Study on Carcass Contaminating *Escherichia coli* in Apparently Healthy Slaughtered Cattle in Haramaya University Slaughter House with Special Emphasis on *Escherichia coli* O157:H7, Ethiopia

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Abstract

A cross-sectional study was conducted from October 2010 to March 2011, on apparently healthy slaughtered cattle in Haramaya University Slaughter House to determine prevalence of *Escherichia coli*, with special emphasis on *Escherichia coli* O157:H7, and its antimicrobial susceptibility pattern. Carcass swab samples were collected and pre-enriched in buffered peptone water and plated on MacConkey agar plate. Presumptive colonies were confirmed by biochemical tests. Further identification of *Escherichia coli* O157:H7 was done by plating the isolated bacteria on Sorbitol MacConkey agar; and then colorless colonies were subjected to *Escherichia coli* O157:H7 Latex agglutination test. From a total of 113 samples collected, *Escherichia coli* was isolated from 35 (30.97%) and out of these, 3 (2.65%) were found to be *Escherichia coli* O157:H7. The difference in prevalence was not statistically significant (P>0.05) between local and cross breeds (χ²=0.11, df =4), among age group of animals (χ²=2.56, df=1) and origin of animals (χ²=2.66, df=2). The isolated bacteria were subjected to antimicrobial susceptibility testing and the majority were found to be susceptible to Chloramphenicol (30 µg), Kanamycin (30 µg), Spectinomycin (SH, 100 µg). The presence of *E. coli* O157:H7 in raw meats reaching to consumers indicated possible risks of infection to people through the consumption of raw (undercooked) meat and cross contamination of other food products. Therefore, control measures at all stages of food chain was recommended.

Keywords: Antimicrobial susceptibility test; Cattle; *Escherichia coli*; Haramaya University slaughter house; Prevalence

Introduction

*Escherichia coli* (*E. coli*) are normal inhabitants of the gastrointestinal tract (lower ileum and large intestine) of animals and human beings [1,2,3]. Some serotypes are closely correlated with certain clinical syndromes [4]. Infection with *E. coli* O157:H7 is a major food borne and zoonotic pathogen responsible for hemorrhagic colitis and hemolytic uremic syndromes in humans. Transmission to human occurs through consumption of undercooked meat, unpasteurized dairy products, and vegetables or water contaminated by feces of carrier animals [1]. Outbreaks of *E. coli* O157 have been reported in different parts of the world and antibiotic use is controversial because of the potential to increase production and secretion of Shiga toxins. Increase in antibiotic resistance has been noted over the last 20 years [5]. Differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics [4].

In Ethiopia, there were studies conducted by few researchers [5,6] to determine the occurrence and proportion of *E. coli* O157:H7 in faeces, skin swabs and carcasses of sheep, goat and cattle in Debere Zeit and Modjo town. Even though little is known about the prevalence and antimicrobial susceptibility pattern of this bacterium in Ethiopia either in humans or animal population or foods, there is no information in eastern Ethiopia generally and in Haramaya University and its surrounding specifically, where large populations of cattle are reared for slaughter. Therefore, the main objectives of this study were to assess prevalence of carcass contamination with *E. coli* and determine the antimicrobial susceptibility pattern of isolates from the apparently healthy slaughtered cattle in Haramaya University slaughter house.

Materials and Methods

Study area and population

The study was conducted in Haramaya University slaughter house from October 2010 to March 2011. Haramaya University is located at an altitude of 1980 meters above sea level between latitude 9° 26” N and longitude 42° 3” E. The mean annual rainfall is 870 mm with a range of 560-1260 mm, and the mean maximum and minimum temperatures are 23.4°C and 8.25°C, respectively [7]. Both local and cross breeds cattle are reared in and around the study area for meat production mostly. Cattle brought from Chalanko, Kulubi, Water, Kersa and Haramaya, and slaughtered in Haramaya University slaughter house were the study population. Since only animals above one year are brought to the slaughter house, different age groups of above one year and both breeds were included in study.

Study protocol

A cross-sectional study was conducted on all animals slaughtered during regular visit (every week) of the slaughter house. Breed, age and origin were considered as risk factors. The age of individual study animal was determined according to De-Lahunta and Habel [8]. Carcass swab samples were collected from all apparently healthy slaughtered cattle using sterile cotton swab dipped in hydrated Buffed Peptone Water (BPW) according to the method described...
by Quinn et al. [9] and sterile gloves were used for each sampling to avoid cross contamination.

