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Escherichia coli O157:H7: Prevalence, Identification and Antimicrobial Resistance in Cattle Slaughter at Addis Ababa Municipal Abattior, Ethiopia

Muhammed Hamid¹, Yalew Tefera³, Tadese Eguale² and Yalelet Worku^{3*}

¹College of Veterinary Medicine, Samara University, P.O.BOX, 132, Semera Ethiopia
 ²Aklilu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia
 ³School of Veterinary Medicine, Wollo University, Dessie, Ethiopia
 *Corresponding Author: Yalelet Worku, School of Veterinary Medicine, Wollo University, Dessie, Ethiopia

E-mail: yaleletworku@yahoo.com

Abstract

Escherichia coli O157:H7 is recognized as an important cause of diarrhoea hemorrhagic colitis and haemolytic-uremic syndrome worldwide. Meat, meat products, dairy products, vegetables and drinking water contaminated with animal faeces are probably the major sources of Escherichia coli O157:H7 infection. A cross sectional study was conducted from October to April 2006 E.C. at Addis Ababa Abattoir with aim of to investigate the prevalence and antimicrobial drug resistance of Escherichia coli O157:H7. The samples used for this study research were 186 faecal samples were collected from cattle slaughtered at Addis Ababa Municipal Abattoir for prevalence investigation. For isolation and identification of Escherichia coli O157:H7 strain isolates 186 faecal samples, 25 carcass swab samples and 3 waste water samples were used for culturing on different medias. Culturing on of these samples on different microbial Medias was conducted used for isolation and identification of Escherichia coli O157:H7. From a total 186 faecal samples 6.4% (n=12/186) were positive for Escherichia coli O157:H7, 12% (n=3/25) of carcass samples were positive and 33.3% (n=1/3) water samples were positive. A total of 16 Escherichia coli O157:H7 positive isolates (12 from faecal, 3 from carcass swaps and 1 from waste water were used for antimicrobial susceptibility testing on 18 antimicrobial drugs. The isolated strains were 100% susceptible to amikacin and gentamicin. Multidrug resistance (MDR) to two or more drugs was detected in 75% (n=12/16) of the strains. In conclusion the occurrence of E. coli O157:H7 strain and the presence of antimicrobial resistant strains indicate as there may be a risk for the consumers' health. Thus, rational use veterinary drugs in livestock production. So based on the data reported by this study it is advisable to carry out large scale research works on the area of meat hygiene and pharmaco-epidemiological surveillance in food animals.

Keywords: Antimicrobial resistance, cattle, E. coli O157:H7, isolation, prevalence, Addis Ababa Municipal Abattoir, Ethiopia

Introduction

The causes of food-borne diseases in human often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria (Nouichi and Hamdi, 2009; Pal, 2012). Among the different zoonotic pathogenic bacteria that have different serotypes are *E. coli*, *Salmonella* and *Camplylobacter* species (Humphrey

and Jorgensen, 2006; Pal, 2007). E. coli O157:H7, is one of the serotype strain that *E.coli* species has (Pal, 2007). Domestic and wild animals are the sources of E. coli O157, but the major animal carriers are domesticated ruminants, primarily cattle and, to lesser extent, sheep, and possibly goat (Sima et al., 2009; Kiranmayi et al., 2010; Rahimi et al., 2012a). Outbreak of human cases due to E. coli O157 have been linked to beef meat and raw milk and also other dairy products, vegetables, unpasteurized fruit juices and water (Sima et al., 2009). There are also traceable links between human infection and ruminant faeces via water or direct contact (Licence et al., 2001; Strachan et al., 2001), and evidence that contact with animal faeces is a strong risk factor for sporadic E. coli O157:H7 infection (Locking et al., 2001). Red meat animals can be infected or carry a wide range of microorganisms, which are potentially pathogenic for man (Pal, 2012). The enteric habitat of E. coli in animals provides easy access to animal-derived meats at slaughter and at points downstream in the food production process (Olatoye et al., 2012). Possible contamination of edible carcass tissue is the most significant challenge to food safety, and the extent and nature of such contamination are related to the E. coli O157:H7 status of the pre slaughter animal, and any processes which distribute the organism within or between carcasses during dressing operations (McEvoy et al., 2003). Antimicrobial resistance has emerged in the past few years as a major problem and many programs have been set up for its surveillance in human and veterinary medicine. These programs are aimed mainly at human pathogens, agents of zoonoses, and indicator bacteria of the normal intestinal flora from animals. However, little attention has been paid to the resistance in specific animal pathogens (Lanz et al., 2003). Limited studies on the ecology of E. coli O157:H7/NM have been reported, particularly from developing countries (Rahimi and Nayebpour, 2012). The magnitude of the public health burden due to resistant food borne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of food borne illness is infection by antimicrobial resistant strains that lead to more intractable and severe disease (Helms et al., 2002; Martin et al., 2004). This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and

