Prevalence and antibiogram of *Escherichia coli O157* isolated from bovine in Jimma, Ethiopia: abattoir-based survey

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https://dx.doi.org/10.4314/evj.v21i2.8

Abstract

E. coli O157 is an important serotype that caused many food borne outbreaks worldwide in the past decades**.** This study was carried out to estimate the prevalence and determine the antimicrobial susceptibility of *E. coli* O157 isolated from bovine carcasses and cecal contents at one abattoir in Jimma. A total of 300 samples from bovine carcass swabs(n=150) and cecal contents(n=150) were examined to identify *E. coli* O157 by ISO 17604:2005 method and by using Dry spot *E. coli* O157 latex test kit. Susceptibility to panels of 9 antimicrobial agents for all 25 *E. coli* O157 isolates was examined The overall prevalence of *E. coli* O157 from bovine carcass swabs and cecal contents were 9.3% and 7.3%, respectively. All *E. coli* O157 isolates were susceptible to chloramphenicol, ceftriaxone, sulfamethoxazole-trimethoprim, tetracycline and 96% of the isolates were susceptible to amoxacillin-clavulanic acid. Twenty-eight, 24% and 20% of the isolates were resistant to amikacin, streptomycin and cephalothin respectively. In conclusion, considerable proportions of bovine carcasses and cecal contents in the current study harbored drug resistant *E. coli* O157 and pose a significant public health risk. Attention must be given during post mortem examination and evisceration to avoid contamination of carcasses.

Keywords: Abattoir; Antimicrobial susceptibility; Carcass; Cecal content; *E. coli* O157*;* Jimma; Prevalence

Introduction

Foodborne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (Nafisa *et al*., 2010). Foodborne zoonotic diseases often occur due to 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).

One of the most significant foodborne pathogens that have gained increased attention in recent years is *E. coli* O157:H7 (Pal, 2007). This pathogen is more significant than other well recognized foodborne pathogens for reasons including the severe consequences of infection that affect all age groups, their low infectious dose, unusual acid tolerance (Robert *et al.,* 1997). From a broader perspective, antimicrobial resistance is considered the 3rd major public health challenge of the 21st century (Omulo *et al*., 2015). Food, including beef, can play an important role as a vehicle for transmission of antimicrobial resistant bacteria to people (Christopher *et al*., 2013).

Gastroenteritis due to foodborne disease is one of the most common illnesses in Ethiopia, and it is a leading cause of death among people of all ages in the country (IHME, 2013). Consumption of raw beef is commonly practiced in Ethiopia. Generally unhygienic slaughter practices in the abattoirs, and widespread consumption of raw meat (Kitfo and Kurt) and traditional practice are potential factors contributing to the risk of exposure of the Ethiopian community to foodborne pathogens. Despite the high risk of exposure to *E. coli* O157, limited studies on the ecology of *E. coli* O157 and antimicrobial susceptibility has been reported, particularly from developing countries (Rahimi and Nayebpour, 2012). Furthermore, it has not been determined well to what extent feces of slaughtered cattle serve as sources of *E. coli* O157 to red meat contamination. The objectives of this study were therefore to estimate the prevalence of *E. coli* O157 in carcasses and cecal contents of bovine slaughtered in an abattoir in Jimma as well as to determine the antimicrobial susceptibility profiles of *E. coli* O157 isolates.

Materials and Methods

Study area

The study was carried out in Jimma Zone, South Western Ethiopia. The study area lies between 36^{o} 10´ E longitudes and 70^{o}40´ N latitude at an elevation ranging from 880 meter to 3360 meters above sea level (Dechassa Lemessa, 2000). Jimma Zone is divided in to 17 districts (hosting a total population of over 2.4 million; (CSA, 2008). The study area has three agro-ecological conditions consisting of highlands (15%), midlands (67%) and lowlands (18%) (Dechassa Lemessa, 2000). The thirteen years mean annual minimum and maximum temperature of the area was 11.3°C and 26.2°C, respectively (CSA, 2008).

Study design and population

A cross-sectional study involving microbiological analysis was employed to isolate and identify *E. coli* O157 from carcass and cecal contents of slaughtered bovine from October 2015-April 2016. The study population comprised apparently healthy bovine slaughtered at an abattoir in Jimma.