All collected swab samples were placed in the universal bottle containing buffered peptone water and transported in an ice box with ice packs to Veterinary Microbiology laboratory of Haramaya University and incubated at 37°C for 24 hours for enrichment. All pre-enriched carcass swab samples were subsequently subcultured onto MacConkey agar for primary screening of E. coli and incubated at 37°C aerobically for 24 hours. Suspected colonies of E. coli (pinkish color appearance) were subcultured onto nutrient agar (non-selective media) and confirmed by Triple Sugar Iron (TSI) and IMViC tests. Then the bacterium that was confirmed as E. coli was subcultured onto Sorbitol MacConkey agar from nutrient agar and colorless colonies (non-sorbitol fermenter) were again subcultured onto nutrient agar and Latex E. coli O157:H7 agglutination test was performed to determine strains.

In vitro antimicrobial susceptibility test was conducted on all confirmed E. coli and E. coli O157:H7 for six antimicrobial agents [Tetracycline (Te30 µg), Kanamycin (K30 µg), Spectinomycin (SH100 µg), Ampicillin (AMP10 µg), Amoxicillin (AML10 µg) and Chloramphenicol (C30 µg)] by using agar disc diffusion technique as described by NCCLS [10].

Data management and analysis

All collected data were entered into Microsoft Excel data sheet and statistical analysis was done by SPSS Version 17 statistical software. The variations between different factors were analyzed using chi-square ($\chi^2$) test. A p-value<0.05 was considered to determine statistical significance using 95% confidence interval.

Results

The overall prevalence of carcass contamination with E. coli was 35 (30.97%) (Table 1) and that of E. coli O157:H7 was 3 (2.65%). The difference in prevalence of carcass contamination with E. coli and E. coli O157:H7 between the two breeds, among the three age groups and origin of animals was not statistically significant (p>0.05).

In vitro antimicrobial susceptibility test was conducted on all isolates. Out of the isolated E. coli, six were 100% susceptible to Tetracycline (Te30 µg), Chloroamphenicol (C30 µg), Kanamycin (K30 µg), Spectinomycin (SH100 µg), Ampicillin (AMP10 µg) and Amoxicillin (AML10 µg). Multidrug resistance (MDR) was also observed both in E. coli and E. coli O157:H7 to different drug disks (Table 2).

Discussion

Estimates of the prevalence of verotoxin-producing E. coli among populations of cattle vary considerably [11]. The overall prevalence of carcass contamination with E. coli species of the present study in the slaughtered animals was 30.97 % (n=113) which is higher than 4.4% (n=885) prevalence reported in Kenya [12]. This might be due to lack of strict hygienic measures taken in Haramaya University slaughter house.

Cattle have been implicated as the principal reservoir of E. coli O157:H7 [13]. Many studies determined the prevalence of E. coli O157:H7 on cattle carcasses which were from 0.0% to 27.8% [13,14,15]. The prevalence of E. coli O157:H7 (2.65%) isolated from carcass in this study was in close agreement with the reported prevalence of 2.9% and 3.2% in the United Kingdom [16, 17], respectively. 2% in Canada [18] and five isolates (2.8%) out of 180 meat and meat products examined in South Africa [19]. The lower carcass contamination might be due to low fecal prevalence of E. coli O157:H7 in these animals and operational activities in the slaughter house, which results in relatively low risk of contamination and cross contamination [5]. The exact contamination rate may be higher than the stated one due to the low isolation rate of culture methods compared to immunological and molecular methods [20].

In comparison to the present study, a higher prevalence of E. coli O157:H7 were reported from different countries; 8% [5] and 8.1% [6] in Ethiopia, 9% in India [21] and 6% in Turkey [22]. A lower prevalence of E. coli O157:H7 were also reported from America (0.8%) [23], Kenya (0.2%) [12] and New York (1.3%) [24]. The overall variations in the prevalence of E. coli O157:H7 might be due to different sampling techniques, laboratory methodologies, areas, time and hygienic conditions used.