other pathogenic bacteria (Olatoye et al., 2012). Several studies have suggested that foods might be a source of human acquired antimicrobial-resistant E. coli. The food supply is an established vehicle for certain other antimicrobial resistant and/or pathogenic bacteria including E. coli O157:H7 (Oliver et al., 2011; Rahimi and Navebpour, 2012). In developing countries of the world, where there is still an alarming rate of insanitary conditions, malnutrition and poor health facilities, there is an urgent need to study this organism and its characteristics with an aim to reduce the human hazard caused by this emerging pathogen (Isibor et al., 2013). It might seem paradoxical to discuss on the subject of food safety when millions are suffering from lack of food and of the most inferior quality. In Ethiopia at a national level however, both food shortage and lack of appropriate food safety assurance systems are problems that have become obstacles to the country"s economic development and public health safety (FAO/WHO, 2007; Ayalew et al., 2013). Food borne diseases commonly occur without being reported and Ethiopia is no exception. The lack of vigorous surveillance of food pathogens in Ethiopia meat and meat products presents a challenge for riskbased approaches to improve food safety, as it becomes difficult to demonstrate the magnitude of contamination with this pathogen. However, in the presence of this situations, little is known about the prevalence, distribution and associated virulent genes of E. coli O157: H7 in in foods of animal origin in Ethiopia (Hiko et al., 2008; Mersha et al., 2010; Taye et al., 2013). In this study for isolation and identification of E. coli serotype culturing method on different Medias were used such as sorbitol MacConhkey (SMAC) agar, cefixime-SMAC agar or SMAC agar supplemented with cefixime and tellurite (CT-SMAC) with subsequent stereotyping. Accurate diagnosis of EHEC O157 infections requires the isolation of the pathogen to clarify the aetiology of disease and the infectiousness of patients as well as to allow sub-typing of strains for epidemiological purposes. Thus, the objectives of this study were to estimate the prevalence of E. coli O157:H7 from faecal samples of cattle slaughtered at E. coli O157:H7, to isolate and identify E. coli O157:H7 serotype from samples taken from cattle faeces, carcass swab and water and also to determine the antimicrobial susceptibility pattern of E. coli isolates at Addis Ababa Municipal Abattoir.

Materials and Methods

Study Area

The study was carried out at Addis Ababa Municipal Abattoir which is located in the capital city of the Federal Democratic Republic of Ethiopia. Addis Ababa has an area of 51,000 hectare in the central highlands. Its altitude ranges from 2000-2560 meters above sea level .The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls is during the long rainy season from June to September. The short rainy season is from February to April. Its annual average minimum and maximum temperature are $10.7^{\circ}c$ and $23.4^{\circ}c$, respectively (ENMSA, 2003).

Addis Ababa Municipal Abattoir was established in 1956. The types of species of animals slaughtered in the abattoir are bovine, ovine, caprine and swine. The source of these animals is from different part of the country. The main purpose of the abattoir are processing of fresh meat for human consumption, hygienic processing and storage of meat and edible by products, exercise close control over environmental condition at all stages of processing and break down the transmission of meat borne disease through meat inspection. In this abattoir on average daily 700 cattle, 250 sheep and 75 goats are slaughtered and 153,000 cattle, 39,000 sheep, 3200 goats and 750 pigs are slaughtered annually.