Sample size determination and sampling technique

Sample size required was determined using the formula indicated in Thrusfield (2005), based on expected prevalence of *E. coli* O157 in bovine, which was estimated at 4.7% following Tizeta Bekele et *al*. (2014). The confidence interval was 95% and the precision was 5%. Thus, the required sample size was 69; however, 150 samples were taken in order to maximize the precision of the study. Therefore, 150 carcass swabs and 150 cecal samples were selected using a systematic random sampling technique from apparently healthy bovine during slaughtering operations.

Sample collection procedure

A total of 150 carcass swabs were collected using the method described in ISO17604 (2005) by placing sterile test tube (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral) and breast (lateral) which are sites with the highest rate of contamination were chosen for sampling. On completion of the rubbing process, the shaft was broken by pressing it against the inner wall of the test tube and disposed leaving the cotton swab in the test tube. A second dry sterile cotton swab of the same type was used as before over the entire sampled area. Finally, the samples were transported for to Microbiology Laboratory, College of Agriculture and Veterinary Medicine, Jimma University for microbiological analysis. Pooled sample were then used for bacterial culture.

Similarly, a total of 150 cecal contents were collected. The cecal samples were collected immediately after evisceration from cecal contents of slaughtered bovine; an aseptic incision was made with surgical blade in the cecum to obtain a representative sample of the cecal content. The fecal material was aseptically compressed and the resultant liquid was decanted in sterile universal bottle. The samples were labeled, transported on ice to the laboratory and held in a cold storage overnight and processed the following day.

Culture and isolation of *E. coli* O157

Approximately 1ml/g of fecal sample and pooled carcass swab samples were suspended into 9 ml of modified tryptone soya broth supplemented with novobiocin (10 mg/l) in a ration of 1:9 (Oxoid Ltd, Hampshire, UK). Samples were vortexed and incubated over night at 37°C. After selective enrichment, 50µl of product was streaked onto sorbitol MacConkey agar (Oxoid Ltd., Hampshire, UK) and the plates were incubated at 37°C for 24 hours. Up to six colorless colonies (non- sorbitol fermenters) were picked and separately sub-cultured on MacConkey agar for 24 hours at 37°C for purification. The purified and intensely red colonies with a pale periphery were tested for indole production. The indole test was carried out as follows. One colony was inoculated into 4 ml of tryptone soya broth, using a straight inoculation wire. Incubation was done for overnight at 37°C. After this, one drop of indole reagent was added to the tryptone soya broth culture to test for indole production (red ring-positive). Indole positive isolates were cultured on nutrient agar for serological confirmation by latex agglutination.

Confirmatory test by latex agglutination for *E. coli* O157 serogroup

Non-sorbitol fermenting (NSF) isolates were inoculated onto nutrient agar for testing. Then, the serogroup of NSF of indole positive colonies was identified using the DrySpot *E. coli* O157 latex agglutination test (Oxoid Ltd., Hampshire, UK). One drop of saline was dispensed to the small ring (at the bottom of each oval) in both the test and control reaction areas ensuring that the liquid did not mix with the dried latex reagents. Using a sterile single use plastic loop, a portion of the colony to be tested was picked and carefully emulsified in the saline drop until the suspension was smooth. Then, using paddle the suspension was mixed into the dry latex spots until completely suspended and spread to cover the reaction area. The test card was picked up and rocked for up to 60 seconds, and checked for agglutination. The result was recorded as positive if agglutination of the latex particles occurred within 1 minute. This indicated the presence of *E. coli* serogroup O157. A negative result was reported if no agglutination occurred and a smooth blue suspension remained after 60 seconds in the test area.

Antimicrobial susceptibility

The antimicrobial susceptibility test was performed following the standard agar disc diffusion method according to CLSI (2008) using commercial antimicrobial disks (Table.1).

Table 1. Antimicrobial discs used to test *E. coli* O157, their respective concentrations and susceptibility cut off points

Antimicrobial disks	Disc code	Concentration(µg)	Diameter of zone of inhibition in (mm)		
			Resistant ≤	Intermediate	Susceptible ≥
Amikacin	AK	30	14	15-16	17
Amoxycillin-Clavulanic acid	AMC	20/10	13	14-17	18
Ceftriaxone	CRO	30	14	15-17	18
Cephalothin	CF	30	14	15-17	18
Chloramphenicol	C	30	12	13-17	18
Nalidixic acid	NA	30	13	14-18	19
Streptomycin	S	10	11	12-14	15
Tetracycline	TE	30	11	12-14	15
Sulfamethoxazole-Trimethoprim	SXT	30	10	11-15	16

