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ORIGINAL RESEARCH

Occurrence of Multi-Drug Resistant *Escherichia Coli* and *Escherichia Coli* O157:H7 in Meat and Swab Samples of Various Contact Surfaces at Abattoir and Butcher Shops in Jimma Town, Southwest District of Ethiopia

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Infection and Drug Resistance

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Background: Raw meat is one of the commonly consumed traditional diets in Ethiopia. However, unhygienic processing and distribution practices are risky for contamination of meat leading to human infection. This study was conducted to assess the presence of multi-drug resistant *E. coli* with special emphasis on *E. coli* O157:H7 from meat of cattle and swab samples at abattoir houses and butcher shops in Jimma town, Southwest district of Ethiopia.
Methodology: A cross-sectional descriptive study was conducted from April to July, 2018. The isolation and identification processes passed through enrichment of samples with modified tryptone soy broth (mTSB), streaked onto MacConkey agar and Cefixime-tellurite sorbitol MacConkey agar, biochemical testing (indole and TSI), followed by latex agglutination testing.
Results: Out of 505 samples, 102 (20.2%) and 27 (5.4%) were positive for *E. coli* and *E. coli* O157:H7, respectively. Of these, 55 (19.3%) and 47 (21.4%) of *E. coli* and 17 (6.0%) and 10 (4.5%) of *E. coli* O157:H7 were isolated from the abattoir and butcher shop samples, respectively. A significant difference in the occurrences was observed among sample sources. Antimicrobial susceptibility test results showed that, 92.2% to 96.1% of *E. coli* and 85.5% to 96.3% of *E. coli* O157:H7 were susceptible to third generation cephalosporin, ciprofloxacin, gentamycin, kanamycin, streptomycin, and chloramphenicol. About 91.2% and 97.1% of *E. coli* and 88.9% and 92.6% of *E. coli* 0157:H7 were resistant to ampicillin and erythromycin, respectively. A total of 57 (44.2%) *E. coli* and *E. coli* O157:H7 isolates were resistant to three or more classes of antibiotics. All abattoir and butcher shop workers did not have any formal education or training certificates on food safety, and unhygienic practices were also observed.
Conclusion: The presence of *E. coli* and *E. coli* O157:H7 including multi-drug resistant isolates in raw meat highlights how the current meat processing and distribution practice was unhygienic. Therefore, strategies in the prevention and control of food-borne infections that could be caused by multi-drug resistant strains will depend greatly on hygienic processing and distribution practices of meat.

Keywords: multi-drug resistant *E. coli* and *E. coli* O157:H7, abattoir and butcher shops, Jimma town

Background

Escherichia coli are a member of the family *Enterobacteriaceae*, and are normal inhabitants of the gastrointestinal tract of animals and humans. Some strains, such

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as shiga toxin-producing enterohemorrhagic *E. coli* (EHEC) O157:H7 is the predominant and most virulent serotype associated with bloody and non-bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS).[1,2] Ruminants are said to be reservoirs, whereby cattle are regarded as principal sources of infections. Ingestion of *E. coli* O157:H7 with contaminated food products of animal origins and following contact with infected animals or the contaminated environment has led to human infection.[3,4]

In the past two decades, isolation of the pathogens, including STEC O157:H7 from animals, food, clinical samples, and the environment has been reported from all continents.[5] Globally, STEC O157:H7 causes 2, 801, 000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 persons per year.[5] Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 people per year.[5] Ruminants, particularly cattle and sheep, seem to be the maintenance hosts for EHEC O157:H7.[6]

In developing countries, animals are commonly slaughtered and dressed under unhygienic conditions and this further compromises the microbiological quality and safety of the meat obtained from the animals.[7,8] For instance, fecal carriage of *E. coli* O157:H7 in animals provides the potential for these organisms to enter the food chain via fecal contamination of milk, contamination of meat with intestinal contents during slaughter or contamination of fruit and vegetables by contact with contaminated manure. During slaughter, the pathogen may be present on the skin or in the feces of the animal, and may get transferred to the carcass during evisceration or skin removal. Therefore, poor slaughter processes, particularly poor hygienic practices during slaughtering, transport and display of meat play a large role in increasing meat contamination. Thus, hygienic management of animal and their food products, especially during slaughtering and display of meat for sale remains a better option in the control of *E. coli* O157 transmission.