Study population

The study animals were cattle brought from different parts of the country especially around Addis Ababa. Cattle of different age groups, mainly male and local breed cattle slaughtered at Addis Ababa abattoir were included in the study.

Study design and sample size calculation

A cross sectional study design was conducted to estimate the faecal prevalence and antimicrobial resistance test on the isolated E coli O157:H7 strains from cattle slaughtered at the abattoir.

The required sample size of the study animal is determined by the formula given by (Thrusfield, 2005) with 95% of confidence interval and 5% desired precision level.

$$N = \frac{1.96^2 x P_{exp} (1 - P_{exp})}{d^2}$$

Where:-

N=number of sample size P_{exp}=expected prevalence d²=Absolute precision CI=Confidence interval (95%)

Expected prevalence of 4.2% reported by (Hiko *et al.*, 2008) was used

Based on this $N = (1.96)^2 \times 0.042 (1-0.042) = 62$ 0.05^2

Therefore the minimum sample size was 62 but in order to increase the precision three fold of the minimum sample size was taken 186 of animals were used for the study.

Sampling strategy and sample collection

Systematic random sampling technique was employed while selecting animals to collect faecal samples from the total number of cattle presented and slaughtered Addis Ababa Municipal Abattoir on daily basis. During ante-mortem examination, the associated risk factors such as age, sex, breeds, body condition, and origin of cattle were assessed and recorded in data recording book prepared for this study. The cattle were grouped into three age groups: young (<2 years), young adult (3 – 6 years) and adult (> 6 years). Body condition score was made according to Nicolson and butter worth and recorded as poor, medium and good (Nicolson and Butterworth, 1986).

Then faecal samples were collected from cattle and collected by using sterile universal bottles. The 25 carcass swabs from 25 slaughtered cattle were collected by using 25 screw cupped test tube containing 10 ml of sterile bacteriological peptone samples. All the collected samples brought to the ALIPB microbiology laboratory using in a cool box with frozen gel packs within twenty four hours of sampling for microbiological analysis. In addition to this, three water samples (10ml) were collected from the bucket of water which was used for knives and carcass.

Study methodologies

Isolation and identification: Immediately after arrival in ALIPB microbiology laboratory, 1gm of faecal sample was suspended into 9 ml of modified tryptone soya broth supplemented with novobiocin (Oxoid) (10 mg/l). Then samples were vortexed and incubated for overnight at 37°C. After selective enrichment, 50µl of product was streaked onto sorbitol MacConkey agar (Oxoid) and the plates incubated at 37°C for twentyfour hours. Up to six colourless colonies (non-Sorbitol fermenters) were picked and separately subcultured on MacConkey agar (Oxoid) for twenty-four hours at 37°C for purification. After overnight incubation, the purified and intensely red colonies with a pale periphery were tested for indole production (Oxoid) and indole forming isolates were seeded onto non selective media (nutrient agar) for serological confirmation. The indole test was carried out by inoculation one colony into 4ml of tryptone soya broth (Oxoid), using a straight inoculation wire. Incubation was done for overnight at 37°C. After this one drops of indole reagent were added to the tryptone soya broth culture to test for indole production (red ringpositive). Then they were tested with E. coli O157 antibody-coated latex and control latex) according to the procedures recommended by the manufacturer (March and Ratnam, 1986). All the 25 carcass swab and water samples subjected to similar tests for bacteriological analysis as faecal samples.

