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Study on Isolation and Identification of *E. coli* from Carcass of Slaughtered Goat and Environmental Samples at Selected Abattoirs, Ethiopia

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Abstract: The present study was conducted from December 2016 to March 2017 with the objective of isolation and identification of *E. coli* at abattoirs in Ethiopia. The swabs of carcass and environmental samples were taken for isolation and identification of *E. coli*. Samples were planted on TSA media and MacConkey agar for 24-48 hours after being diluted. Consequently, the suspected colonies were confirmed as *E. coli* by using cultural characteristic and standard biochemical tests. Out of 192 purposively collected carcass and environmental samples, 64(33.33%) were found to be positive for *E. coli* and from these positive tested samples, 17(26.56%) and 47(36.72%) were from carcass swab and environmental samples respectively. Furthermore, the overall percentage of contamination at abattoir (A) and Abattoir (B) located in Bishoftu were 13 (36.11%) and 10 (27.78%) respectively and at abattoir (O), abattoir (L) and abattoir (F) located in Modjo were 11 (30.56%), 14(38.89%) and 16 (33.33%) respectively. This difference might be due to sanitation and hygienic difference among those abattoirs. Higher percentage of *E. coli* contamination was recorded from hands and apron from dirty area. *E. coli* particularly pathogenic strains have public importance which has been transmitted through consumption of raw meat. So, each abattoir should improve the sanitation and hygiene practices of their workers.

Key words: *Escherichia coli* · Abattoir and Ethiopia

INTRODUCTION

Bacteriological quality of meat products is strongly influenced by the prevailing hygiene condition during their production and handling [1]. Food borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs [2]. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food borne illnesses. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) [3].

Despite the extensive scientific progress and technological developments achieved in recent years in developed countries, microbial food borne illness still remains a global concern. Numerous epidemiological reports have implicated food of animal origin as the major vehicles associated with illness caused by food borne pathogens, such as *Escherichia coli* (*E. coli*), *Salmonella, Shigella and Campylobacter* for human infection and this problem is highly aggravated in the developing world [4, 5, 28].

The *Enterobacteriaceae* group of bacteria is the most challenging bacterial contaminant to raw and processed meat products worldwide. *Salmonella*, *E. coli*, *Proteus* and *Klebsiella* species are the most predominant species in all food poisoning cases associated with some meat products [6, 29].

E. coli is a Gram-negative, facultative anaerobic, non-spore-forming rod, which belongs to the *Enterobacteriaceae* family (30) and it is one of the

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mesophilic commensal organisms found in the gastro intestinal tract of man and animals. *E. coli* O157:H7 is one of the most significant food-borne pathogens that have gained increased attention in recent years. This bacterium is often termed as an enterohemorrhagic *E. coli* (EHEC) and causes food borne illness [5]. An enterohemorrhagic *E. coli* (EHEC) are responsible for intestinal diseases (Gastroenteritis) and extra intestinal infections, which include urinary tract infections (UTI), bacteremia and neonatal meningitis. *E. coli* accounts for more than 90% of all uncomplicated UTIs [7]. STEC **(Put full name in the first mentioning??)** form the majority of the *E. coli* implicated in food borne disease [8] and they are a heterogeneous group of *E. coli* linked by a single feature; the ability to produce Shiga toxins (Stx) [9].

Contamination of edible carcass tissue is the most significant challenge to food safety; the extent and nature of such contamination may be 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 [10]. The habit of consuming raw and/or undercooked meat is one of the factors that exacerbate the transmission of food borne pathogens including *E. coli* in the country. Sufficient heating of meat kills these organisms [11]. Poor hygiene and slaughter practice can result in contaminated meat. However, in Ethiopia, most people prefer to eat raw or undercooked beef (Locally called kitfo, dulet and kurt). In Ethiopia studies concerning the prevalence of the pathogenic *E. coli* have been conducted on different types of samples from cattle, sheep and goat meat at abattoirs in East Showa [12] and abattoir in Modjo [13]. But a study to estimate the source of contamination in the abattoirs is not well addressed (Are you sure??). Consequently, this study was designed to address the information gap of the source of contamination of carcass and isolation and identification of *E. coli* at selected abattoirs, Ethiopia.