Additionally, antimicrobial resistance among enteric bacteria is an increasing global public health concern. In Ethiopia also, the occurrence of *E. coli*, including multidrug resistance in foods of animal origin is arguably high due to many reasons like unhygienic slaughtering practices in the abattoirs, illegal slaughtering of animals in open fields, poor meat transport, and display conditions at butcher shops.[9,10] In addition, indiscriminate use of antimicrobial agents for growth promotion in livestock and for treatment of diseased animals may lead to the development of considerable resistant bacterial strains, in which it can be transmitted to humans through the food chain.[11] This may pose a potential risk for the occurrence of foodborne disease because of a widespread tradition of raw meat consumption in our country.

There is a need to investigate the possible sources of STEC O157:H7 and to quantify risk factors to ensure that, prevention and control strategies are appropriate. However, in Ethiopia, the precise attribution of animals and their food products as the sources of resistant strains, and the consequences of it on human health have not yet been seriously evaluated. Therefore, this study was conducted to assess the presence of multi-drug resistant *Escherichia coli* with special emphasis on *Escherichia coli* O157:H7 from meat of cattle, cecal contents of slaughtered animals and swabs of contact surfaces at abattoir houses and butcher shops in Jimma town, Southwest district of Ethiopia.

Methodology

Study Area and Period

This study was conducted in Jimma town, Southwest district of Ethiopia from April to July, 2018. Jimma town is located at 346 km, Southwest of Addis Ababa, capital city of Ethiopia. The town is divided into 17 administrative kebeles. According to the information obtained from Jimma town trade and industry office, at the time of this study, there was one municipality abattoir which gives a slaughtering service to provide raw meat for around 80 officially registered butcher shops and for one government university cafeteria in the town.

Study Design and Sampling

A cross-sectional study was conducted and apparently healthy slaughtered cattle (n= 90) in Jimma municipal abattoir were selected randomly. Samples collected at the abattoir included; meat (n= 90) and cecal contents of slaughtered animals (n= 90), swabs from abattoir environments (eviscerator's knife (n= 20) and hands (n= 30), cutting boards (n= 20), transporter clothes (n= 20) and meat transport vehicles (n=15)), whereas from the butchers shops, meat (n= 90) and swab samples of knife (n= 30), butcher's hand (n= 40), cutting board (n= 30) and protective cloths (n= 30) were collected over a period of 8 weeks. Sampling points in the abattoir and at butcher shops were selected according to the problem areas in the process (after dehiding, after evisceration and after

display for sale). To collect the data, one visit to the municipal abattoir and all officially registered butcher shops (n=80) were made per week (on Saturday) for a consecutive 8 weeks. The reason is that the numbers of slaughtered animals were increased on Saturday; because, most local people prefer to buy and eat raw meat at the weekend.

Collection of Samples

Meat Sample Collection

During the visit, a total of 180 meat samples (90 from abattoir house and the other 90 from butcher shops) were collected from different parts of the carcasses (abdomen [flank], thorax (lateral) and breast (lateral), brisket and crutch) at abattoir house and from whole cuts of raw meat at butcher shops as per the International Organization for Standardization (ISO).[12] A total of 25 g of meat were taken from the relevant places of the carcasses at abattoir house and from whole cuts of raw meat at butcher shops using sterile scalpels and forceps and put into a sterile, separately labeled plastic bag and each pooled meat sample was thoroughly homogenized. Meat samplings were made after the removal (evisceration) of the gastrointestinal tract and after displayed for sale at butcher shops. After each sampling, separate scalpel and forceps were cleaned with pieces of gauze dipped in 70% ethanol to minimize cross-contaminations.

Cecal Content Collection

Ten gram (10 g) of cecal content was collected from 90 slaughtered cattle using sterile, wide mouthed and leak proof containers immediately after evisceration. Incision of the cecum was made with sterile surgical blade and fecal material was aseptically compressed to obtain a representative sample of the cecal content. All the collected samples were transported to the laboratory in a cool box on ice and analyzed within 24 hours of sampling.

