East African Medical Journal Vol. 90 No. 3 March 2013
MOLECULAR CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI* ISOLATES FROM GOATS SLAUGHTERED IN PARTS OF KENYA
S. Njoroge, Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200, Nairobi, Kenya, A. W. T. Muigai, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000-00200 Nairobi, Kenya, P. N.Njiruh, Division of Microbiology, Department of Biochemistry & Biotechnology, The Technical University of Kenya, Nairobi, Kenya, S. Kariuki, Center for Microbiology Research, Kenya Medical Research Institute, PO Box 54840 00200 Nairobi, Kenya

Request for reprints to: Stephen Njoroge, Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

MOLECULAR CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI* ISOLATES FROM GOATS SLAUGHTERED IN PARTS OF KENYA

S. NJOROGE, A. W. T. MUIGAI, P. N.NJIRUH and S. KARIUKI

ABSTRACT

***Objective*: To determine the antibiotic resistance patterns of pathogenic Escherichia coli on goat meat carcass at Huruma and Kiserian abattoirs in Kenya.**
***Design*: Laboratory based study.**
***Setting*: Huruma and Kiserian abattoirs in Kenya,**
***Subjects*: 400 slaughtered goats inspected by veterinary health officers and approved for human consumption.**
***Methods*: A Total of 400 slaughtered goats which were inspected by veterinary health officers and approved for human consumption were sampled from Huruma and Kiserian abattoir. Goat carcass swabs were collected by passing each swab tissue on four parts of the carcass mainly neck , right and left forelimbs, right and left hind limbs, and brisket.**
***Results*: A total of 54 *E. coli* isolates were isolated and confirmed to be pathogenic. The percentage of isolates resistant to various microbial agents was recorded as follows: ampicillin (26%), amoxycillin-clavulanic acid (17%), tetracycline (15%), chroramphenicol (4%), and ceftrixone (2% each). All Escherichia coli isolates were susceptible to gentamicin sulphamethaxazole-trimethomprin, kanamycin, cetriazididine (CAZ, 30µg), ciproxacin, nalidixic acid and chloramphenicol. Isolates were resistant to one or more of the antibiotics tested. Among the drugs tested, resistance was more frequently observed against ampicillin, amoxycillin-clavulanic acid, tetracycline, ceftrixone and chroramphenicol antibiotics.**
Among the isolates 26(48%) were positive for the stx1 gene, 19(35%) had the eae gene, 10(19%) possessed est gene, while 8(15%) harboured elt gene. Overall five isolates (10%) possessed aspu gene and two (4%) had aggR gene. No isolate possessed ipah gene.
***Conclusion*: This study demonstrated that there is a significant level of antimicrobial resistance in pathogenic *E. coli* isolated from goat meat from Huruma and Kiserian abattoir. This indicates that goat meat from abattoirs could pose a risk of transmission of pathogenic antibiotic resistant strains to human. Poor hygienic standards and indiscriminate use of antimicrobials are the two main reasons for the presence of resistant pathogens in goat carcasses.**
Recommendations: Implemention of appropriate hygiene measures to control contamination of meat with pathogenic E. coli.
***Key words*: *Escherichia. coli* pathotypes, Antibiotic resistance, Goat meat, Abattoirs,**

INTRODUCTION

Food borne diseases often follow the consumption of contaminated food-stuffs especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as Salmonella spp and pathogenic *Escherichia coli*.

The majority of these germs result from contamination occurring at the slaughterhouse (1, 2), where conventional veterinary inspection cannot detect the presence of these bacteria on apparently healthy carcasses (3).

During the slaughtering process, the Superficial bacterial contamination of goat carcasses at goat abattoirs main sources of contamination are the slaughtered animals themselves, the staff and the work environment (4). Infection caused by resistant strains usually lead to a high fatality rate especially among immuno-comprised individuals (5). The identification of pathogenic organisms is highly crucial for surveillance, prevention, and control of food-borne diseases. In addition, studying antimicrobial resistance in humans and animals is important in order to detect changing patterns in resistance, implement control measures on the use of antimicrobial agents, and prevent the spread of multidrug-resistant strains of bacteria (6).

The contamination of equipment, material, and workers hands can spread pathogenic bacteria to non-contaminated carcasses. Microbial population that comes in contact with fresh meat during slaughtering, dressing and processing presents a challenging problem to the meat industry.

