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ORIGINAL RESEARCH PAPER

# Occurrence and Antibiogram of Non-Sorbitol Fermenting *Escherichia coli* in Marketed Raw Meat of Dharan, Eastern Nepal

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## Abstract

*This study aimed to explore the distribution of non-sorbitol fermenting Escherichia coli (E. coli) from meat marketed in Dharan city and study its susceptibility to antibiotics. This study was the laboratory based cross-sectional study conducted from December 2016 to May 2017 at Microbiology laboratory of Central Campus of Technology. A total of 24 meat samples from butcher's retail shop of Dharan were taken for study that included 6 chickens, 6 buffalo, 6 pork, and 6 goat meat sample. The bacterial isolates from meat samples were isolated by routine microbiological procedures and identified by colony characteristics on selective medium, Gram's staining and biochemical tests. The antibiotic susceptibility test (AST) of the isolated bacteria was performed by Kirby–Bauer disc diffusion method. Results reported 41.66% (10/24) prevalence of non-sorbitol fermenting E. coli in meat samples. However, this distribution was not statistically significant (p=0.877). The prevalence of E. coli was 3 (50%) in chicken, 3 (50%) in buffalo, 2 (33.33%) in pork and 2 (33.33%) in goat meat. All the isolated E. coli were subjected to the antibiotic susceptibility test using 17 different antibiotics and all the strains showed 100% resistance against ampicillin, amoxicillin and ceftazidime and the highest sensitivity towards gentamycin (90%), ceftriaxone (80%), amikacin (80%) and chloramphenicol (80%). The 100% multidrug resistance was observed in all the isolates. This study concludes that the meat consumers of Dharan are at higher risk of infection by pathogenic strain of E. coli. The increasing incidence of multi drug resistance of pathogenic strains may pose serious health ailment among semi-processed meat consumers whilst the cooked meat consumers too are at risk of toxin-mediated food poisoning.*

**Keywords:**
Multidrug resistance
*Escherichia coli* O157:H7
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## Introduction

Food safety is one of the leading issues for the agricultural industry, including livestock production sector (Bousfield and Brown, 2011). The global burden of foodborne diseases has caused rapid morbidity and mortality (WHO, 2015). The microbial food-borne illness still remains a global concern in developing countries (Odeyemi, 2016). Such problems occur because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory system, lack of financial resources to invest in safer equipment and lack of education for food-handlers (Haileselassie et al., 2013). Foodborne disease outbreaks are caused by undercooked meat, raw meat handling, and cross contamination of ready-to-eat items with microbial contaminants from raw poultry or

others introduced during food preparation (Khalafalla et al., 2015).

Cattle have been recognized as a key reservoir of *E. coli* O157:H7 for human infection, but less is known about the organism's ecology in sheep and goats (Abrehan et al., 2019). In underdeveloped nations, food-borne infections are the major cause of disease and death, costing billions of dollars in medical care and medical and societal expenses. Changes in eating patterns, mass catering, complicated and long food supply systems associated with greater international migration, and inadequate sanitary standards are all key contributors for food-borne diseases. One of the most common causes of food poisoning is contaminated raw meat. Contaminated meat is also linked to the spread of zoonotic illnesses (Ali et al., 2010). Raw meat, salads, and unpasteurized dairy products are all high-risk food sources, increasing the risk of food-borne illness. As a result, Lebanon recorded 1926 instances of food-borne disease in 2003 and Libya reported 779 cases in 2004, while Oman reported 112,904 cases of acute gastroenteritis and diarrhea in 2002 (Al-Ajmi et al., 2020).

Among the pathogenic strains identified, the commonest is the Enterohaemorrhagic *E. coli* (EHEC), linked to serious outbreaks and sporadic cases of enterohaemorrhagic diseases (Bolton et al., 2011). Human infections with *E. coli* O157:H7 have been mostly associated with the consumption of contaminated and improperly cooked meat (Doyle, 1991). Contaminated raw meat and retail meat shops are potential vehicles for transmitting food borne illness (Regunath and Salza, 2016). Meat sold in stores has gone through a protracted process of killing and shipping, with each stage posing a danger of germ contamination (Upadhayaya and Ghimire, 2018). When these organisms colonize a piece of meat, they begin to break it down leaving behind toxins that can cause enteritis or food poisoning. They do not survive a thorough cooking, but several toxins and spores can survive (Lawrie and Ledward, 2006). *E. coli* O157:H7, termed as an enterohaemorrhagic *E. coli* (EHEC) is one of the most significant food-borne pathogens. It differs from most other strains of *E. coli* in being unable to ferment sorbitol. Typical illness because of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including hemolytic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura (Ojeda et al., 1995; Rahal et al., 2012; Chileshe and Ateba, 2013).