Environmental Samples Collection

A total of 235 swab samples (105 from the abattoir and 130 from butcher shops) were collected from the hands of meat handlers, their protective clothing, knives and chopping boards after eviscerations at abattoir house and during the beginning of the operation at the butcher shops. In addition, swab samples were taken from vehicles used for distribution of meat to different butcher shops. The swab samples were taken from 15–20 cm^2 of meat surface contact using sterile, buffered peptone water moistened cotton swabs. All the collected samples were labeled, packaged in sterile, separate containers and carried to the microbiology laboratory of Jimma University in a cold box immediately after collection for processing.

Data on Hygienic Practices of Abattoir and Butcher Shop Workers

Data on hygienic practices of abattoir and butcher shop workers were collected with a structured checklist and by direct observations. During the visits, about 15 from abattoir house and 30 butcher shop workers were checked as to whether they had educational and training certificates on food safety or not. In addition, the workers were checked as to whether their meat processing and handling practices were safe or not by using observational checklists. Data related to educational levels of meat processors and availability of clean tap water, regular hand washing and disinfection practice of hands, the floor and processing tools and vehicles before and during their work, whether they used clean protective cloths, whether they used separate, washable chopping boards and knives for processing of abdominal organs and other parts of meat, whether the same buckets of water were used for cleaning knives, washing hands, and whether fisting was done with most care to avoid carrying dirt were checked.

Isolation and Identification Process

Enrichment Processes

From 25-g meat samples, 10 g each was weighed and enriched into an Erlenmeyer flask containing 90 mL of modified tryptone soya broth (mTSB) (Oxoid Ltd., Hampshire, UK) and incubated at 37°C for 24 hours. Similarly, all swab samples and 1 g of cecal contents from the slaughtered cattle was homogenized in 9 mL of mTSB and was incubated at 37°C for 24 hours.[13]

Identification of the Isolates

The overnight broth cultures were spread onto MacConkey agar and Sorbitol MacConkey agar (Oxoid Ltd., Hampshire, UK) supplemented with 0.05 mg/l Cefixime- 2.5 mg/l potassium tellurite.[13] The inoculated plates were incubated at 37°C for 24 hours. The isolated colonies first were screened by colony morphology, color production (lactose fermenting pink colonies) and identified as *E. coli* by relevant biochemical tests, such as indole and triple sugar iron (TSI) tests. Three to five non- sorbitol fermenter, colorless with a small, round, weak pale brownish appearance *E. coli* O157:H7 suspect colonies were streaked onto non-selective media

(ie, nutrient agar) and incubated at 37°C for 24 hours for confirmation by indole production and latex agglutination test kits (Oxoid Ltd., Hampshire, UK), following the manufacturer's instructions. From a pure culture on nutrient agar plates 1 µL loop full were inoculated onto tryptophane medium and after overnight incubation of the plate at 37°C, 1 mL of the Kovacs reagent was added to the medium to confirm indole production (pink ring). Similarly, using a sterile wire loop, a single colony was taken and carefully emulsified in the drop of saline and then the suspension was mixed into the dry latex spots and spread to cover the reaction area. A result was positive if agglutination of the latex particles occurred within 1 minute. To be sure of the test results, first the isolates were tested with the control O157 latex reagent provided with the kit and then with H7 latex reagent. *E. coli O157* (CCUG 29889) and *E. coli (*ATCC25922) used as positive and negative control for reproducibility of Sorbitol MacConkey agar plates, respectively.

Antibiotic Sensitivity Testing

Antimicrobial susceptibility testing were done for all confirmed *E. coli* and *E. coli* O157:H7 isolates by using Kirby-Bauer disc diffusion technique for the following 13 antimicrobial agents (Oxoid Ltd., Hampshire, UK): cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CTZ, 30 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), ampicillin (AMP, 10 µg), gentamicin (GEN,10 µg), ciprofloxacin (CIP, 5 µg), erythromycin (ERY, 15 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), tetracycline (TET,30 µg) Kanamycin (KAN, 30 µg), chloramphenicol (CHL, 30 µg), and spectinomycin (STR, 100 µg). First, the suspension was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Mueller-Hinton agar (MHA) and the above antimicrobial agents were placed using sterile forceps and pressed gently to ensure the contact of a medium. After overnight incubation of the plate at 37°C, the zone of inhibition was measured by using sliding calipers and interpreted by comparing the zone of inhibition with the Kirby–Bauer chart as recommended by CLSI guidelines.[14] *E. coli* ATCC*25922 were used as control strains to monitor accuracy and precision of identification and susceptibility testing procedures.