Escherichia coli is a common, usually harmless, bacteria of the human intestinal flora. However, five groups of E. coli-causing diarrhoea in humans and other warm-blooded animals have been identified (7). These include enterotoxinogenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC). The later includes Shiga Toxin (Stx)-Producing *E. coli* (STX-EC). Shiga toxin (Stx)-producing *E. coli* (STX-EC), also known as Verotoxin-producing *E. coli*, is associated with infant diarrhea, haemorrhagic colitis, thrombiotic thrombocytic purpura, and hemolytic uremic syndrome in humans (8).

Different molecular markers are now being used for the detection of *E. coli* strains by use of the Polymerase chain reaction (PCR) (9). The following genes are used as markers for PCR amplification for detection of various *E. coli* strains: *stx* for Shiga toxin (10), *E. coli eae* for enteropathogenic *E. coli* (11), *est* (12) and *elt* for enterotoxinogenic *E. coli* (13), Aspu (14) and AggR for enteroaggregative *E. coli* (15) and ipah for Enteroinvasive *E. coli* (16), using different sets of oligonucleotide primers.

Considering the marked importance of *E. coli* organisms as food borne pathogens, this study aimed at characterising the different *E. coli* strains that are present in goat meat from abattoirs in Huruma and Kiserian and to evaluate their susceptibility to twelve commonly used antimicrobial agents.

MATERIALS AND METHODS

Sample collection: Swabs from the goat carcass were collected using dry sterile transport swabs 30-45 minutes after evisceration of goat. The samples were collected as per the procedure recommended by International Commission on Microbiological Specification for Food (17).

Swabbing was done from surface area 100 cm2 from each of the five sites, viz. neck, right forelimbs and hind limbs and left forelimbs, hind limbs and brisket (18). After sampling, swabs were returned into the tubes containing peptone buffered water medium, packed in a cool box and transported to the laboratory for testing within 1 hour but never more than 24 hours after sampling.

Bacterial isolation and bacteriological analysis: Swabs were suspended in 2 ml normal saline in tubes. One ml was inoculated on 3 M Petrifilm E.coli/Coliform Count Plate and incubated in humid incubator at 37°C over night in a humid environment. After overnight incubation colonies were identified and counted as per manufacturer's instructions for both presumptive *E. coli* and coliforms. Identified presumptive *E. coli* on petrifilm (3 M) media was sub-cultured on MacConkey agar plates (Oxoid Ltd, Basingstoke, United Kingdom).

From each plate one isolate of the lactose fermenting colonies was picked from each plate and inoculated on Eosin Methylene Blue (EMB) (Oxoid Ltd,) agar medium for preliminary characterization. Colonies showing characteristic metallic sheen on EMB agar were considered as presumptive E. coli and picked for further characterisation.

The isolates were gram stained and confirmed by biochemical tests including, ndole, Methyl red, Voges Proskauer, Citrate utilisation, (IMViC), gas and acid production on TSI agar as per Edwards and Ewing (19). The purified cultures confirmed as E. coli were stored in Tryptone soy broth (Oxoid Ltd,) for further identification.

DNA extraction: Total bacterial DNA was extracted as described by Ausubel et al. (20). A loopful of the colonies was suspended in 0.5 ml of sterile water and heated at 95°C for 10 min. Centrifugation was then carried out at 5,000 rpm for 5 min at 4°C. The DNA-containing supernatant was used as the source of template for further amplification.

PCR Assay: The supernatant containing a crude DNA extract was used as a DNA template on a multiplex PCR for identification of E. coli pathotypes, namely, EPEC, STEC, EAEC, and ETEC, EIEC. The detection of *stx1*, *stx2*, *est*, *elt*, *Aspu*, *Ipah* and *eae* genes was performed using multiplex PCR following the protocol of Toma C. *et al*., (14) using primer sequences listed in table 1

A multiplex PCR amplification was performed in a total volume of 25 μl containing 2 μl of DNA template, 2 μl primer mixer (each primer had a concentration of 5-10 pmol) and 21 μl of primer water that reconstituted PCR bead to 200 μM of dNTP(BSA,dATP,dCTP,dGTP,dTTP), 2.5 unit of puReTaq DNA polymerase, 10mM Tris-HCL(PH 9.0 at RT),50 mM Kl and 1.5 mM $MgCl_2$. Tubes containing the mixture were vortexed to mix and spinned slightly at low speed to concentrate reaction mixture at the bottom of the tube.