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^{o}C for 6 hours. The turbidity of the culture broth was adjusted using sterile saline solution or more isolated colonies added to obtain turbidity usually comparable with that of 0.5 McFarland standards (approximately $3x10^8$ CFU per ml). Mueller-Hinton agar (Oxid, England) plates were prepared according to the manufacturer's instruction. 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 were dried, antimicrobial disks were placed on the inoculated plates using sterile forceps. The antimicrobial disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37^oC for 24 hours. The diameter of inhibition zone formed around each disk was measured using a black surface, reflected light and transparent ruler by lying it over the plates. The results were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the manufacturer (CLSI, 2008). *E. coli* ATCC 25922 type strains was used as a positive control.

Statistical analysis

Data was analyzed using SPSS statistical software version 20. Descriptive statistics such as frequency and percentage were used to present the data. Difference between prevalence of *E. coli* O157 from carcass and cecal samples was tested using chi-square test. A p-value < 0.05 was considered indicative of a statistical significance

Results

Prevalence of *E. coli* O157

The prevalence of *E. coli* O157 in carcass swabs and cecal content were 14 (9.3%) and 11 (7.3%), respectively (Table 2). There was no significant difference in the rate of recovery of *E. coli* O157 between carcass swab and cecal content (p= 0.54).

Table 2. Prevalence of *Escherichia coli* O157 in carcass and cecal content

Type of sample	Total samples examined	Number positive	Prevalence (%)	χ^2	p-value
Carcass swab	150	14	9.33	1.3	0.54
Cecal content	150	11	7.33		
Total	300	25	8.33		

Antimicrobial susceptibility of the isolates

The result of antimicrobial susceptibility test of 25 *E. coli* O157 isolated from carcass swabs and cecal content samples to 9 selected antimicrobial agents is shown in table 5. All isolates were susceptible to chloramphenicol, ceftriaxone, sulfamethoxazole-trimethoprim and tetracycline. On the other hand, 96%, 72%, 72% and 72% of the isolates were susceptible to amoxycillin-clavulanic acid, cephalothin, nalidixic acid, and amikacin respectively. Fifty-two percent of the isolates were susceptible to streptomycin. Intermediate susceptibility was also observed in streptomycin (24%), nalidixic acid (8%), and cephalothin (8%). Similarly, 12%, of the isolates were resistant to amoxycillin-clavulanic acid.

Table 3. Antimicrobial susceptibility pattern of *E. coli* O157isolates

Antimicrobial used	Type of samples and *E. coli* O157 isolates								
	Carcass (n=14)			Cecal content(n=11)			Total (n=25)		
	S No. (%)	I No. (%)	R No. (%)	S No. (%)	I No.(%)	R No.(%)	S No.(%)	I No.(%)	R No.(%)
AK	9(64.3)	0(0)	5(35.7)	9(81.8)	0(0)	2(18.2)	18(72)	0(0)	7(28)
AMC	14(100)	0(0)	0(0)	8(72.7)	0(0)	3(27.3)	24(96)	0(0)	1(4)
CRO	14(100)	0(0)	0(0)	11(100)	0(0)	0(0)	25(100)	0(0)	0(0)
CF	12(85.7)	2(14.3)	0(0)	6(54.5)	0(0)	5(45.5)	18(72)	2(8)	5(20)
C	14(100)	0(0)	0(0)	11(100)	0(0)	0(0)	25(100)	0(0)	0(0)
NA	14(100)	0(0)	0(0)	9(81.8)	2(18.2)	0(0)	23(92)	2(8)	0(0)
S	3(21.4)	6(42.9)	5(35.7)	10(90.9)	0(0)	1(9.1)	13(52)	6(24)	6(24)
TE	14(100)	0(0)	0(0)	11(100)	0(0)	0(0)	25(100)	0(0)	0(0)
SXT	14(100)	0(0)	0(0)	11(100)	0(0)	0(0)	25(100)	0(0)	0(0)

See Table 1 for abbreviations of antimicrobial agents; S= Sensitive, I= Intermediate, R= Resistant

Of the 25 *E. coli* O157 isolates, 4 (16%) were resistant to three or more drugs tested (Table 4). Out of the multiple drug resistant isolates, 3 (21%) were recovered from carcass samples and 5 (45.5%) were from cecal content.