Multi Drug Resistance (MDR)

MDR is defined as a resistance of a bacterial strain for at least one agent in three or more antimicrobial classes.[15]

Data Analysis

The data were analyzed by using comprehensive meta-analysis version 3.3.070 software (www.Meta-analysis.com). Prevalence were expressed as the percent positive samples from total samples tested. Differences in the proportions of positive samples and multidrug resistance isolates between the samples sources (abattoir and butcher shops) and sample types (meat, cecal content and swab samples) were assessed statistically by using Z- testing and p-value < 0.05 were considered as statistically significant.

Results

Proportions of *E. Coli* and *E. Coli* O157: H7 Positive Samples

In this study, from the total of 505 samples, 102 (20.2%) were confirmed as positive for *E. coli* and 27 (5.4%) were positive for *E. coli* O157: H7 strains. With regard to sample sources, 55 (19.3%) of *E. coli* and 17 (6.0%) of *E. coli* O157: H7 were detected in abattoir house samples and 47 (21.4%) and 10 (4.5%) were detected from butcher shop samples, respectively. Accordingly, from abattoir house samples, *E. coli* was detected in 18 (20.0%) of meat samples whereas, *E. coli* O157: H7 was detected in 4 (4.4%) of the meat samples. From cecal content samples, *E. coli* was detected in 19 (21.1%) and *E. coli* O157: H7 was detected in 9 (10.0%). From the butcher shop samples, *E. coli* was detected in 26 (28.9%) of meat samples, whereas *E. coli* O157: H75 was found in 5 (5.6%) of the meat samples. In addition, *E. coli* was detected in 5 (16.7%) of the knife and 7 (23.3%) of the cutting board swabs, whereas *E. coli* O157: H7 was detected in 1 (5.0%) of the knife and 3 (10.0%) of the cutting board swab samples, respectively. There was a statistically significant difference in the occurrence of *E. coli* and *E. coli* O157:H7 between sample types and sample sources ($p \leq 0.001$) (Table 1).

Hygienic Practices of Abattoir and Butcher Shop Workers

In this study, none of the slaughter staff and butchers had any form of formal educational certificate on food safety and any kind of short course training on safe practices in meat processing. In addition, none of abattoir and butcher shop workers wore clean protective clothing, and none of them were washing and disinfecting their hands, the processing tools and the floor after each working interval. The same cutting boards and knives were used for cutting of meat and abdominal organs. Slaughtering, flaying/dehiding and evisceration/fisting of animals were also taking

Table 1 Proportions of *E. Coli* and *E. Coli* O157: H7 Positive Samples Obtained from the Abattoir and Butcher Shops in Jimma Town, Southwest of Ethiopia

Samples Sources	Types of Samples	No. of Samples Tested	No. of Positive Samples		Positive/ Sample Tested	Prop. of Positives [95% CI]	*p*-value
			E. coli N (%)	*E. coli* O157: H7 N (%)			
Abattoir house	Meat sample	90	18 (20.0)	4 (4.4)	22/90	0.244 [0.167,0.344]	0.001
	Ceacal content	90	19 (21.1)	9 (10.0)	28/90	0.311 [0.224,0.414]	0.001
	Hand swabs	30	4 (13.3)	1 (3.3)	5/30	0.167 [0.071,0.343]	0.001
	Knives swabs	20	4 (20.0)	1 (5.0)	5/20	0.250 [0.108,0.478]	0.033
	Cutting board swabs	20	6 (30.0)	2 (10.0)	8/20	0.400 [0.214,0.620]	0.374
	Protective cloth swabs	20	2 (10.0)	0	2/20	0.100 [0.025,0.324]	0.003
	Transport vehicles swab	15	2 (13.3)	0	2/15	0.133 [0.034,0.504]	0.014
	Sub-total N (%)	285	55(19.3%)	17 (6.0%)	72/285	0.253 [0.206,0.306]	0.001
Butcher shops	Meat sample	90	26 (28.9)	5 (5.6)	31/90	0.344 [0.254,0.448]	0.004
	Hand swabs	40	5 (12.5)	0	5/40	0.125 [0.053,0.567]	0.001
	Knie swabs	30	5 (16.7)	1(5.0)	6/30	0.200 [0.093,0.379]	0.002
	Cutting board swabs	30	7 (23.3)	3 (10.0)	10/30	0.333 [0.190,0.516]	0.074
	Protective cloth swabs	30	4 (13.3)	1 (5.0)	5/30	0.167 [0.071,0.343]	0.001
	Sub-total N (%)	220	47 (21.4%)	10 (4.5%)	57/220	0.259 [0.205, 0.321]	0.001
Total N (%)		505	102 (20.2%)	27 (5.4%)	129/505	0.255 [0.219,0.295]	0.001