PCR amplification was performed using a DNA Engine DYADTM Peltier Thermal cycler (MJ Research). Primary denaturation was conducted for 3 min at 96ºC followed by 25 cycles of denaturing for 1 min at 96oC, annealing for 1 min at 54ºC and extension for 1 min at 72ºC. Final extension was carried out for 3 min at 72ºC and the reaction brought to a hold at 4ºC.

Reference positive *E. coli* control strains used in this study were characterized in previous studies and confirmed to have the relevant gene by single PCR. Negative control was primer water only. Negative and positive controls were included with every PCR assays. After the PCR, 10 μl aliquots were analyzed by agarose gel electrophoresis using 2% agarose gel containing 0.05 μl ethidium bromide per ml. DNA was visualised on a UV transilluminator, and results recorded by photography.

Table 1
Primers used in identifying E. coli strains

Target gene	Designation	Sequence (5′ – 3′)	Amplicon size (bp)	Reference
stx	VTcom-u	F: GAGCGAAATAATTTATATGTG	518	10
	VTcom-d	R: GATGATGGCAATTCAGTAT		
eae	SK 1	F: CCCGAATTCGGCACAAGCATAAGC	881	11
	SK2	R: CCCGGATCCGTCTCGCCAGTATTCG		
est	AL65	F:TTAATAGCACCCGGTACAAGCAGG	147	12
	AL125	R:CCTGACTCTTCAAAAGAGAAAATTAC		
elt	LTL	F:CCATACTGATTGCCGCAAT	322	13
	LTR	R:TCTCTATGTGCATACGGAGC		
aspU	aspU-3	F:GCCTTTGCGGGTGGTAGCGG	282	14
	aspU-2	R:AACCCATTCGGTTAGAGCAC		
aggR	aggRks1	F:GTATACACAAAAGAAGGAAGC	254	15
	aggRkas2	R:ACAGAATCGTCAGCATCAGC		
ipaH	IpaIII	F:GTTCCTTGACCGCCTTTCCGATACCGTC	619	16
	ipaIV	R:GCCGGTCAGCCACCCTCTGAGAGTAC		

Antimicrobial susceptibility testing: Isolates were tested for their susceptibility to twelve antimicrobial agents using the disk diffusion method as set by Clinical and Laboratory Standards Institute (CLSI) (21). Briefly, organisms were grown in a shaking water bath at 370 C until a 0.5 McFarland turbidity standard was obtained. A volume of 0.1 ml of the culture was then spread over Mueller-Hinton agar (Oxoid Ltd, Basingstoke, United Kingdom) plates. Antimicrobial disks impregnated with either of the following

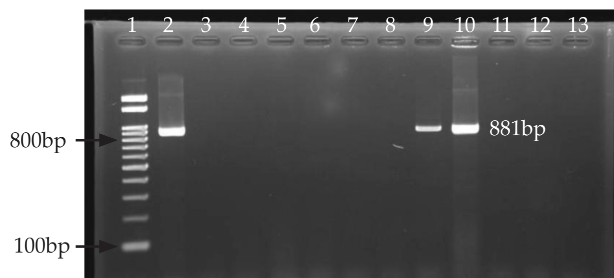
antimicrobials were placed on the innoculated medium; Ampicillin (A, 10 μg), Cetriazididine (CAZ, 30 μg), Sulphamethaxazole-Trimethomprin (SXT, 25μg), Amoxycillin-Clavulanic acid (AMC, 30 μg), Ciproxacin (CIP, 5 μg), Chroramphenicol (CAF, 30 μg), Streptomycin (S, 25 μg), Kanamycin (K, 30 μg), Nalidixic acid (Na, 30 μg), Tetracycline (TE, 30 μg), Ceftrixone (CRO, 30 μg) and Gentamycin (CN, 30 μg) (all from Oxoid Ltd). Plates were inocubated for 18-24 hrs at 37ºC. After incubation, Zones of inhibition around each antimicrobial disk were measured after the incubation period. Using NCCLS guidelines, each isolate was classified either as resistant or susceptible to the antimicrobial agents. Intermediate-resistant and resistant strains were grouped together.