Table 4. Resistance pattern of multidrug resistant *E. coli* O157 isolates

MDR Pattern	Source of resistant isolates		
	Carcass (No.)	Cecal content (No.)	Total (No.)
AK,S	3	-	3
AMC, S	-	1	1
AK,CF,S	-	2	2
AK,AMC,CF,S	-	2	2
Total MDR No. (%)	3 (21)	5 (45.5)	8 (32)

See Table 1 for abbreviations of antimicrobial agents; S= Sensitive, I= Intermediate, MDR= Multidrug resistance

Discussion

The present study revealed an overall *E. coli* O157 prevalence of 9.3% and 7.3% from carcass and cecal contents of cattle, respectively. The present prevalence in carcass was similar with reports from other researchers from Iran 6.4% by Rahmi *et al*. (2008), from Ethiopia 8% by Adem Hiko *et al*. (2008), from Iran 8.3% by Hashemi *et al*. (2010) and 9.6% by Tahamta *et al*. (2006). The higher prevalence recorded in carcass swab in this study is in agreement with suggestion given by some researches that during transporting cattle to the slaughter facility that cattle *E. coli* O157:H7 might be shred and aid as a method for cross-contamination (Minhan *et al*., 2003) evaluated the influence of lairage and transportation in fecal shedding of *E. coli O157* in cattle. According to Minihan *et al*. (2003) findings even positive cohorts of cattle may be slaughtered and processed to produce clean carcasses when hygienic practices are followed.

The current finding 9.33% *E. coli* O157 prevalence in bovine meat swab is by far greater than the reports of Tizeta Bekele *et al*. (2014**)** (4.7%) in Addis Ababa from bovine, 3% by Gashaw Mersha *et al*. (2009) in Modjo, Carney *et al*. (2006) from Ireland, 2 % by Adem Hiko *et al*. (2008) in DebreZeit and Modjo towns and Fitsum Dulo *et al*. (2014) in which a prevalence of 4.2%, and 3.2 % *E. coli* isolation from bovine, ovine and goats meat, respectively. On the other hand, it is lower than the culture based prevalence of *E. coli* isolates (22.2%) from meat samples collected from Mekelle Municipality abattoir in northern Ethiopia (Mekonnen Haileselassie *et al*., 2012). This varied prevalence in above studies might be due to the sampling techniques employed and laboratory methodologies used, and ecological variations among the study sites.

The current study on antimicrobial sensitivity testing of *E. coli* O157 recovered from bovine feces and carcass revealed a varying degree of susceptibility to antimicrobial agents tested. All of the 25 isolates were susceptible to tetracycline, sulfamethoxazole-trimethoprim, amoxycillin-clavulanic acid and chloramphenicol. On the contrary, study reported that tetracycline resistance was frequently detected among *E. coli* in Ethiopia (Adem Hiko *et al.,* 2008) and (Melaku Taye *et al*., 2013). Frequent use of tetracycline to treat animal diseases in other areas in the country might have contributed to the higher rate of resistance in previous reports. On the other hand, low level of resistance to chloramphenicol has been reported in other studies (Mulugeta Kibret and Million Tadesse, 2013) which is in agreement with the current finding.

Multidrug resistance has been common problem among Gram negative bacterial species (Ahemed *et al*., 2005). Concurrent resistance of *E. coli* O157 to some antimicrobials may complicate the therapeutic management of infection. In the present study, multidrug resistance was observed to amikacin, amoxycillin-clavulanic acid, cephalothin, streptomycin and tetracycline antimicrobials. From the above mentioned antimicrobials, resistance to streptomycin was observed in all MDR *E. coli* O157 isolates. This finding is in agreement with the previous report (Adem Hiko *et al*., 2008).Unlike the previous report by Adem Hiko *et al.* (2008) where high rate of sensitivity to amikacin was reported, in the current study, 28% of the isolates were resistant to amikacin. Another possible reason for difference in degree of susceptibility and resistance may be the result of temporal and geographical differences between the current and previous studies (Galland *et al*., 2001).

Conclusion

Detection of drug-resistant *E. coli* O157 in bovine carcasses and cecal contents shows the possible public health risk. This is particularly important in consumers who have habit of eating raw or undercooked meat. Attention must be given during post mortem examinations and evisceration to avoid contamination of carcasses with fecal contents. Further investigation should be conducted on other pathogenic serogroups and virulence factors of *E. coli* and the emergence of multidrug-resistant strains.

Acknowledgement

This study was supported by the Addis Ababa University Thematic Research Project entitled "Pneumonia, diarrhea and mastitis in food animals".

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