place on the same floor without taking cares to avoid cross-contaminations. Moreover, the visceral organs were put very near to the carcass when displayed for sale or when meat was cut into pieces for selling or consumption. Most slaughter staff indicated that inadequate supplies of clean water posed a challenge towards maintaining hygiene (Table 2).

Antibiotic Resistance Profile

In this study, antimicrobial resistance testing was done against 13 different antimicrobial agents. Accordingly, *E. coli* and *E. coli* O157: H7 showed higher resistance against erythromycin and ampicillin with the prevalence of (97.1%) and (92.6%) and (91.2%) and (88.9%), respectively. Moreover, (38.2%) and (36.3%) *E. coli* and (40.7%) and (44.4%) *E. coli* O157: H7 were resistant to trimethoprim-sulfamethoxazole and tetracycline, respectively. In contrast, (94.1%) and (85.2%) of *E. coli* and *E. coli* O157: H7 isolates were susceptible to cefotaxime, (95.1%) and (88.9%) to ceftriaxone, (92.2%) and (85.2%) to ceftazidime, (96.1%) and (96.3%) to ciprofloxacin, (95.1%) and (88.9%) to kanamycin, (94.1%) and (92.6%) to gentamycin, (94.1%) and (92.6%) to streptomycin and (95.1%) and (85.2%) to chloramphenicol, respectively (Table 3).

Table 2 Summary of Observational Checklist Results on Educational Level and Hygienic Practice of the Abattoir and Butcher Shop Workers in Jimma Town, Southwest Ethiopia

Activities Observed and Checked	Abattoir House Workers	Butcher Shop Workers
Having formal education certificate on food safety	None of them	None of them
Having short course training on safe meat handling	None of them	None of them
Use of protective clean clothing	None of them	None of them
Regular washing of hand and processing tools	None of them	None of them
Regular disinfection of hand, the floor and processing tools	None of them	None of them
Is slaughtered animals flaying/dehiding and evisceration/ fisting on separate surface?	No	Not checked
Fisting done with most care to avoid carrying dirt	No	Not checked
Is there adequate supply of clean water?	No	Not
Use of separate knives for cutting of meat and abdominal contents	None of them	None of them
Use of separate chopping boards for cutting of carcass and abdominal organs	None of them	None of them
Use of the separate buckets of water for cleaning knives, washing hands	None of them	None of them

Table 3 Antimicrobial Resistance Patterns of *E. Coli* and *E. Coli* O157: H7 Isolates in Different Samples from Abattoir and Butcher Shops in Jimma Town, Southwest of Ethiopia

Antimicrobial Disc (Code)	Resistance Patterns					
	E. coli (n=102)			*E. coli* O157: H7 (n=27)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ampicillin (AMP)	9 (8.8)	0	93 (91.2)	2 (7.4)	1 (3.7)	24 (88.9)
Amoxicillin -clavulanic acid (AMC)	66 (64.7)	3 (2.9)	33 (32.4)	17 (63.0)	2 (7.4)	8 (29.6)
Cefotaxime (CTX)	96 (94.1)	3 (2.9)	3 (2.9)	23 (85.2)	1 (3.7)	2 (7.4)
Ceftriaxone (CRO)	97 (95.1)	2(2.0)	4 (3.9)	24 (88.9)	1 (3.7)	2 (7.4)
Ceftazidime (CTZ)	94 (92.2)	3 (2.9)	5 (4.9)	23 (85.2)	2 (7.4)	2 (7.4)
Chloramphenicol (CHL)	97 (95.1)	0	5 (4.9)	23 (85.2)	1 (3.7)	3 (11.1)
Ciprofloxacin (CIP)	98 (96.1)	0	3 (2.9)	26 (96.3)	0	1 (3.7)
Erythromycin (ERY)	3 (2.9)	0	99 (97.1)	2 (7.4)	0	25 (92.6)
Gentamicin (GEN)	96 (94.1)	1(1.0)	5 (4.9)	25 (92.6)	1 (3.7)	1 (3.7)
Kanamycin (KAN)	97 (95.1)	0	5 (4.9)	24 (88.9)	1 (3.7)	2 (7.4)
Streptomycin (STR)	96 (94.1)	1 (1.0)	5 (4.9)	25 (92.6)	0	2 (7.4)
Trimethoprim- sulfamethoxazole (SXT)	63 (61.8)	0	39 (38.2)	16 (59.3)	0	11 (40.7)
Tetracycline (TET)	64 (62.7)	1 (1.0)	37 (36.3)	15 (55.6)	0	12 (44.4)