RESULTS

Total coliform and E.coli counts: The mean value of the TCC of goat meat from slaughterhouses was 4.05 $\log_{10}$ cfu/cm^2 mean coliform count at Huruma and 3.4 $\log_{10}$ cfu/cm^2 mean coliform count at Kiserian. A total of 286(72%) out of the 400 samples were positive for coliforms and *E. coli* were isolated from 210 (52.5%) of the samples. The total mean coliform and *E. coli* counts were 3.19 and 2.68 $\log_{10}$ cfu/cm^2 respectively.

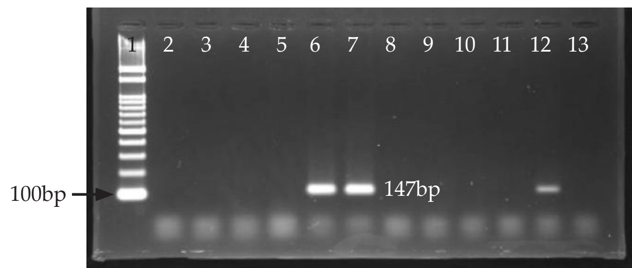
Molecular characterisation of E. coli: Of all *E. coli* isolated from goat carcasses, 54(25%) possessed pathogenic genes Of these, 26(48 %) were positive for *stx1* gene, 19(35%) had *eae* gene, 3(6%) possessed *est* gene, 2(4%) harboured elt gene, while four isolates (7%) possessed both *aspu* and *aggR*. None of the isolates haboured *ipah* gene.

Figure 1
Agarose gel showing amplified product (881bp) for eae (EPEC)



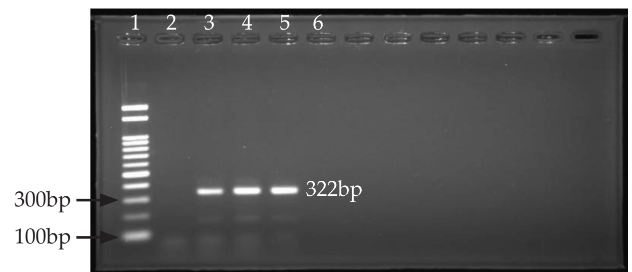
Lane 1: 100 bp DNA marker, Lane 9: Positive control, Lane 2 &10: Positive samples
Lane 8: Negative control Lane 3-7: Negative samples.

Figure 2
Agarose gel showing amplified product (147bp) for est (ETEC)



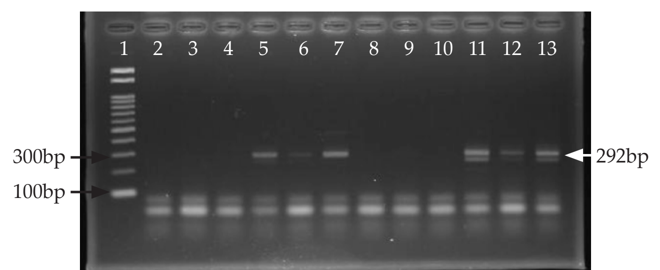
Lane 1: 100 bp DNA marker, Lane 13: Negative control, Lane 2-5: Negative samples,
Lane 12: Positive control; Lane 6 & 7: Positive samples

Figure 3
Agarose gel showing amplified product elt (322) ETEC



Lane 1: 100 bp DNA marker, Lane 2: Negative Control, Lane 6: Negative sample,
Lane 3, Positive control Lane 4 & 5: Positive samples

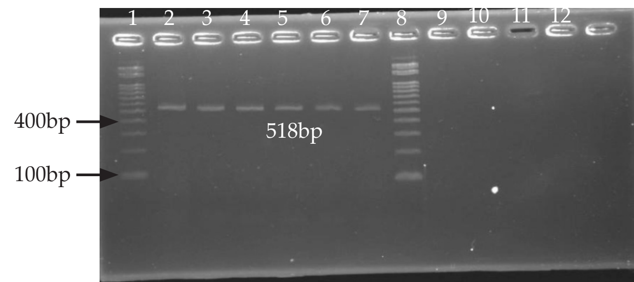
Figure 4
Agarose gel showing amplified product (292 and 254bp) for aspU /aggR (EAEC)



Lane 1: 100 bp DNA marker, Lane 10: Negative control Lanes 2, 3, 4, 8 &9: Negative samples; Lanes 5, 6,& 7: Positive sample Lane 12 &13, Positive control samples; Lane 11: Both aspu and AggR gene are co-harboured in one strain.

