

Full Length Research Paper

Antibiogram of *Escherichia coli* strains disconnected from nourishment of bovine Origin in selected Woredas of Tigray, Ethiopia

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Escherichia coli is a food borne pathogen causing a major public health problems. The use of antimicrobials in food animals produces resistant bacteria. To determine antimicrobial resistance of *E. coli* species isolated from food of bovine origin, a total of 384 of milk samples (n=192) and meat samples (n=192) were collected from different sources in 1:1 ratio in selected Woredas of Tigray, Ethiopia. Samples were cultured on sheep blood agar and sub-cultured on Eosin Methylene and further sub-cultured on Biolog Universal Growth Agar (BUG media). Pure colonies were taken and suspension was made and inoculated into micro plates. The bacteria were identified by BiOLOG Identification system. Antimicrobial resistance of *E. coli* isolates was done by disk diffusion method using twenty antimicrobials and minimum inhibitory concentration was determined for resistant isolates. The study revealed that out of 384 samples of milk and meat, *E. coli* 0₁₅₇:H₇ (10.4%), *E. coli*, Non 157 STEC (2.6%) and *E. coli* enterotoxigenic (10.7%) were isolated. Antimicrobial resistance pattern of *E. coli* isolates (n=91) revealed high resistance against cephalothin (84.6%), chloroamphenicol (83.3%), tetracycline (88.9%), gentamicin (65.9%), but low resistance for sulphoxazole-trimethoprim (16.5%), neomycin (15.4%), streptomycin (29.7%), kanamycin (30.8%), ciprofloxacin (10%), nitrofurantoin (3.3%), norfloxon (3.3%) and ceftriaxone (9.9%). Multidrug resistance was observed in 82 (93.2%) of species. The high prevalence of 0₁₅₇:H₇ and enterotoxigenic and high rates of multiple drug resistance indicate there is a need for timely designing prevention and control strategies.

Key words: Antimicrobial, *Escherichia coli*, meat, milk, resistance, zoonoses.

INTRODUCTION

Food safety, safety of products of animal origin in particular, is an increasingly important issue with regards

to human health. With increasing consumption of products of animal origin, the risk of food borne

diseases of humans also increases. One product that is commonly distributed in raw form is milk. Raw milk is a known vehicle and medium for pathogens like *Escherichia coli*. Milk can become contaminated in many ways. There are mammary gland infection (mastitis) or a systemic infection, and contamination through the faeces of the animals and the hand of the milker usually during hand milking procedure or by equipment used for milk collection and storage (Leedom, 2006).

Similarly, meat and its products are important reservoirs for many of the food-borne pathogens, including *E. coli* O157:H7. Foodborne diseases remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food (WHO, 2000). Reliable statistics on food-borne diseases are not available due to poor or non-existent reporting systems in most developing countries.

Besides its high prevalence, the rising antimicrobial resistance (AMR) is partly due to the overuse and misuse of antimicrobials (e.g. as growth promoters for food animals) in food animal production, becoming a major problem.

In some countries, up to 70% of antibiotics are used for animals raised in industrial farms that are not sick, to offset the effects of crowding and poor sanitation. This practice promotes the development of drug-resistant bacteria that can spread to humans. Thus, food-borne diseases, when associated with resistant bacteria, are harder to treat, resulting in longer hospitalization, higher mortality and morbidity, decreased productivity, and increased costs (WHO, 2011). Likewise, antimicrobial resistance is constantly evolving challenge. Further transfer of antimicrobial resistant bacteria to humans via food chain has been reported (Angulo et al., 2004). A limited number of investigations have been studied regarding the presence of antimicrobial resistance in food animals in Ethiopia (Mekonnen et al., 2005; Hundera et al., 2005). The finding of the present study on antimicrobial resistance of food-borne pathogens will provide useful information on the development of public health policy in food animal production. Thus, the study was carried out with the aim to isolate *E. coli* species and to determine its antimicrobial resistance from food of bovine origin.

MATERIALS AND METHODS

Study area

The study was conducted in three districts of Tigray, Mekelle; Alamata and Adigrat. These districts were selected mainly because of their difference in the altitudes that may help us to obtain reliable evidence on the magnitude and epidemiology of disease in the region (RSITBARD, 2009).