Abbreviations: S, sensitive; I, intermediate; R, resistance.

Multi-Drug Resistance Profiles

In this study, a total of 57 (44.2%) *E. coli* and *E. coli O157: H7* isolates were resistant to three or more classes of antibiotics. Multi-drug resistance profiles against three, four and five antibiotic classes were 34 (26.4%), 19 (14.7%) and 4 (3.1%), respectively. The frequency of resistant phenotype was more common for ampicillin and erythromycin, co-trimoxazole and tetracycline (Table 4). Statistically significant difference was observed in multi-drug resistance pattern between *E. coli* and *E. coli* O157: H7, sample types and sample sources ($p \leq 0.001$) (Table 5).

Discussion

Infections related to contaminated foods are major health problems, especially in developing countries, including Ethiopia. However, limited information was available on incidence and antimicrobials susceptibility pattern of the causative agents to helps policy makers to develop appropriate strategies in terms of prevention, treatment, and control. In this study, different samples such as meat of cattle, cecal content of slaughtered animals, swab samples taken from hands of meat handlers, and different surfaces that have contact with meat were tested for the presence of *E. coli* and *E. coli* O157: H7. Accordingly, *E. coli* were detected in 102 (20.2%) of the samples tested, whereas *E. coli* O157: H7 were detected in 27 (5.4%) of the samples. The proportion of positive samples in this study was higher than the previous study finding in different parts of Ethiopia.[9,16–22] However, this finding is lower than the study finding in Ethiopia and other African countries.[10,23–25] Another meta-analysis study showed that the overall prevalence of *E. coli* O157: H7 was 31.20% in Africa and 1.65–7.35% in other continents.[26] The observed differences might be due to the use of different methods of detections of isolates, differences in sample size, the type of sample and how and when it was collected. For instance, the use of immuno-magnetic separation and PCR technique may improve the sensitivity of the detection, which was not done in this study methodology.

With regard to sample source, 55 (19.3%) and 17 (6.0%) of samples from the abattoir house and 47 (21.4%) and 10 (3.7%) of samples from the butcher shops were positive for *E. coli* and *E. coli* O157: H7, respectively. Despite the differences in proportion of positive samples, the presence of *E. coli* and *E. coli* O157: H7 in abattoir and butcher shops samples were reported in many studies in Ethiopia.[16–18,22,27–29] As compared to developed countries, this finding is higher than a finding in USA,[30] in which the prevalence of *E. coli* O157: H7 on meat goat carcasses was 2.7%. Moreover, other study on the safety and quality of pork and poultry meat imports for the common European market received at border inspection post Hamburg Harbour reported that, *E. coli* was present in 50% and 67% of all pork and poultry samples, respectively, and thereof 33 isolates were confirmed as extended-spectrum β-lactamase-producing *E. coli*.[31] The expected slight differences might

Table 4 Multi-Drug Resistance Profiles of *E. Coli* and *E. Coli* O157: H7 Isolates in Samples Obtained from Abattoir and Butcher Shops in Jimma Town, Southwest of Ethiopia