Figure 5

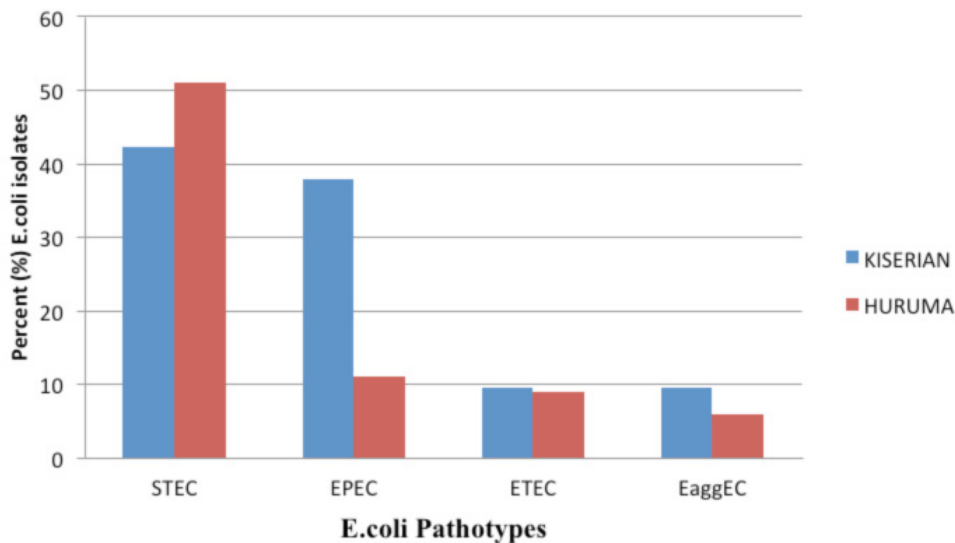
Agarose gel showing amplified product stx1 (518) STEC



Lane 1& 8: 100 bp DNA marker, Lane 2: Positive control
Lane 3 -7: Positive samples: Lane 9: Negative control: Lane 10: Negative sample

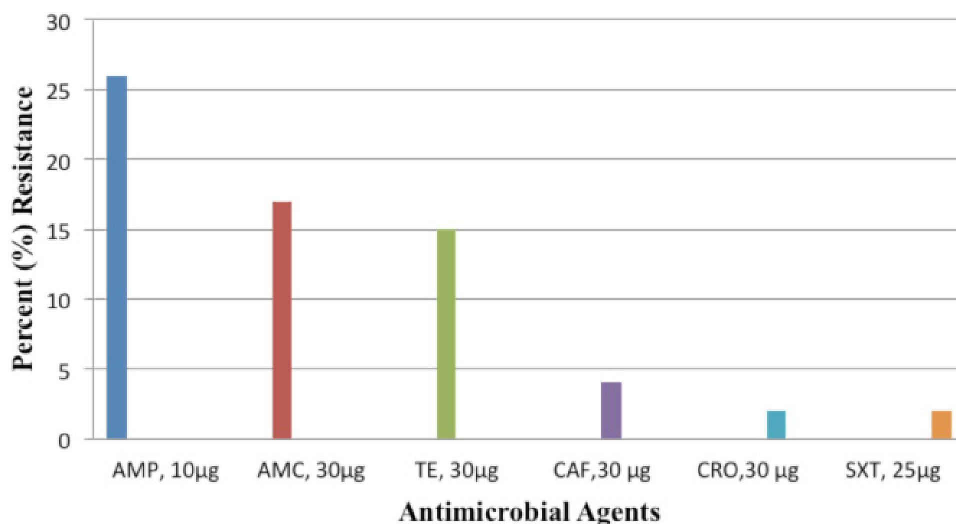
Figure 6

Distribution of Pathogenic E. coli pathotypes at Kiserian and Huruma abattoirs.



Antimicrobial resistance of the E. coli isolates: All 54 E. coli, isolates confirmed positive by PCR were tested for their susceptibility to various antimicrobial agents using the agar diffusion method. In vitro antibiotic resistance pattern against 12 antibiotics were detected. The percentage of isolates resistant to various antibacterial agents was recorded as follows: ampicillin (27%), amoxycillin-clavulanic acid (17%), tetracycline (15%), chroramphenicol (4%), Sulphamethaxazole-Trimethomprin and ceftrixone 2% each. All isolates were sensitive to gentamicin kanamycin, cetriazididine, ciproxacin, nalidixic acid and streptomycin which showed maximum sensitivity.