The majority of *E. coli* are typical commensals present in both people and animal's digestive tracts, whereas some are dangerous to humans. *E. coli* O157: H7 is a well-known serotype that has pathotypes that may infect people through food. In the intestines of healthy cattle, deer, goats, and sheep, *E. coli* O157:H7 pathotypes have been discovered (Bekele et al., 2014). Despite the existence of other serotypes, O157:H7 is the most usually reported in outbreaks (Lupindu et al., 2014). The pathogenicity of *E. coli* O157:H7 is affected by several virulence factors. The main factor contributing to the pathogenicity is its ability to produce potential cytotoxins called Shiga-toxins (Stx), encoded by stx1 and stx2 genes (Gyles, 2007).

Cattle are thought to be the main reservoirs of Shiga toxin producing *E. coli* (STEC). However, STEC has been isolated even from other species (Terajima et al., 2017). The presence of antibiotic resistance isolates and the discovery of *E. coli* O157:H7 in raw meat emphasize the potential hazard to public health (Bekele et al., 2014)

Treatment of enterohemorrhagic *E. coli* infection with anti-microbial agents may worsen the infection, apparently by breaking up the bacteria with the discharge of toxins. However, early use of some antimicrobials is effective. Improper ways of antimicrobial use have contributed to the increment in antimicrobial drug resistance (Beyi et al., 2017). Even though agricultural settings account for the bulk of antibiotic usage, little emphasis has been made to how antibiotic use in farm animals contributes to the general problem of antibiotic resistance (Landers et al., 2012).

Meat can be a source of antibiotic-resistant bacteria, which can then spread across the community via the food chain (Gousia et al., 2011). Antibiotic-resistant *E. coli* 0157:H7 strains discovered are of public health concern (Goncuoglu et al., 2010). Although *E. coli* infections often cause mild to moderate self-limiting gastroenteritis, invasive illnesses and complications can arise, leading to more serious cases (Zhao et al., 2001). Multi drug resistance obstructs disease control by increasing the risk of resistant microorganisms spreading, lowering treatment efficacy and, as a result, prolonging the duration of infection in the patient (Tanwar et al., 2014).

In Dharan, only few small-scale studies estimating the prevalence and/or assessing the antimicrobial sensitivity profile of *E. coli* were conducted. The studies at the level of butcher shops and enterohemorrhagic *E. coli* O157:H7 are lacking. Therefore, this study was designed to address the information gap pertaining to the prevalence and antibiotic susceptibility profiles of non-sorbitol fermenting *E. coli* O157:H7 isolated from meat marketed in Dharan city.

## Materials and Methods

### Study site and study sample

This was a laboratory based cross-sectional study carried out in Dharan Sub-metropolitan city from December 2016 to May 2017. Dharan is a small city located in Sunsari district (Latitude: 26° 48' 44.93" N; Longitude: 87° 17' 0.78" E), Sunsari Nepal with 2112-hectare area and located in the eastern Terai of Nepal stretching from the edge of northern Mahabharat hill range up to the Charkoshe Jhadi in south separating from the southern Terai (Figure 1). The samples sites were selected by simple random sampling following lottery method from meat retail shops of different locations of Dharan Sub metropolitan city. The study samples were a total of 24 meat samples (6 chicken, 6 buffalo, 6 pork and 6 goat) collected from respective 24 meat shops of local market of Dharan. The average meat consumption by Nepalese people is high (ABPSD, 2005). Apparently the hygienic, condition of meat sold in Dharan market is very poor. Despite the wide presence of the problems, there is still lack of comprehensive survey of food borne pathogens. Therefore, Dharan was selected as study site to assess microbiological quality of meat.

### Sample collection and transport

Each sample of mass 25 g was collected in sterile plastic bags avoiding possible contamination. Meat samples were collected between 8:00 to 9:00 AM, maintained in ice cold box and were transported to the microbiology laboratory of Central Campus of Technology, Tribhuvan University, Dharan, Nepal and processed within 2 h of collection. In case of delay the samples were preserved at 4℃ for not more than 24 h (U.S FDA, 2015).