Antimicrobial susceptibility testing: The Antimicrobial susceptibility test of 16 isolates were performed according to the National Committee for Clinical Laboratory Standards (NCCLS, 2008) method using Kibry-Bauer disk diffusion test on Muller-Hinton agar (Oxoid CM0337 Basing stoke, England). The isolated E. coli O157:H7 strains were tested for antibiotic resistance to 18 antimicrobial agents obtained from Oxoid. The selection criteria of antibiotics testing discs depended on the regularly use of antimicrobials in the ruminants, potential public health importance and recommended from the guideline of antimicrobial susceptibility. Resistance testing discs contained Tetracycline (30µg). Chloramphenicol (30µg), Sulfamethoxazole-trimithoprim (25µg), Amoxycilline and clauvlonic acid $(30 \mu g),$ Nitrofurantion (100µg), Ciprofloxacine $(5\mu g)$, Naldixic acid Kanamycine (30µg), (30µg), Streptomycine (10µg), Cefoxitin (30ug). Amikacin (30µg), Ampicillinm (10µg), Gentamycin (10µg), Cephalothin (30µg), Ceftriaxone (30µg), Sulfisoxazole (1000µg), Trimethoprim (5µg) and Neomycin (30µg). The isolates were considered resistant if the diameter of inhibition zone was less than or equal to the resistance breakpoint provided by (CLSI guidelines, 2013) (Table 1).

NO. Antibiotic discs		Disc Concentratio	Concentration	Diameter of Zone of inhibition in mm			
		code		Resistant	Intermediate	Susceptible	
1	Tetracycline	TE	30µg	11	12-14	15	
2	Chloramphenicol	С	30µg	12	13-17	18	
3	Sulfamethoxazole- trimithoprim	SMT	25µg	10	11-15	16	
4	Amoxycilline and clauvlonic acid	AMC	30µg	13	14-17	18	
5	Nitrofurantion	F/M	100µg	14	15-16	17	
6	Ciprofloxacine	CIP	5µg	20	21-30	31	
7	Naldixic acid	NA	30µg	13	14-18	19	
8	Kanamycine	Κ	30µg	13	14-17	18	
9	Streptomycine	S	10µg	11	12-14	15	
10	Cefoxitin	FOX	30µg	14	15-17	18	
11	Amikacin	AN	30 µg	14	15-16	17	
12	Ampicillin	AM	10 µg	13	14-16	17	
13	Gentamycin	GM	10 µg	12	13-14	15	
14	Cephalothin	CF	30 µg	14	15-17	18	
15	Ceftriaxone	CRO	30 µg	14	15-17	18	
16	Sulfisoxazole	G	1000 µg	12	13-16	17	
17	Trimethoprim	TRM	5 µg	10	11-15	16	
18	Neomycin	Ν	30 µg	12	13-16	17	

Table 1: Antibiotic disks used to test E. coli O157:H7 and their respective concentrations

Each isolated bacterial colony from pure fresh culture was transferred in to a test tube of 5 ml Tryptone Soya Broth (TSB) (Oxid, England) and incubated at 37°C for 6 hours. The turbidity of the culture broth was adjusted using sterile saline solution or added more isolated colonies to obtain turbidity usually comparable with that of 0.5 McFarland standards (approximately $3x10^8$ CFU per ml). Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville plates were prepared according USA) the manufacturer's instruction (March and Ratnam, 1986). A sterile cotton swab was immersed into the suspension and rotated against the side of the tube to remove the excess fluid and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic discs were placed on the inoculated plates using sterile forceps. The antibiotic discs was gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37°C for 24 hours. Following this the diameter of inhibition zone formed around each disc was measured using a black surface, reflected light and transparent ruler by lying it over the plates (NCCLS, 2008). The result of the test was interpreted as sensitive, intermediate and resistant by using the breakpoints for each antimicrobial according to (CLSI, 2013). The results were classified as sensitive. intermediate. and resistant according to the standardized table supplied by the manufacturer (CLSI, 2012). For the results and discussion, we used the terminology of (Knezevic and Petrovic, 2008): very high rate of resistance (>75% resistant isolates); high rate (50-75%); moderate rate (30-50%); low rate (10-30%); and very low resistance rate (0-10%).

Data analysis

The data obtained from the study was entered to the Microsoft excel spreadsheet and analyzed by version 20 SPSS. Chi-square statistics were used to test the association between variables. The prevalence of *E. coli* O157: H7 was calculated by dividing the number of positive samples to the total number of samples examined multiplied by 100. Pearson chi-square test and Fisher's exact two-tailed test analyses were performed and differences were considered significant at P < 0.05.