Study design

A cross-sectional study was conducted from November 2012 to June 2013 in the selected districts of Tigray, Ethiopia.

Sample size and sampling technique

A total of 384 samples were collected from bovine raw milk and meat in the selected Woredas of Tigray, Ethiopia. The sample size was determined according to the formula given by Thrusfield (2005) by taking prevalence of 50% so that the maximum sample size could be achieved. Accordingly, the calculated value for sample size was 384. Then, equal number of milk (n1=192) and meat (n2=192) samples were included purposely. In sampling of milk and meat samples, simple random sampling technique was applied until sample size was achieved.

Sample collection, transport and handling

Milk samples

Milk samples were collected according to the National Mastitis Council Guideline (1990) by principal investigator. Milk samples were aseptically collected directly from teats of lactating cows (n=64) and from distribution sites (shop=64 and restaurant=64) using sterile sample bottles. Samples were transported using icebox to Microbiology Laboratory of College of Veterinary Medicine, Mekelle University. Milk samples were immediately cultured or stored at 4°C for a maximum of 24 h until the samples were cultured.

Meat samples

Raw meat from slaughter house (n=64) during slaughtering and non pre-packed meat samples from beef were purchased randomly from selected butcher shops (n=64) and restaurant (n=64). Sections of meat (10 × 10 × 3 cm) from neck of each carcass were aseptically removed and placed in separate sterile plastic bags to prevent spilling and cross contamination. It was immediately transported to Microbiology Laboratory of College of Veterinary Medicine, Mekelle University in a cooler with ice packs. After culture, the prepared samples were transported with icebox to Microbiology Laboratory of Institute of Biodiversity Conservation, Addis Ababa for further confirmatory identification.

Culture and identification

Milk sample

Bacteriological examination was done according to the National Mastitis Council Guideline (1990). A 0.1 ml of milk was spread on tryptose blood agar base (Oxoid, UK) enriched with 7% defibrinated sheep blood using spread plate after centrifugation and discarding the supernatant. Blood agar plates were incubated aerobically at 37°C for 24 - 48 h. Then Gram staining was done for all suspected cultures of *E. coli* and Gram negative bacillus were sub-cultured into Eosin Methylene blue agar. Then, pure colony was taken and sub-cultured on BUG (BiOLOG Universal Growth Media) at 37°C for 18-24 h as a primary and secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media were inoculated into 18-20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into micro plates with multi-channel pipettes. The micro plates were loaded into Omnilog tray to be

incubated, analyzed and interpreted for 18-24 h as per BiOLOG Users Guideline (2008) and finally identified bacteria were printed out.

Meat sample

The microbiological examination of each meat sample, 25 g was homogenized with 1 g of the homogenate and added to 5 mL of buffered peptone water (BPW- HiMedia Laboratories, Mumbai, India) and incubated. Cultures spread on tryptose blood agar base (Oxoid, UK) enriched with 7% defibrinated sheep blood using the spread plate techniques and the plates were incubated overnight at 37°C. From each plate (one plate for each meat sample), 5 to 10 suspected bacterial colonies were selected and sub-cultured onto Eosin Methylene Blue agar. Then pure colony was further sub-cultured on BUG at 37°C for 18-24 h as primary and secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media were inoculated into 18-20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into micro plates with multi-channel pipettes. The micro plates were loaded into Omnilog tray and incubated, analyzed and interpreted for 18-24 h as per BiOLOG Users Guideline (2008) and finally identified bacteria were printed out.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed for all isolates according to the criteria of the Clinical and Laboratory Standards Institute (2008). For susceptibility test, a pure culture of all identified *E. coli* was taken from BUG media and transferred to a tube containing 5 ml of sterile normal saline and mixed gently to make homogenous suspension which was adjusted to a turbidity equivalent to a 0.5 Mc Farland standard as measured by turbidity meter. The bacterial suspension was inoculated on to Muller-Hinton agar (Oxoid, UK) with the sterile swab to cover the whole surface of the agar. The inoculated plates were left at room temperature to dry. The plates were prepared as per the manufacturer's instructions and checked for sterility before inoculation by incubating the plates over night at 37°C. Before using the antimicrobial disks, they were kept at room temperature for one hour and then dispensed on the surface of media. Following this, the plates were incubated aerobically at 37°C for 24 h.