MATERIALS AND METHODS

Study Area and Study Population: The study was conducted in the Oromia regional state at abattoirs located in Modjo and Bishoftu town from December 2016 to March 2017. In these abattoirs only male small ruminant's sheep and goats are slaughtered. The abattoir operates in all days a week and each abattoir slaughters 500-1500 goats daily depending on the demand from customers, availability of supply of animals. The study goats are Ethiopian indigenous goat types sourced from lowland and mid highland areas of Ethiopia including Borena, Afar, Metahara, Jinka, Hararghe and Wollo. These goats were kept under traditional extensive management condition and these goats were apparently healthy at the time of slaughter.

Study Design: Cross sectional study was conducted from December 2016 to March 2017 with the aim of isolation and identification of *E. coli* at genus level at abattoirs located in Bishoftu and Modjo. Carcass (Before wash, after wash spray and chilled) and environmental samples (Washing water, hands, knife, loading table and apron) were tested for the isolation of *E. coli* and the abattoirs were selected purposively to determine the source of contamination and on each sampling day, usually once a week, 10-13 samples were collected. A total of 192 samples were collected purposively from those abattoirs in a given period of time based on voluntary of abattoir management, availability of distance and transportation and the size of sample was limited due to limitation of equipment, reagent and budget for doing and processing more samples in microbiology.

Carcass Sampling: A total of 64 carcass swab samples (Carcass before and after wash, chilled and after acetic acid spray) were collected from different sites of the carcass and the selected site were swabbed using the method described in Mersha *et al.* [14] by placing sterile template (10 x 5 cm) on specific sites of a carcass. For each sampling area, a sterile cotton tipped swab (2 X 3 cm) fitted with shaft moistened in 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, India) was rubbed first horizontally and then vertically several times on the sampling site. After completion of rubbing, the different swabs used for the different sites were put into a different universal bottle.

Environmental Sampling: Furthermore, a total of 128 environmental samples consisting of hand (n=32), apron (n=32) knife (n=32), loading table (n=16) and carcass wash water samples (n=16) were collected. They were collected by swabbing the hands of abattoir workers, the slaughter knives, table and apron in the same principle used in swabbing carcass. In addition, carcass washing water samples (10ml) at using point were collected. On completion of the process samples were transported using an insulated ice box at 4°C to National Veterinary Institute bacteriology laboratory.

Laboratory Work: After arrival each sample was placed in biosafety cabinet and swab samples were placed in zero dilution and mixed by vortex and homogenized to give 10-fold dilutions in the 1:9 ratios. Homogenate sample obtained were shaken to mix thoroughly and then 1ml was pipetted into a test tube containing 9ml of tryptone soya broth (TSB) and mixed further to give a 10^{-2} dilution. This procedure was repeated through serial dilutions ranging from 10^{-2} to 10^{-4} by adding 1 ml of homogenate to 9 ml sterilized TSB according to method described by Harrigan [15]. The universal bottles were labeled respectively and selected dilutions of the samples were appropriately mixed by overtaxing. By using pipette, 1 ml of sample per plate was poured on two sterilized TSA plate and streaking by using plaster loop and the inoculated media were incubated at 37°C for 48h according to Quinn *et al.* [16]. The inoculation methods used were adopted from the compendium of methods for microbiological examination of foods [17]. After that colony morphology on the plate was observed and Gram staining was done on the different colonies to differentiate Gram negative bacteria. Those revealed gram negative, short rods and pink colony samples were subcultured on to MacConkey agar (Oxoid Ltd., Hampshire, India) and incubated at 37°C for 24 hours to obtain pure colonies for biochemical tests. Primary biochemical test viz Oxidase and Catalase were done on rode shape pink colonies isolated from MacConkey agar plate. Secondary biochemical tests namely, Indole, Methyl red, Vogesproskauer reaction and Citrate utilization tests were done To check for their ability to ferment glucose, lactose and sucrose sugars, gas and H_2S production isolated colonies were inoculated onto Triple Sugar Iron (TSI) agar slants by stabbing and biochemically confirmed as *E. coli* following standard bacteriological procedures described in Quinn *et al*. [16]. Those isolates giving a result of yellow slant and butt with gas but no H_2S production on TSI slant agar after incubation of the media at 37°C for 24 hours and exhibited the IMViC pattern of +, +, -and -, respectively were identified as *E. coli*.