Figure 7
Percentage of E. coli isolates resistant to various antimicrobial agents.



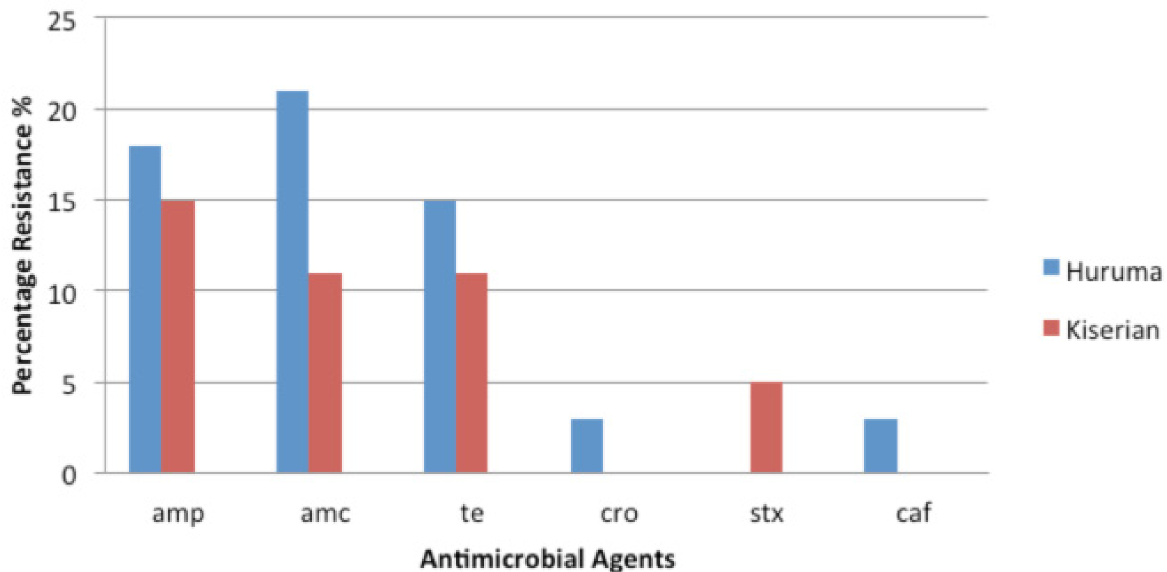
AMP – Ampicillin, SXT- Sulphamethaxazole-Trimethomprin, AMC- Amoxycillin-Clavulanic acid, CAF-Chroramphenicol, TE- Tetracycline, CRO- Ceftrixone

Table 2
Distribution of the antimicrobial resistance phenotypes among the pathogenic Escherichia coli strains isolates

Bacteria		Resistance	Phenotype	observed		Number
EPEC						
	amp					2
	amp	amc				2
	amp	amc	caf	te	stx	2
STEC						
	amp					2
	amc					2
	amp	amc				2
	cro					1
	amp	amp	cro			2
	amp	cro	te			2
EAEC						
	amp					1
	amp	amc				1
ETEC						
	amp					1
	amc					1
	amc	amp				1

Multiple drug resistance was demonstrated by most of the isolates. All but one strain were resistant to one drug. EPEC isolates had the high multidrug resistance with two isolates showing resistance to five drugs while EAEC and ETEC exhibited resistance to only two drugs.

Figure 8
Antimicrobial resistance of E. coli isolates within abattoirs



Higher percentage of resistance was observed for amp, amc te in Huruma compared to Kiserian. While, resistance to cro and caf was only observed in isolates from Huruma and stx is isolates from Kiserian only.

DISCUSSION

This study is the first to be undertaken in Kenya to investigate the occurrence of virulence genes and antimicrobial resistance specific for *E. coli* from slaughtered goats.

The results of this study showed that the mean value of the TCC of goat meat from slaughterhouses was 4.05 $\log_{10}$ cfu/cm^2 mean coliform count at Huruma and 3.4 $\log_{10}$ cfu/cm2 mean coliform count at Kiserian. Gill and Jones (22) showed that the meat contacting surfaces of cleaned equipment demonstrated less recovery of coliforms. Total mean coliform and *E. coli* count was 3.19 and 2.68 $\log_{10}$ cfu/cm^2 respectively. These findings have proximal relationship with the findings of Rahman et al. (23) who found the mean coliform count to be 1.18 $\log_{10}$ cfu /ml in lamb carcasses processed in United States. McEvoy et al (24) found mean Enterobacteriaceae counts of log 2.75 ± 0.64 in beef carcasses.