### Experimental Design

### Processing of meat sample

Processing of sample and isolation of *E. coli* was done as described by Sheikh et al. (2013). Meat sample (25 g) was aseptically transferred into a sterile beaker and was disinfected with 70% v/v ethanol and then it was later rinsed with 100 ml double distilled sterile water. The sample was homogenized with help of meat mincer (Philips Ltd., India). Then the homogenate was poured into the conical flask containing 225 ml of EC0157:H7 Enrichment Broth (HiMedia, India) and incubated at 37℃ for 24 h.

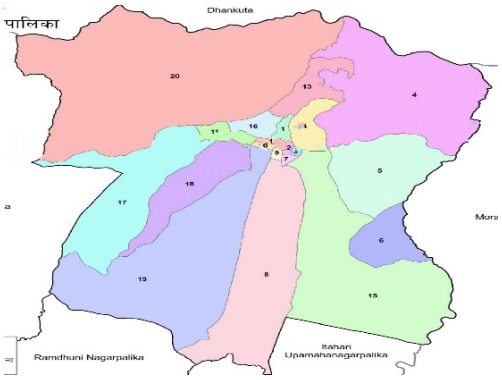


**Figure 1**
Map of study site, Dharan Sub-Metropolitan city

### Screening of non-sorbitol fermenting *E. coli*

After the end of incubation, a 100 µl of suspension was inoculated and streaked on surface of Sorbitol MacConkey agar (SMAC) (HiMedia, India) and were incubated at 37℃ for 24 h. After incubation, the plates were observed for the round, smooth and colorless colonies. Suspected non-sorbitol fermenting colonies were selected and streaked on Eosin Methylene Blue (EMB) (HiMedia, India) and plates were incubated at 37℃ for 24 h. After 24 h of incubation, colonies producing green metallic sheen were streaked on Nutrient Agar (NA) (HiMedia, India) and incubated at 37℃ for 24 h. Further, the pure culture was inoculated in the test tube containing sorbitol broth and incubated at 37℃ for 16-18 h for reconfirmation of non-sorbitol fermenting *E. coli*. After incubation, the test tubes with no color change suspected of non-sorbitol fermenting *E. coli* isolates were selected and streaked again on Nutrient Agar. After obtaining the pure culture, the organisms were identified by using Standard Microbiological Techniques as described in Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005). *E. coli* isolates were identified based on evidence from colony characteristics, Gram's staining and biochemical tests like Indole test, Methyl red test, Voges-Proskauer test, Simon Citrate Agar test, Urease production test, and SIM test (Sulfur, Indole, Motility).

### Antibiotic Susceptibility Testing

Antimicrobial Susceptibility Test of isolated bacteria was done as described by the Modified Kirby Bauer Disk Diffusion method following Clinical and Laboratory Standards Institute guidelines (CLSI, 2015). Fresh 4-5 colonies were selected and transferred into the 5 ml Nutrient Broth (NB) (HiMedia, India) to obtain turbidity equivalent to 0.5 McFarland standards ($1.5x10^8$ CFU/ml). Mueller Hinton Agar (HiMedia, India) plates were inoculated with bacterial suspension

through cotton swabs. Then antibiotic discs were placed with sterile forceps and allowed to stand at room temperature for 15 min for pre-diffusion followed by incubation at 37℃ for 24 h. A wide range of antibiotics namely Amoxicillin, Ampicillin, Cefotaxime, Cefixime, Amikacin, Tetracycline, Ceftriaxone, Ceftazidime, Ciprofloxacin, Chloramphenicol, Nalidixic acid, Erythromycin, Nitrofurantoin, Teicoplanin, Imipenem, Cotrimoxazole and Gentamycin were used for antibiotic susceptibility test. The zones of inhibition were interpreted as susceptible, intermediate and resistant.

### Quality Control

Aseptic condition was maintained during media preparation, sample collection, sample processing and culture identification. Strain of *E. coli* ATCC 25922 was obtained from Department of microbiology, Central Campus of Technology, Dharan and was used as a positive control. Before data collection, 5% of pretest was done at the meat shops. Reagents and culture media were regularly monitored for expiry date and proper storage. Laboratory equipment like incubator, refrigerator, autoclave and hot air oven were regularly monitored.

### Data Analysis

The data was documented and tabulated in MS Excel 2010. The data were statistically analyzed by Chi-square test to establish the association between variables at 95% level of confidence by SPSS version 16.0 and $p<0.05$ was considered to be statistically significant.