For susceptibility test, antimicrobials which were used for treatment of bovine mastitis or considered as important antimicrobial agents for human was selected for antibiogram based on the criteria of Clinical and Laboratory Standards Institute (2008). Thus, antimicrobials used in this study were cephalothin (30 µg), sulphoxazole-trimethoprim (25 µg), neomycin (5 µg), streptomycin (10 µg), kanamycin (30 µg), chloramphenicol (30 mg), tetracycline (30 µg) and gentamicin (10 µg) (Oxoid, UK). Antimicrobials not used for treatment of bovine mastitis but important for human were ciprofloxacin (5 µg), nitrofurantoin (300 µg), norfloxon (10 µg), ceftriaxone (30 µg) (Oxoid, UK). The diameters of the zone of inhibition around the disks were measured to the nearest millimeter using calibrated rulers, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of Clinical and Laboratory Standards Institute (2008). In addition, minimum inhibitory concentration (MIC) was determined using broth dilution method with an antimicrobial concentration ranging from 0.25-512 µg/µL, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). Those isolates with minimum inhibitory concentrations (MIC) higher than the breakpoint for the respective antimicrobial agents were regarded as resistant, while those with MIC equal to or lower than the breakpoint were regarded as susceptible. Moreover,

isolates showing resistance to three or more antimicrobial subclass were considered as multidrug resistant.

Quality control

Confidence in the reliability of test results was increased by adequate quality assurance procedures, and the routine use of control strains. Thus, *E. coli* ATCC-25922 was taken as an important part of quality control for culture, BiOLOG identification and antimicrobial susceptibility through this study.

Variables

Independent variables such as types of samples were interpreted against dependent variable of species isolates and antimicrobial sensitivity pattern of each isolates.

Ethical issues

Verbal consent was obtained from dairy farms, abattoirs and butcher shop owners/managers.

Statistical analysis

The collected data was entered into EPI data version 3.1 and exported to SPSS version 16 computer software then the data was analyzed. Accordingly, descriptive statistics such as percentages and frequency distribution were used to describe/present bacterial isolates and antimicrobial susceptibility which were expressed as percent of resistant and susceptible. In addition, the proportion of bacteria resistant to at least one of the antibiotics and resistant to two or more were calculated.

RESULTS

Prevalence of subspecies isolated from milk and meat samples of bovine origin

The total number of species isolated from milk and meat samples of bovine origin are indicated in Figure 1.

O157:H7 (10.4%) , *Non 157 STEC* (2.6%) and *E. coli enterotoxigenic* (10.7%) were detected in all the samples tested.

Antimicrobial resistance profile of species isolated from milk and meat samples

Analysis of subspecies specific resistance rates indicated for isolates from milk and meat are shown in Table 1. All *E. coli* strain showed high percentage resistance to cephalothin, chloramphenicol, tetracycline and gentamicin. On the other hand, most *E. coli* isolates were susceptible to sulphoxazole-trimethoprim, neomycin, streptomycin, kanamycin, ciprofloxacin, nitrofurantoin, norfloxon and ceftriaxon

The overall multiple antimicrobial resistance rate was 93.2%. The resistances against two or more antimicrobial