The difference between age groups, breed and origin of animals were not statistically significant (P>0.05). This disagrees with work

| Risk factors | Number of animals examined | No. of isolates (Prevalence) | E. coli | E. coli O157:H7 from isolated E. coli |
|--------------|---------------------------|-----------------------------|--------|-------------------------------------|
| Breed        |                           |                             |        |                                     |
| Local        | 109                       | 32 (29.35%)                 | 0      |                                     |
| Cross        | 4                         | 3 (75%)                     | 3 (100%)|                                     |
| p-value      |                           | 0.73                        | 0.73   |                                     |
| $\chi^2$     |                           | 0.11                        | 0.11   |                                     |
| Age          |                           |                             |        |                                     |
| 1-3 years    | 21                        | 9 (42.85%)                  | 2 (22.2%)|                                     |
| 4-6 years    | 56                        | 16 (28.6%)                  | 0      |                                     |
| ≥7 years     | 36                        | 10 (27.7%)                  | 1 (10%)|                                     |
| p-value      |                           | 0.425                       | 0.07   |                                     |
| $\chi^2$     |                           | 1.71                        | 5.28   |                                     |
| Origin       |                           |                             |        |                                     |
| Chalanko     | 31                        | 7 (22.6%)                   | 0      |                                     |
| Kulubi       | 34                        | 9 (26.8%)                   | 2 (22.2%)|                                     |
| Water        | 8                         | 4 (50%)                     | 0      |                                     |
| Kersa        | 34                        | 12 (35.3%)                  | 1 (8.3%)|                                     |
| Haramaya     | 6                         | 3 (50%)                     | 0      |                                     |
| p-value      |                           | 0.404                       | 0.644  |                                     |
| $\chi^2$     |                           | 4.011                       | 2.56   |                                     |
| Total        | 113                       | 35 (30.97%)                 | 3 (8.57%)|                                     |

Table 1: Prevalence of carcass contamination with E. coli and E. coli O157:H7 and associated risk factors.

| Antimicrobials tested | Susceptible E. coli | Intermediate E. coli | Resistant E. coli |
|-----------------------|---------------------|----------------------|-------------------|
| Te(30µg)              | 51.4%               | 66.67%               | 0                  | 48.57%             | 33.33%             |
| C (30µg)              | 74.3%               | 100%                 | 2.86%              | 0                  | 22.86%             | 0                  |
| K(30µg)               | 85.5%               | 100%                 | 8.57%              | 0                  | 8.66%              | 0                  |
| SH(100µg)             | 85.7%               | 100%                 | 8.57%              | 0                  | 5.67%              | 0                  |
| AMP(10µg)             | 28.6%               | 0                    | 5.7%               | 0                  | 65.7%              | 100%               |
| AML(10µg)             | 31.4%               | 0                    | 0                  | 0                  | 66.67%             | 100%               |

Table 2: Antimicrobial susceptibility profile of E. coli and E. coli O157:H7 isolates.
of Luga et al. [25] in which the seroprevalence of E. coli O157:H7 in cattle is significantly associated with sex, diarrhea status and age. The variation between these studies may be due to climatic variation which plays important role [26].

There was variation in antimicrobial susceptibility of E. coli in the present study. Similarly, tests conducted on stool samples collected from diarrheic patients in Korem, Ethiopia showed that 53% of E. coli strains were found to be resistant to ampicillin, 47% to Chloramphenicol, and 67% to Tetracycline [27]. Although there is significant variation between this study and the present study, there were resistant nature of E. coli species to ampicillin and tetracycline so long years back.

All isolated strains in the present study were found susceptible to Kanamycin (K30 µg), Chloramphenicol (C30 µg) and spectinomycin (SH100 µg) and 100% resistant to Ampicillin (AMP10 µg) and Amoxicillin (AML10 µg) and 33.33% resistant to Tetracycline (Te30 µg). Similar findings were reported by other researchers [5,28]. Multidrug resistant was also detected in 66.67% of the strains. This agrees with results of many workers report [5].

Conclusion and Recommendations

The present result revealed prevalence of carcass contamination with E. coli and E. coli O157:H7 from apparently healthy slaughtered cattle in Haramaya University slaughter house. The obtained higher prevalence indicated poor hygienic practice in the house. Age group, breed and origin of animals as potential predisposing factors were found to be not significantly associated with the occurrence of E. coli contamination. The isolated bacteria showed variety of susceptibility pattern against different antimicrobials used for in vitro test. Therefore, the following recommendations were forwarded:

1. Proper and strict hygiene is very important for equipments and personnel working in the house.
2. Creating public awareness about the bacteria and thorough cooking of products from the slaughter house is essential to reduce consumers' infection rate.

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