Bacteriological analysis performed showed a differential *E. coli* contamination levels between Huruma and Kiserian abattoirs. Higher levels of bacterial contamination was observed in Huruma than in Kiserian abattoirs. The fact that Huruma had a higher level of contamination than Kiserian can be attributed to many reasons. Huruma abattoir is located Huruma slum area were water is not sufficiently available and there is poor drainage system. At Kiserian water is plenty and abattoir worker wash the slaughter surface and their equipment too. Limited water supply at Huruma abattoir contributed to poor sanitation. Incidence of coliform count in examined samples was lower in Kiserian abattoir 3.4 $\log_{10}$ cfu/cm^2 mean coliform count. The variation in the results obtained from both abattoirs may be due to difference in handling practices. The differences in superficial bacterial loads depending on the anatomic sampling sites have been reported (25).

Among the *E. coli* isolates 26 (48.1%) possessed stx1 gene that is associated with shiga-toxin producing *E. coli*. Similar results h have been reported by Khan *et al*., (26), Salvadori *et al*., (27), Irino *et al.* (28) and Zweifel et al., (29). However, lower isolation rates for Stx1 positive *E. coli* isolates have also been reported by Chattopadhyay et al., (30). STEC have been found in the faecal flora of a variety of animals including cattle, sheep, goats, pigs, cats and dogs (31, 32). The most important animal species in terms of human infection is cattle and goats, but the prevalence of STEC in cattle varies significantly from country to country (33). The main route of STEC into the food chain is through contamination of meat by intestinal contents and faeces in the abattoir (34).

EPEC is an important category of diarrhoeagenic E. coli, which is a cause of infant diarrhoea in both industrialised and developing countries.

The *eae* gene is responsible for the pathogenicity of EPEC strains. The eae is only one of many genes located on the 35 kb pathogenicity island called the locus of enterocyte effacement (LEE), which confers the A/E phenotype for EPEC. In this study, eae gene was found in 19(35%) of E. coli isolates. Although several outbreaks of diarrhoea due to EPEC have been reported in healthy adults in industrialised countries (35), very little is known about the current status of EPEC as a diarrhoeagenic agent in developing countries.

Even though some serotypes of STEC and EPEC have been associated with diarrhoea in young animals, diarrhoea caused by STEC has only been demonstrated in experimentally infected newborn ruminants. Young animals between 2 and 4 months, and sometimes up to two years of age, tend to shed more STEC and EPEC of all serotypes. In humans, infection is associated with younger age groups and older individuals (36). The findings of this study are in partial agreement with the finding of Mathew et al. (36). However, comparison is difficult because of difference in modes of evaluation, hygienic standards followed in abattoirs, and geoclimatic conditions. Higher frequency of isolation of STEC in the present study from dressed goat meat might be due to contamination from the intestinal contents of a slaughtered animal since the intestinal tract of sheep and cattle has been shown to be a major reservoir of STEC (37).

ETEC strains were among the first pathogenic micro-organism for which molecular diagnostic techniques were developed. Moseley *et al*. (38) found DNA probes useful in the detection of LT and ST encoding genes in stool and environmental samples. ETEC produce heat labile (LT) and heat stable (ST) toxins and cause diarrhoea. ETEC is defined as *E. coli* strains that produce at least one of the two defined groups of enterotoxins: *est* and *elt* enterotoxins genes (39). Thus, detection of ETEC has long relied on detection of the enterotoxins. Three isolates (5.5%) possessed est gene while two isolates (3.7%) harboured elt gene. Presence of ETEC on goat meat is likely to be associated with contamination from persons handling meat during slaughter.

Food and water are the most common vehicles for ETEC infection. Thus, faecal contamination is the principal reason for the high incidence of ETEC infection throughout the developing world. ETEC infections in areas of endemic infections tend to be clustered in warm, wet months, when multiplication of ETEC in food and water is most efficient (40).
EAEC strains are *E. coli* strains that adhere to HEp-2 cells in an aggregative adhesion (AA) pattern (41).