Sources of Isolates	Resistance Patterns	Antibiotics Classes	Sources of MDR (%)			MDR Isolates (%)		Total MDR
			Meat Samples	Ceacal Content	Swab Samples	*E. coli*	*E. coli* O157: H7	
Abattoir house (n=72)	**Abattoir house (N=72)**		(n=22)	(n=28)	(n=22)	(n=55)	(n=17)	(n=72)
	Amp, ERY, SXT	R3	3	3	3	7	2	9
	AMP, ERY, TET	R3	3	3	3	7	2	9
	AMP, ERY, SXT, TET	R4	2	2	1	4	1	5
	AMP, ERY, AMC, CXT, SXT, TET	R4	1	2	1	3	1	4
	AMP, ERY, AMC, SXT, TET, STR, GEN	R5	0	2	0	1	1	2
	Sub-Total MDR		9	12	8	22	7	29/72
Butcher shops (n=57)	**Butcher shops (N=57)**		(n=31)	—	(n=26)	(n=47)	(n=10)	(n=57)
	AMP, ERY, SXT	R3	5	—	3	6	2	8
	AMP, ERY, TET	R3	4	—	4	6	2	8
	AMP, ERY, SXT, TET	R4	3	—	3	4	2	6
	AMP, ERY, AMC, CXT, SXT, TET	R4	2	—	2	3	1	4
	AMP, ERY, AMC, SXT, TET, STR, GEN	R5	1	—	1	2	0	2
	Sub-Total MDR		15	—	13	21	7	28/57
Overall MDR (%)			24 (45.3%)	12 (42.9%)	21 (43.8%)	43 (42.2%)	14 (51.8%)	57 (44.2%)

Abbreviations: R3-R5, resistance to three, four and five classes of antibiotics; AMP, ampicillin, CXT, cefotaxime, ERY, erythromycin, GEN, gentamicin, STR, streptomycin, SXT, trimethoprim-sulphamethoxazole, AMC, amoxicillin-clavulanic acid, TET, tetracycline.

Table 5 Analysis of MDR Profiles Based on Strain of Isolate, Samples Sources and Samples Types in Jimma Town, Southwest of Ethiopia

Categories		Number of Antibiotic Classes			MDR/ Total	Prop. of MDR [95% CI]	*p*-value
		R3	R4	R5			
Sample types	Meat isolates (n=53)	15	8	1	24/53	0.453 [0.663, 0.881]	0.001
	Cecal isolates (n=28)	6	4	2	12/28	0.429 [0.561, 0.876]	0.012
	Swab sample isolates (n=58)	13	7	1	21/58	0.362 [0.473, 0.720]	0.118
Total MDR [95% CI]		34 (26.4%)	19 (14.7%)	4 (3.1%)	57/129	0.442 [0.575, 0.821]	0.003
Sources of samples	Abattoir house (n=72)	18	9	2	29/72	0.403 [0.579, 0.790]	0.001
	Butcher shops (n=57)	16	10	2	28/57	0.491 [0.724, 0.916]	0.001
Total MDR [95% CI]		34 (26.4%)	19 (14.7%)	4 (3.1%)	57/129	0.442 [0.593, 0.885]	0.004
Strains of isolates	*E. coli* (n=102)	26	14	3	43/102	0.422 [0.662, 0.829]	0.001
	E. coli O157: H7 (n=27)	8	5	1	14/27	0.518 [0.586, 0.897]	0.007
Total MDR [95% CI]		34 (26.4%)	19 (14.7%)	4 (3.1%)	57/129	0.442 [0.678, 0.826]	0.001

Abbreviations: R3-R5, resistance to three, four and five classes of antibiotics.

be due to the difference in hygienic conditions that could be risky for cross-contamination of meat and different contact surfaces with fecal materials during slaughtering, processing, transportation, and displaying at abattoir house and retailer shops. At all these stages, strict adherence to standard operating measures must be practiced.

With regard to sample types, higher proportions of cecal contents of slaughtered animals were positive for *E. coli* and *E. coli* O157: H7 followed by meat and other different swab samples obtained from the abattoir and butcher shops. The presence of *E. coli* and *E. coli* O157: H7 in meat samples, cecal content and swab samples were also reported in different studies.[9,16–23,27–32] Many reasons might be contributed for cross- contaminations of meat and surface contacts with cecal contents and other sources. Previous work in cattle suggests that the prevalence of *E. coli* O157:H7 in the feces is correlated to the prevalence on the hide and carcasses of animals at slaughter.[33,34] In the present study, poor hygienic practices of the abattoir house and butcher shops' workers observed were found to be risky for contamination of meat with *E. coli* and *E. coli* O157: H7. This condition is more risky in our situation; because of the widespread practice of raw meat consumption throughout the country. Studies concluded that, raw meat can harbor harmful pathogenic *E. coli* O157: H7 causing diarrhoea and systemic manifestations such as hemorrhagic colitis, hemolytic uremic syndrome (HUS).[6,35,36]

