawoot Mooljuntee *et al*., 2010) demonstrated that all isolated *E. coli* strains from poultry meat exhibited antibiotic resistance rates of 100%, 100%, 73.3%, and 26.7% against tetracycline, ampicillin, erythromycin, and sulfonamide+trimethoprim, respectively. Also, another study revealed that 82.4% of *E. coli* isolates from poultry meat were resistant to tetracycline (Miles *et al*., 2006).

Abbasi (2012) conducted a study in Isfahan, examining the antibiotic resistance profile of *E. coli* O157:H7 found in different poultry meats. Within the O157 isolates, there was the greatest resistance to sulfamethoxazole and vancomycin, and the least resistance to gentamicin and ciprofloxacin.

In a study conducted by Akond *et al*. (2009), it was found that varying percentages of *E. coli* strains from poultry sources were resistant to different antibiotics, ranging from 88% resistance to penicillin to 20% resistance to chloramphenicol and neomycin. Notably, no strains exhibited resistance to norfloxacin or gentamicin.

Environmental and husbandry conditions, such as feeding ostriches with vegetables or fruit waste contaminated with STEC strains, as well as indiscriminate use of antibiotics, are significant risk factors for the transmission of antibiotic-resistant STEC. Various mechanisms contribute to antibiotic resistance:

1. Possession of resistance genes.

2. Production of specific enzymes: Some bacteria produce enzymes that can inactivate or degrade active antibiotics. A notable example is beta-lactamase (penicillinase) production in bacteria resistant to penicillin and cephalosporins.

3. Alterations in bacterial permeability to antibiotics.

4. Active efflux of antibiotics.

5. Changes in drug receptors.

6. Metabolic pathway alterations in resistant bacteria.

7. Modification of target enzymes (Davies and Davies, 2010, Viazis *et al*., 2011, Reygaert, 2018).

In addition to the findings of this study, previous research also indicated a high prevalence of antibiotic resistance in *E. coli* O157:H7 strains isolated from ostriches, particularly against tetracycline, erythromycin, and other common antibiotics. It's not surprising that *E. coli* isolated from ostriches exhibit resistance to these antibiotics, likely due to prolonged use in the ostrich industry.

According to the information gathered from a questionnaire administered to ostrich farm managers in Lorestan province, the only antibiotic used in these farms was tetracycline. However, tetracycline not only lacks efficacy but also leads to widespread resistance against other common antibiotics such as vancomycin, erythromycin, and clindamycin. The indiscriminate use of antibiotics, especially in poultry farming, poses a significant challenge in increasing antimicrobial resistance nationwide. Excessive and inappropriate antibiotic use has resulted in high levels of resistance across various bacterial species. In the present region, ostriches could potentially serve as asymptomatic carriers and disseminators of STEC O157:H7 through direct contact and fecal-oral transmission. Although documented reports of serotype O157:H7 prevalence in humans via food are lacking in Iran, this issue should not be overlooked. Establishing a national monitoring and surveillance program for both O157:H7 and non- O157:H7 STEC strains producing Shiga toxins within food supply systems, especially those of animal origin and hospitals, is essential. As mentioned, *E. coli* O157:H7 strains isolated in this study exhibited resistance to most antibiotics, serving as a serious warning against the inappropriate and incorrect use of antibiotics in these animals. Inadequate antibiotic treatment, leading to a reduction in gut flora, facilitates the survival of *E. coli* O157:H7. To address this dilemma, updating antibiotic prescription methods, implementing antibiotic rotation, and using simple disk diffusion methods are crucial strategies for reducing antibiotic resistance in food-producing animals. The observed differences in results compared to other researchers may be attributed to variations in husbandry practices, climatic conditions, and isolation methods, all of which significantly impact pathotype prevalence in a given geographical area. Further studies and epidemiological analyses are necessary to identify the importance and role of ostriches in the distribution of STEC pathotypes and antibiotic-resistant strains in the environment.

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Conflict of Interest

No conflict of interest has been reported among the authors.

References

Cabassi, CS; Taddei, S; Predari, G; Galvani, G; Ghidini, F; Schiano, E and Cavirani, S (2004). Bacteriologic findings in ostrich (*Struthio camelus*) eggs from farms with reproductive failures. Avian Dis., 48: 716-722.

Capita, R and Alonso-Calleja, C (2013). Antibiotic-resistant bacteria: a challenge for the food industry. Crit. Rev. Food Sci. Nutr., 53: 11-48.

Carneiro, C; Araujo, C; Goncalves, A; Vinue, L; Somalo, S; Uliyakina, I; Rodriques, J; Igreias, G; Poeta, P and Torres, C (2010). Detection of CTX-M-14 and TEM-52 extended-spectrum beta-lactamases in fecal *Escherichia coli* isolates of captive ostrich in Portugal. Foodborne Pathog. Dis., 7: 991-994.