Results

Prevalence and identification of *E. coli* O157:H7

Out of 186 faecal samples examined, 12 (6.4%) were found to be have *E. coli* O_{157} :H₇. The Prevalence of *E. coli* O157:H7 infection was totally from male cattle and all the female cattle were negative for *E. coli* O157:H7 (Table 2) and the difference between sex was not statistically significant (P>0.05).

The prevalence of *E. coli* O157:H7 was higher in adults than in young's but it was 0% prevalence in old cattle (Table 3); furthermore the variation in the prevalence among age groups was not statistically significant (P>0.05).

Statistically significant difference was not observed (P>0.05) in the Prevalence of *E. coli* O157:H7 among those animals with different body condition scores but The Prevalence of *E. coli* O157:H7 was higher proportion in medium cattle 9(4.8%), then in low cattle 2(1.07%) and finally 1(0.53%) in fat cattle (Table 4).

Sex	examined	No of positives	Prevalence	\mathbf{X}^2	P value
Male	174	12	6.4%	0.885	0.348
Female	12	0	0%	0.885	0.548
Total	186	12	6.4%		

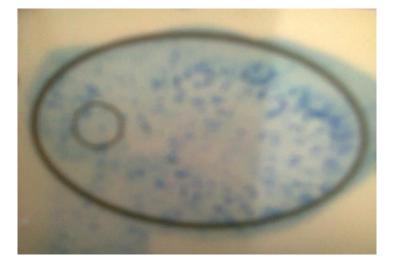
Table 2: Prevalence of E. coli O157:H7 Based on Sex

Age group	No of examined	No of positives	Prevalence	X^2	P value
Adult	125	8	4.3%		
Old	26	0	0%	3.23	0.199
Young	35	4	2.1%		
Total	186	12	6.4%		

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Body condition	No of examined	No of positives	Prevalence	\mathbf{X}^2	P value
Fat	41	1	0.5%		
Low	43	2	1.1%	2.276	0.321
Medium	102	9	4.8%		
Total	186	12	6.4%		

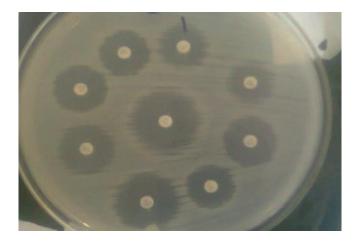
Table 4: Prevalence of *E. coli* O157:H7 Based on Body Condition Scores



Antimicrobial susceptibility patterns

The isolated strains *E. coli* O157:H7 were susceptible (100%) to amikacin and gentamicin. Multidrug resistance (MDR) to two or more drugs was detected in 12/16 (75%) of the strains. Of the 10 MDR strains,

2 were resistant to 3 drugs, 3 were resistant to four drugs, 2 were resistant to five drugs and 3 strains were resistant to twelve and above drugs. From the isolated strains 8(50%), 9(56.25%) and 11(68.75) of they were resistant to Amoxycillin and clauvlonic acid (AMC), ampicillin (AM) and cephalothin (CF) respectively.



Discussion

The prevalence of E. coli determined by the present study 12 (6.4%) is found to be greater than values reported by (McEvoy et al., 2003) 2.4% from United Kingdom and (Conedera et al., 1997) 3% from Irish abattoir in faeces. On the other hand, the findings of the present study is lower than 8.6% and 7.5% reported by (Synge et al., 2000; Omisakin et al., 2003) respectively from United Kingdom, and 11.3% from United State (Callaway et al., 2006). there is also significantly higher reports 23.7%, (40.4%) and 28% by Strachan et al., 2005; Paiba et al., 2002) from United Kingdom and (Elder et al., 2000) from United State respectively.The observed variation in prevalence of E. coli among the above studies could be attributed to difference in sampling and isolation procedures, variability in sampled populations, diverse geographical origins of cattle, study design, season and use of antibiotics in the cattle population.