## Results

### Prevalence of non-sorbitol fermenting *E. coli*

In this study, the incidence of non-sorbitol fermenting *E. coli* in total meat samples was found to be 10 (41.66%). The incidence of non-sorbitol fermenting *E. coli* was 3 (50%) in chicken meat, 3 (50%) in buffalo-meat, 2 (33.33%) in pork and 2 (33.33%) in goat meat ($p=0.877$). The highest occurrence of non-sorbitol fermenting *E. coli* was found in chicken and buffalo-meat followed by lower incidence in pork and goat-meat (Table 1).

**Table 1**
Occurrence of sorbitol non-fermenting *E. coli*

| *Meat sample (n)* | *Prevalence of non- sorbitol fermenting E. coli (%)* | *Chi-square value (p-value)* |
|---|---|---|
| ***Chicken (n=6)*** | 3 (50%) | 0.686 (0.877) |
| ***Buffalo-meat (n=6)*** | 3 (50%) | |
| ***Pork (n=6)*** | 2 (33.33%) | |
| ***Goat-meat (n=6)*** | 2 (33.33%) | |
| ***Total (n=24)*** | 10 (41.66%) | |

### Colonial characteristics and biochemical reactions

The non-sorbitol fermenting *E. coli* isolates were colorless, round configured, smooth margin, raised elevation, Gram-negative (Table 2).

**Table 2**
Colonial characteristics and biochemical reactions of non-sorbitol fermenting *E. coli*

| *Organism* | *Colony characteristics* | | | | *Biochemical reactions* | |
|---|---|---|---|---|---|---|
| ***E. coli*** | *Configuration* | *Margin* | *Elevation* | *Color* | *Test* | *Result* |
| | Round | Smooth colonies with entire edge | Raised | Colorless and yellowish white | Gram's stain | - |
| | | | | | Catalase | + |
| | | | | | Oxidase | - |
| | | | | | Indole | + |
| | | | | | MR | + |
| | | | | | VP | - |
| | | | | | Citrate | - |
| | | | | | SIM | + |

### Antibiotic Susceptibility Pattern of *E. coli*

All *E. coli* isolates exhibited 100% resistance to amoxicillin, ampicillin and ceftazidime and highest sensitivity towards gentamycin (90%), amikacin (80%), ceftriaxone (80%) and chloramphenicol (80%) (Table 3).

**Table 3**
Antibiotic susceptibility pattern of non-sorbitol fermenting *E. coli*

| *Antibiotics* | *Resistant* | | | | *Intermediate* | | | | *Sensitive* | | | | *Chi-square (p-value)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | B | P | G | C | B | P | G | C | B | P | G | |
| ***Amoxicillin (AMX)*** | 30% | 30% | 20% | 20% | - | - | - | - | - | - | - | - | - |
| ***Ampicillin (AMP)*** | 30% | 30% | 20% | 20% | - | - | - | - | - | - | - | - | - |
| ***Ceftazidime (CAZ)*** | 30% | 30% | 20% | 20% | - | - | - | - | - | - | - | - | - |
| ***Cefotaxime (CTX)*** | 30% | 10% | 20% | 20% | - | 20% | - | - | - | - | - | - | 11.52 (0.001) |
| ***Ceftriaxone (CTR)*** | 10% | - | - | - | - | - | - | 10% | 20% | 30% | 20% | 10% | 14.70 (0.001) |
| ***Cefixime (CFM)*** | 10% | - | - | - | 10% | 10% | - | 20% | 10% | 20% | 20% | - | 3.90 (0.142) |
| ***Clotrimazole (COT)*** | 10% | 10% | - | 10% | - | 10% | - | 10% | 20% | 10% | 20% | - | 5.7 (0.058) |
| ***Amikacin (AK)*** | 10% | - | - | - | - | - | - | 10% | 20% | 30% | 20% | 10% | 14.70 (0.001) |
| ***Gentamycin (GEN)*** | - | - | - | - | 10% | - | - | - | 20% | 30% | 20% | 20% | 21.9 (p<0.001) |
| ***Tetracycline (TE)*** | 10% | 10% | - | 10% | - | - | - | - | 20% | 20% | 20% | 10% | 11.10 (0.004) |
| ***Nalidixic acid (NA)*** | 20% | 30% | - | 10% | - | - | - | - | 10% | - | 20% | 10% | 8.4 (0.015) |
| ***Ciprofloxacin (CIP)*** | 10% | 20% | - | 10% | 20% | 10% | 20% | 10% | - | - | - | - | 0.800 (0.371) |
| ***Chloramphenicol(C)*** | - | 10% | - | - | - | - | - | 10% | 30% | 20% | 20% | 10% | 14.70 (0.001) |
| ***Imipenem (IPM)*** | 20% | 10% | - | - | - | 10% | 10% | 20% | 10% | 10% | 10% | - | 0.30 (0.861) |
| ***Erythromycin (E)*** | 20% | 20% | - | 10% | 10% | 10% | 20% | 10% | - | - | - | - | 0.00 (1.00) |
| ***Teicoplanin (TEI)*** | 30% | 20% | 20% | 20% | - | - | - | - | - | 10% | - | - | 12.80 (p<0.001) |
| ***Nitrofurantoin (NFT)*** | 20% | - | - | 10% | 10% | 20% | - | 10% | - | 10% | 20% | - | 0.300 (0.861) |