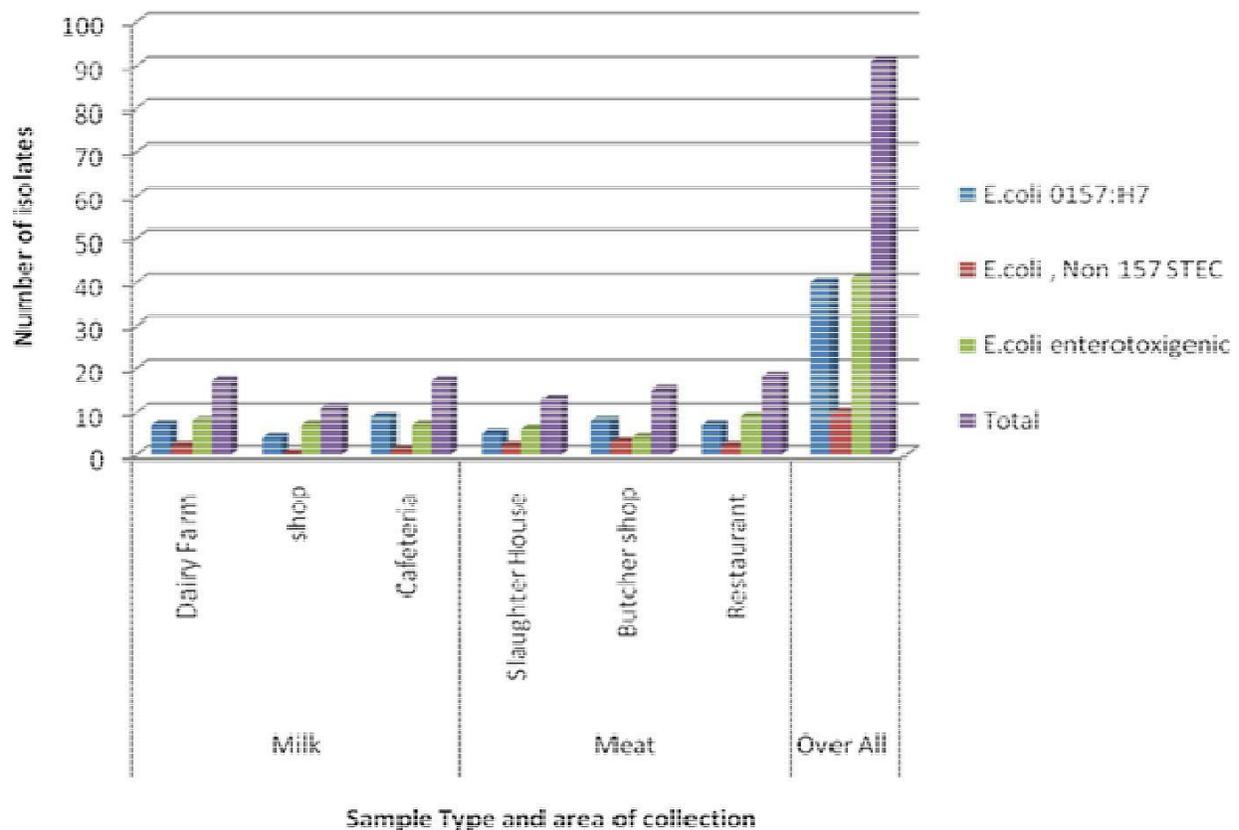


Figure 1. Prevalence of strains from milk and meat of bovine origin.

agents were observed in all 0₁₅₇:H₇ and non 157 STEC and 95% enterotoxigenic isolated from milk showed multiple drug resistance (Table 2). 89.5% 0₁₅₇:H₇, 71.4% of Non 157 STEC and 94.7% enterotoxigenic isolated from meat samples showed multiple drug resistant.

DISCUSSION

In the present study, the presence of strains in food of bovine origin indicated that the bacteria originated from infected animals or unhygienic conditions during processing, handling and distribution. It did not only originate from infected animals but more likely as an indicator of poor hygiene and sanitary practices while handling food of animal origin.

The isolation rate of *E. coli* in the present study was 23.7% and it was mainly isolated from meat samples from restaurant (28.5%) and milk sample from cafeteria (26.6%). These findings are in conformity with reports by other researchers (Yismaw et al., 2010; Al-Tawfiq, 2006; Gangoué et al., 2004). Higher prevalence was reported by Ali and Abdelgadir (2011) 63% and Lingathurai and Vellathurai (2010) 70%. In fact, if the methods of production, transportation, handling and sale of milk are

entirely unhygienic there is high prevalence (Yismaw et al., 2010).

Antibiotic resistance development among the bacteria poses a problem of concern. In all food samples of bovine origin in the present study, *E. coli* showed high resistance rates (greater than 80%) to cephalothin, chloramphenicol, tetracycline and (greater than 60%) to gentamicin. The results of this study are in line with the findings of other studies conducted in different parts of the world (Bharathi et al., 2008; Briscoe et al., 2005). However, antimicrobial resistance rates obtained in this study were higher as compared to susceptibility patterns reported from previous studies (Zhanet al., 2006; Karlowsky et al., 2002; Barrett et al., 2000).