The differences in isolation rate of *E. coli* O157:H7 from faecal samples from abattoir different studies are possibly due to differences in husbandry practices, agro climatic variation, seasonal differences, sampling times, breeds and age of animals, sampling technique and so on. A number of studies have also showed that prevalence of *E. coli* O157:H7 shed from animal faeces can vary significantly in relation to time, age of animals, nature of feeds etc. (Chapman, 1994; Chapman *et al.*, 2001; Reid *et al.*, 2002).

Although the focus of the current study is on the prevalence of E. coli O157:H7 in faces, there are different reports signifying the presence of high risk of contamination of carcasses by E. coli O157:H7 from faces of slaughtered animals. Association of several risk factors with carcass contaminations has been reported by several researchers. Associations with feces (Elder et al., 2000; Gansher off and O'Brien, 2000) and with skin (Reid et al., 2002) and E. coli O157:H7 has been reported to spread easily on to carcass surfaces from the hide or during evisceration (Arthur et al., 2007; Elder et al., 2000; Gun et al., 2003). Meat is frequently found to be contaminated due to poor sanitary environment during slaughter, transportation and handling. According to the report by (Bassam et al., 2012), the infective dose of the pathogen is < 10 cells for humans.

In the study it was tried to see if there is statistically significant difference in the isolation rate of *E. coli* O157:H7 between age, sex and body condition score of cattle. But statistically significant difference was

not seen in any of the above assumed risk factors although higher prevalence was found in medium body condition animals than low and fat animals and all the isolated strains were from adult 8 (4.3%) and 4 (2.1%) from young.

Antimicrobial resistance pattern of *E. coli* O157:H7 isolates from animal and human sources have been reported in Ethiopia by (Hiko *et al.*, 2008), In the present study, all of the 16 isolates of animal sources were totally susceptible to amikacin (AN) (30 μ g) and gentamicin (GM) (10 μ g), and 13(81.25%) of the isolates were susceptible Ceftriaxone (CRO) (30), chloramphenicol (C) (30 μ g), Sulfisoxazole (G) (1000 μ g) and sulphamethoxazole and trimethoprim (SMT) (23.75 μ g and 1.25 μ g).

On the other hand, the current study revealed that all isolates were highly resistant to Amoxycillin and clauvlonic acid (AMC), ampicillin (AM) and cephalothin (CF). This might be due to inappropriate use of antibiotics for treatment of diseases (Sharada *et al.*, 2010) and also excessive use of antimicrobials for therapeutic and prophylactic treatment (Majalija *et al.*, 2010). This variation probably attributed to the expression of resistant gene code by the pathogen which is associated with emerging and re emerging aspects of the isolates with the regards of different agro ecology (Reuben and Owuna, 2013).

In the present study, majority of the isolates 12 (75%) have multiple drug resistance to two or more drugs. This finding comparable related with previous findings (Guerra *et al.*, 2003; Zhao *et al.*, 2005; Salehi and Bonab, 2006; Akond *et al.*, 2009). The occurrence of this multi drug resistance might be due to administration of multiple antibiotics for prophylaxis or infection, in indiscriminate use of antibiotics in the farms and the other possibility is that, cattle are being treated with antibiotics for other conditions, thereby selecting for resistant populations of *E. coli* O157:H7. Such multi drug resistant organisms may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van De Bogard and Stobberingh, 2000).

In conclusion, this study shows the presence of *E. coli* O157: H7 strain isolates from the faeces and carcass swab cattle slaughtered at Addis Ababa Abattoir. And majority of the isolated strains of *E. coli* O157:H7 have developed multi drug resistance to two or more antibiotics. Thus, consumers can have high risk of infection by this pathogen. Therefore, based on this

research output remarks care should be taken during evisceration in order to prevent contamination of the carcass by the gastrointestinal contents, awareness creation should be done for abattoir workers about the risk of food borne diseases and appropriate slaughter procedures. In general, it is advisable to use the information obtained from this study so as to conduct large scale and detailed investigations on farm risk factors that lead to the development of drug resistance bacteria and for food safety risk assessment modelling in all of Abattoirs of Ethiopia to minimize public health risk.

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Conflict of interest

There is no any conflict of interest among the authors.

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