***Abbreviation***. C-Chicken meat, B-Buffalo-meat, P-Pork and G-Goat-meat

## Discussion

Semi-processed meat and its products are commonly consumed in traditional Nepalese diets. Different *E. coli* strains such as enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli*, enteroaggregative *E. coli* (EAggEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) cause diarrhea in humans. Although other serotypes exist, the most commonly reported enterohaemorrhagic *E. coli* in epidemic outbreaks is non-sorbitol fermenting *E. coli* O157:H7 (Lupindu et al., 2014). EHEC also referred as Shiga toxin (verotoxin)-producing *Escherichia coli* (STEC) resides in the gastrointestinal tract of cattle and other ruminants where contamination of meat with STEC during slaughter is a principal route by which these pathogens enter the food supply (Terajima et al., 2017). Sorbitol negative *E. coli* was commonly encountered from several clinical infections. Ojeda et al. (1995) described that all the EHEC strains from patients with Hemolytic-uremic syndrome, irrespective of O:H serotype or shiga-like toxin genotype, were sorbitol negative.

In the present study, the prevalence of non-sorbitol fermenting *E. coli* was found to be high 10 (41.66%) in comparison to other studies performed by Bekele et al. (2014), Gousia et al. (2011) and Zhao et al. (2001). The distribution of *E. coli* in different meat samples of the present study was not statistically significant (p=0.877). Study by Khalafalla et al. (2015) reported highest prevalence of *E. coli* from meat samples which was not in agreement to this study.

Antimicrobial resistance emerges from the use of antimicrobials in animals and human, and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products and the environment (Goncuoglu et al., 2010). With regard to the antibiogram of *E. coli* in the current study, all the 10 *E. coli* isolates subjected to antimicrobial sensitivity test were found to be 100% resistant against amoxicillin, ampicillin and ceftazidime which is in agreement with the result of Ali et al. (2010). According to Al-Ajmi et al. (2020), isolated *E. coli* were 100% susceptible to cefotaxime and chloramphenicol but in this study the isolates were 80% susceptible to chloramphenicol and 80% resistant to cefotaxime. In this study, highest prevalence of antibiotic drug resistance pattern was observed in *E. coli* isolates from chicken meat. The possible reason is the unauthorized use of antibiotics in poultry feed in order to improve livestock production. Irrational use of antibiotics drugs induces drug resistance in gut flora. Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans (Landers et al., 2012).

Multidrug resistance (MDR) is defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it (Tanwar et al., 2014). Multidrug resistance *E. coli* was dominant (100%) in the present study. This finding was similar to the findings made by Bekele et al. (2014). According to Abreham et al. (2019), *E. coli* isolates were moderately susceptible to ceftazidime, which did not agree to the results of current study.

Lack of meat inspection, infrastructure, well-trained workers, use of unhygienic water and selling of meat in open places and poor sanitation of meat shop and meat animals play additive role to increase the rate of contamination in meat and increase the risk of acquiring the food borne illness via the consumption of meat (Upadhayaya and Ghimire, 2018). Microbiological quality is important from public health point of view. These findings are able to demonstrate the current status of hazards associated with contaminated meat consumption.

## Conclusion

In conclusion, the present study reported high prevalence of non-sorbitol fermenting *E. coli* O157:H7 in meat samples with high antibiotic drug resistance pattern to wide range of antibiotics. Poor hand hygiene, use of untreated water, lack of sanitation and equipment can function as a source for bacterial contamination of meat. In order to prevent such contamination butchers, have to improve their hygiene practice.

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## Compliance with Ethical Standards

### Conflict of Interest

The authors declare no conflict of interest.

### Ethical approval

The study did not involve any inhumane animal study.

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