Davies, J and Davies, D (2010). Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev., 74: 417-433.

Furtula, V; Farrell, EG; Diarrassouba, F; Rampel, H and Pritchard, J (2010). Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. Poult. Sci., 89: 180-188.

Ge, H; Wang, Y and Zhao, X (2022). Research on the drug resistance mechanism of foodborne pathogens. Microb. Pathog., 162: 105306.

Giraffa, G (2002). Enterococci from foods. FEMS Microbiol. Rev., 26: 163-171.

İnanc, A and Mustafa, AS (2018). Antibiotic resistance of *Escherichia coli* O157: H7 isolated from chicken meats. KSÜ Doğa Bilimleri Dergisi, 21: 7-12.

Kazemnia, A; Ahmadi, M and Dilmaghani, M (2014). Antibiotic resistance pattern of different *Escherichia coli* phylogenetic groups isolated from human urinary tract infection and avian colibacillosis. Iran. Biomed. J., 18: 219.

Lee, JH and Choi, SJ (2006). Isolation and characteristics of sorbitol-fermenting *Escherichia coli* O157 strains from cattle. Microb. Infect., 8: 2021-2026.

Levy, SB (1998). The challenge of antibiotic resistance. Sci. Am., 278: 46-53.

Ley, EC; Morishita, TY; Brisker, T and Harr, BS (2001). Prevalence of *Salmonella*, *Campylobacter*, and *Escherichia coli* on ostrich carcasses and the susceptibility of ostrich-origin *E. coli* isolates to various antibiotics. Avian Dis., 45: 696-700.

Manges, A (2016). *Escherichia coli* and urinary tract infections: the role of poultry-meat. Clin. Microbiol. Infect., 22: 122-129.

Marangoni, F; Corsello, G; Cricelli, C; Ferrara, N; Ghiselli, A; Lucchin, L and Poli, A (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. Food Nutr. Res., 59: 27606.

Miles, TD; McLaughlin, W and Brown, PD (2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC Vet. Res., 2: 1-9.

Mooljuntee, S; Chansiripornchai, P and Chansiripornchai, N (2010). Prevalence of the cellular and molecular antimicrobial resistance against *E. coli* isolated from Thai broilers. Thai J. Vet. Med., 40: 311-315.

Mushtaq, H; Bakht, J and Bacha N (2015). Screening of the novel colicinogenic gram-negative rods against pathogenic *Escherichia coli* O157: H7. Indian J. Med. Microbiol., 33: S67-S72.

Nataro, JP and Kaper, JB (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.

Newell, DG; 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. Int. J. Food Microbiol, 139: S3-S15.

Reygaert, WC (2018). An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol., 4(3): 482.

Rezaei Far, A; Peighambari, SM; Sadrzadeh, A and Askari Badouei, M (2013). Bacterial contamination of dead-in-shell embryos in ostrich hatcheries and antimicrobial resistance patterns of isolated *Escherichia coli*. Iran. J. Vet. Med., 7: 169-175.

Romanus, I (2012). Antimicrobial resistance of *Escherichia coli* isolated from animal and human clinical sample. Global Res. J. Microbiol., 2: 85-89.

Schoeni, JL and Doyle, MP (1994). Variable colonization of chickens perorally inoculated with *Escherichia coli* O157: H7 and subsequent contamination of eggs. Appl. Environ. Microbiol., 60: 2958-2962.

Shecho, M; Thomas, N; Kemal, J and Muktar, Y (2017). Cloacael carriage and multidrug resistance *Escherichia coli* O157: H7 from poultry farms, eastern Ethiopia. J. Vet. Med., 2017.

Simonsen, GS; Tapsall, JW; Allegranzi, B; Talbot, EA and Lazzari, S (2004). The antimicrobial resistance containment and surveillance approach-a public health tool. Bull. World Health Organ., 82: 928-934.

Singh, OV (2017). Food borne pathogens and antibiotic resistance, John Wiley & Sons.

Stampi, S; Caprioli, A; De Luca, G; Quaglio, P; Sacchetti, R and Zanetti, F (2004). Detection of *Escherichia coli* O157 in bovine meat products in northern Italy. Int. J Food Microbiol., 90: 257-262.

Sun, DS; Kissler, SM; Kanjilal, S; Olesen, SW; Lipsitch, M and Grad, YH (2022). Analysis of multiple bacterial species and antibiotic classes reveals large variation in the association between seasonal antibiotic use and resistance. PLoS Biol., 20: e3001579.

Viazis, S; Akhtar, M; Feirtag, J and Diez-Gonzalez, F (2011). Reduction of *Escherichia coli* O157: H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. Food Microbiol., 28: 149-157.