E. coli O157: H7 carriage in the intestinal tract of healthy animals, particularly cattle, represents a source of direct and indirect infection to humans. Contamination of meat with fecal material in the slaughtering process and during displaying of meat for sale is the main transmission route of bacteria. It is a well documented fact that, lack of education and training on food safety may contribute for unhygienic practices such as improper handling, processing and display of meat at the slaughtering places and at butcher shops.[18,27–29,37] In this study, none of the abattoir and butcher shop workers had a formal education certificate on food safety or short course training on safe meat handling. Moreover, workers at the slaughter house were not well supplied with materials that would enable them to maintain general hygiene. For instance, inadequate supply of clean water is one of the greatest challenges to maintain hygiene. That is why; risky practices during the slaughtering, transportation and display of meat were observed and documented in the present study. Thus, the presence of *E. coli* and *E. coli* O157:H7 on meat might be due to transfer of fecal material onto the carcass during the slaughter process or from different contaminated materials and hands of meat handlers. This may occur with currently available dressing procedures at the abattoir house and further meat processing at butcher shops cannot be reliable to prevent fecal and cross-contamination of meat. Therefore, the range of activities should be carried out with the appropriate training on knowledge and hygienic practices of meat handlers.

In this study, the antimicrobial resistance pattern of isolates were checked with different classes of antibiotics. Accordingly, a total of 57 (44.2%) *E. coli* and *E. coli O157: H7* isolates were resistant to three or more classes of antibiotics. The prevalence of multi-drug resistant isolates was also reported in many previous studies,[9,16,21,22,29,38] in which about (33.2% to 100%) of isolates were showed multi-drug resistance. The occurrence of multidrug resistance may be linked with indiscriminate utilization of antimicrobial agents or genetic mutation, which was not elucidated with the present study methodology. Moreover, transmission of multi-drug resistant bacteria via consumption of meat and meat products has been suggested as a potential source in Africa.[39,40]

In this study, third generation cephalosporin (cefotaxime, ceftriaxone and cefotaxime), gentamicin, ciprofloxacin, kanamycin, streptomycin and chloramphenicol showed best activities against our isolates. In contrast, higher resistance was observed against erythromycin and ampicillin, followed by tetracycline, co-trimoxazole and amoxicillin-clavulanic acid. The best efficacies and high resistance rate against the above antibiotics were also reported in previous studies in Ethiopia and Iran.[9,16–18,29,32,38,41] The higher resistance rate might be due to inappropriate and excessive use of these antibiotics for therapeutic and prophylactic purposes both in human and animal infections. In fact, the frequent and misuse of antibiotics in humans and food animals is closely linked to the recent emergence of multi-drug resistant bacteria. This may lead to increased load of antibiotics, poor clinical outcome, and limited therapeutic options.

In the current study, although molecular techniques such as PCR is more accurate for identification of *E. coli* and *E. coli* O157:H7 and antimicrobial resistance testing, due to unavailability, phenotypic methods were used for identification and antimicrobial resistance testing of isolates.

Conclusion

This study indicated that, multi-drug resistant *E. coli* and *E. coli* O157: H7 isolates were more common. In addition, poor hygienic practices of meat handlers were observed, which may have implications for cross-contaminations of meat. Therefore, improving knowledge and practice of abattoir and butcher shop workers about safe meat handling and distribution as well as monitoring antibiotics use in human and animal health may have great implications

in prevention and control of food-borne infections that may be caused by antibiotic-resistant strains.

Data Sharing Statement

All the data supporting our findings were incorporated within the manuscript.

Ethical Approval and Consent to Participate

Ethical approval was obtained first from Jimma University Review Board, rather than from my own institution, because the study was conducted when I was an MSc student at Jimma University by the invitation of One-health project. At the time, One-health project office was found in Jimma University and invited MSc students to submit animal-related proposals and the project was facilitated by the ethical approval issues by communicating with Jimma University Review Board. Official permission was also sought from Jimma town municipal administration. All abattoir workers, butchers and meat retailer shop owners provided informed consent prior to participating in this study which was performed in accordance with the Declaration of Helsinki.

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Author Contributions

Authors (MA and ET) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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