Data Management and Statistical Analysis: After coding, data collected from selected abattoirs were transferred to a Microsoft Excel spreadsheet and analyzed using SPSS 20. The results were summarized using descriptive statistics; frequencies and percentages.

RESULTS

Out of the total 192 (Carcass swab and environmental) samples examined 64 (33.33%) were found positive whereas the rest 128 (66.67%) were negative for *E. coli* contamination (Table 1).

As shown in table 2 out of the total of 192 samples examined 23 (31.94%) and 41 (34.17%) positive samples were found from selected abattoir of Bishoftu and Modjo respectively. Abattoir (A) and abattoir (B) were abattoirs located in Bishoftu town and whereas Abattoir (O), Abattoir (L) and Abattoir (F) were abattoirs located in Modjo town.

From the total positive samples tested (33.33%), 26.56% and 36.72% of carcass swabs and environmental sample were positive which indicated that the most contamination was from environmental samples (Table 3).

DISCUSSION

Ruminants are considered the primary reservoir for *E. coli*, where the organism typically colonizes the lower gastrointestinal tract [18] and is shed in the feces. Microorganisms of concern to meat processors may originate from the faeces and skin of animals and also include environmental sources like working utensils presented for slaughter that can be transferred to the carcass during skin removal and evisceration [19]. Moreover, during hide stripping, some bacteria originating from the animal hide become suspended in the abattoir atmosphere. The objective of this study was to isolate and identify *E. coli* from carcass samples and environmental samples at selected export abattoirs found in Bishoftu and Modjo.

In the present study, 17(26.56%) carcass were found positive for *E. coli*. In contrast of this finding Taye*et al.* [20] reported prevalence of 30.97% at slaughtering house in Haramaya University and the reason behind this difference was hygienic practices for example poor hygiene of the workers and improper use of disinfection at abattoirs. As Manna *et al*. [21] described basically, hygienic status of dressed carcasses is largely dependent upon the general slaughterhouse hygiene and the skills of the workers.

As the present finding implied that contamination of *E. coli* was highly found in environmental samples (Water, hand, knife, table and apron 47(36.72%) when compared to samples directly taken from carcass

Table 1: Overall percentage of tested samples in export abattoirs

Item	No. of examined sample	Percentage (%)
No. of positive samples for *E. coli*	64	33.33
No. of negative samples for *E. coli*	128	66.67
Total	192	100

Table 2: Percentage isolation of *E. coli* from the five export abattoirs

Origin	Abattoirs	No. of sample examined	No. of samples with *E. coli*	Percentage (%)
Bishoftu	A	36	13	36.11
	B	36	10	27.78
Modjo	Total	72	23	31.9
	O	36	11	30.56
	L	36	14	38.89
	F	48	16	33.33
	Total	120	41	34.17