E. coli isolates were sensitive to sulphoxazole-trimethoprim, neomycin, streptomycin, kanamycin ciprofloxacin, nitrofurantoin, norfloxon and ceftriaxone. Similar studies conducted in Ethiopia by Tesfaye et al. (2009) and in Nigeria by Wariso and Ibe (2006) have reported comparable susceptibility rates. In this study, sulphoxazole-trimethoprim, neomycin, streptomycin, kanamycin ciprofloxacin, nitrofurantoin, norfloxon, and ceftriaxone were found to be the most effective antimicrobials against *E. coli* isolates. Furthermore in this study, a high rate of multiple antimicrobial resistance

Table 1. Antimicrobial susceptibility pattern of stains isolated from milk and meat sample of bovine origin.

Antimicrobial	O₁₅₇:H₇				Non 157 STEC				Enterotoxigenic				Overall (n=91)		MIC(μg/μl)	
	Milk (n=20)		Meat (n=20)		Milk (n=3)		Meat (n=7)		Milk (n=22)		Meat (n=19)		S (%)	R (%)	MIC 50	MIC 90
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	128	512
cephalothin (30 μ g)	10	90	10	90	33.3	66.7	28.6	71.4	27.3	72.7	5.3	94.7	15.4	84.6	32	128
SXZ (25 μ g)	80	20	85	15	33.3	66.7	85.7	14.3	95.5	4.5	78.9	21.1	83.5	16.5	64	512
Neomycin (5 μ g)	80	20	95	5	100	0	71.4	28.6	181.8	18.2	84.2	15.8	84.6	15.4	128	512
streptomycin(10 μ g)	55	45	70	30	100	0	85.7	14.3	72.7	27.3	73.7	26.3	70.3	29.7	512	512
kanamycin (30 μ g)	55	45	70	30	100	0	85.7	14.3	72.7	27.3	68.4	31.6	69.2	30.8	128	512
Chloramphenicol (30 mg)	10	90	25	75	0	100	28.6	71.4	22.7	77.3	5.6	94.4	16.7	83.3	128	512
Tetracycline (30 μ g)	0	100	20	80	0	100	14.3	85.7	13.6	86.4	10.5	89.5	11.1	88.9	512	512
Gentamicin (10 μ g)	25	75	30	70	0	100	57.1	42.9	40.9	59.1	36.8	63.2	34.1	65.9	64	128
Ciprofloxacin (5 μ g)	94.7	5.3	85	15	100	0	85.7	14.3	95.5	4.5	84.2	15.8	90	10	16	64
Nitrofurantoin (300 μ g)	95	5	95	5	100	0	100	0	100	0	94.7	5.3	96.7	3.3	4	128
Norfloxon (10 μ g)	95	5	95	5	100	0	100	0	100	0	94.7	5.3	96.7	3.3	4	64
Ciftriaxone (30 μ g)	90	10	95	5	100	0	71.4	28.6	100	0	89.5	10.5	90.1	9.9	2	32

S: Susceptible R: resistant MIC: minimum inhibitory concentration, SXZ: sulphoxazole-trimethoprim, n=number of positive isolate.

Table 2. Percentages of number of antimicrobials resistant strains isolated from milk and meat sample of bovine origin.

Number of antimicrobials	O₁₅₇:H		Non 157 STEC		Enterotoxigenic		Overall (n=91)
	Milk (n=20)	Meat (n=20)	Milk (n=3)	Meat (n=7)	Milk (n=22)	Meat (n=19)	
One (%)	0	10.5	0	28.6	5	5.3	6.8
MDR (%)	100	89.5	100	71.4	95	94.7	93.2

MDR: multi-drug resistance n=number of positive isolate.

(93.2%) was recorded, which is consistent with the reports of studies done elsewhere by other scholars (Orrett and Shurl, 2001; Kurutepe et al., 2005). Increases in rate of resistance to different antimicrobials have been reported from previous studies conducted in different parts of the world (Orrett and Shurl, 2001; Kurutepe et al., 2005).

Conclusions

Results clearly indicated that there is a possibility

of potential public health threat of species originating from food of bovine origin. The high prevalence *O*₁₅₇:*H*₇ (Shiga toxin producing) and enterotoxigenic, and high rates of multiple drug resistance indicates alarming situation for designing prevention and control methods.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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