Table 1: The overall *E. coli* isolation carcass and environmental samples

Source of sample	Sample type	No. of sample examined	No. of positive sample (%)
Carcass Swab	CHC*	16	2(12.5)
	CASP*	16	1(6.25)
	CBW*	16	10(62.5)
	CAW*	16	4(25)
	Sub total	64	17(26.56)
Environmental samples	HAD*	16	14(87.5)
	APDA*	16	13(81.25)
	HCLA*	16	3(18.75)
	APCLA*	16	6(37.5)
	KBSt*	16	5(31.25)
	KASt*	16	2(12.5)
	Water	16	1(6.25)
	Table	16	3(18.75)
	Sub total	128	47(36.72)

* = CHC- chilled carcass, CASP- Carcass after spray, CBW-Carcass before wash, CAW-Carcass after wash, HDA- hand dirty area, HCLA- hand clean area, APCLA- apron clean area, APDA-apron dirty area, KBst- knife before sterilization and KAst- knife after sterilization

17(26.56%) and this indicated that *E. coli* contamination in the abattoirs mostly occurs as a result of lack of good hygiene in environmental samples and sanitation of slaughtering house. From environmental samples, worker's hand and apron from dirty area were considered as the major sources of contamination and this indicated that workers in the abattoirs did not keep their own hygiene including protective cloth they use and also improperly disinfect their hands while working. These problems were arising in the abattoirs due to the weakness of abattoir management so manager should follow up the hygiene of each worker and equipment used in the abattoir.

The percentages of *E. coli* isolation among abattoirs found in Modjo were 34.17% and Bishoftu 31.94% and this difference could have a number of reasons. Abattoir located in Bishoftu was well equipped including hand-wash stations with hot running water and stations to sanitize knives. Unlike abattoirs located in Modjo, abattoirs in Bishoftu provided with sanitized lairage and enough water before slaughter for 24 hours with aim of reduction of normal intestinal flora and subsequently reduction on meat contamination with *E. coli* from feces. Furthermore, good sanitation status as well as hygiene of the workers present in abattoirs located in Bishoftu supposed to minimize contamination of carcass with feces and other dirty things during slaughtering process. Samuel *et al*. [22] and Maja [23] reported that feces and soil adhering to the animals were carried into the abattoir on the hair, hide, hooves and tail of the animal and become a major source of carcass contamination.

The present findings concerning carcass contamination by *E. coli* nearly agree with findings of 26.6% by Haimanot *et al*. [24] and 24.89% by Mohammed *et al*. [25] and slightly disagree with report from Modjo 30.2% by Mersha *et al*. [14]. But the present findings of *E. coli* on carcass (26.56%) are higher than the study conducted by Sudhakar *et al.* [26] on the isolation of *E. coli* from meat in Indian modern abattoir (13.4%), Mohammed *et al.* [25] at Dire Dawa ELFORA abattoir

(17.29%), Mersha *et al*. [13] at Modjo export abattoirs (16.9%) and Mohamed and Mebrouk (31) in frozen Bovine meat in Algeria (0.44%). The highest percentage of *E. coli* isolated in this study is probably associated with the poor hygiene practices involved in meat processing like lack of good hygiene management of workers, as well as materials used in slaughtering house and sanitation of slaughtering plant and also due to difference in sampling and isolation procedures, variability in sampled populations [27].

CONCLUSIONS AND RECOMMENDATIONS

Poor hygiene and slaughter practice can result in contaminated meat, which is especially risky in Ethiopia because of the common practice of eating raw meat and they become infected with pathogenic *E. coli* from contaminated meat through eating of undercooked meat. The present study showed that the presence of relatively high percentage of carcass and environmental samples contamination with *E. coli* at abattoirs locating in Bishoftu and Modjo. The obtained higher percentage contamination was found on environmental samples in comparison to samples directly taken from carcass. Worker's hand and apron from dirty area were highly contaminated environmental samples with *E. coli* that may be a potential source of carcass contamination. In line with the above conclusion the following recommendations were forwarded

- Implementation of appropriate hygiene measures to control contamination of meat with *E. coli* that generated from slaughtering house workers and equipment.
- Creating public awareness not to eat raw meat and improperly cooked meat.

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