The pathogenesis and the site of EAEC infection in the human intestine are not well understood. However, a characteristic histopathological lesion and several candidate virulence factors have been described. Only two isolates (3.7%) possessed both aspu gene and aggR gene that confer pathogenicity to Enteroaggregative *E. coli*. In the United Kingdom, four outbreaks of diarrhoea have been reported as being due to EAEC strains. Each of the outbreaks was associated with consumption of a restaurant meal, but no single source could be implicated (42).

Several authors like Sharma *et al*., (43), Mukhopadhyay *et al*., (44) identified different organisms like *Staphylococcus aureus*, *E.coli*, *Bacillus spp*. etc. from chevon and beef carcasses. Similarly isolation of virulent *E. coli* from the meat samples signifies the public health importance of this study.

Antimicrobial drug resistance of the E. coli isolates: The indiscriminate and uncontrolled use of antimicrobial drugs exerts a selection pressure and encourages the proliferation of drug resistant strains of E. coli in animal population. When this is coupled with poor environmental sanitation and low personal hygiene, the situation may constitute a danger to public health. The frequencies of bacterial strains resistant to antimicrobial agents have increased dramatically in the environment as a consequence of the wide spread use of drugs (45). A significant public health concern and the possibility of transfer of resistant genes between bacteria in the natural habitats have attracted attention. In the present study in vitro antibiotic resistance patterns of the *E. coli* isolates were determined by disc diffusion method of Bauer *et al*. (46). In this study 26% of isolates were resistant to ampicillin, however higher percentage have been reported by Indu Sharma and B. Bist (47). Such differences may well be related to the source, frequency and type of *E. coli* isolates encountered in different geographical areas. In this study, 15 per cent of the isolates were found to be resistant to tetracycline. Higher per cent of (50 per cent or more) isolates resistant to tetracycline was reported by Hariharan *et al*., (48). Although tetracyclines initially were useful for treatment of infections with aerobic Gram negative organisms, many enterobacteriaceae are now becoming relatively resistant to them. Resistance to tetracyclines in *E. coli* and related species is principally plasmid mediated and an inducible trait. Mechanisms of resistance include decreased accumulation of tetracycline due to either acquisition of an energy-dependant efflux pathway or to decreased influx, or to decreased access of tetracycline to the ribosome (site of action) due to acquisition of ribosome protected proteins and

enzymatic inactivation (49). Micro organisms that have been resistant to one tetracycline frequently exhibit resistance to the others. Tetracyclines were found initially to be highly effective against ETEC, but resistance has been emerging in the recent past and becoming a constraint in the treatment (50).

In the present study lesser number (4%) of E. coli isolates were found to be resistant to chloramphenicol. Previous studies on drug sensitivity of *E. coli* isolates observed a higher resistance to chloramphenicol at 67% (51)

In addition, the study showed that 5% of E. coli isolates were resistant to ceftrixone antibiotic. High sensitivity of ceftrixone against E. coli isolates might be attributed to its uncommon use in routine work.

Location wise prevalence of antimicrobial drug resistance of the E. coli isolates: E.coli from samples collected from both Kiserian and Huruma abattoirs showed resistance against ampicillin, amoxycillin-clavulanic acid, tetracycline and ceftrixone antibiotics (Figure 8). The highest resistance was observed against amoxycillin-clavulanic acid at 21% for Huruma and 11% for Kiserian. This was followed by ampicillin with resistance between 15-17 % in both abattoirs, and tetracycline at 11-15%. The lowest resistance was observed with ceftrixone (2%) at Huruma abattoir.

Predominant resistance pattern was observed for the following antibiotics viz. ampicillin, amoxicillin-clavulanic acid, tetracycline and ceftriaxone. In this study isolates revealed multiple drug resistance to various antibiotics ranging from two antibiotics up to five antibiotics. Multi drug resistance was observed in 35% E. coli isolates. EPEC isolates strains showed multidrug resistance to five drugs. STEC isolates showed multi resistance to four drugs. Both ETEC and EAEC isolates showed resistance to two drugs each (Figure 8). Ampicillin and Amoxycillin-Clavulanic acid were the most resisted by all four *E.coli* pathotypes.

Antimicrobial resistant bacteria from animals may colonize human population through the food chain; it is possible that resistant bacteria may be readily transferred to humans from animals used as food sources (52).

Due to use of antimicrobial agents for diseases prevention, farm animals are often exposed to antimicrobial substances, so resistance phenotype can give a selective advantage to bacte¬ria. As a result, humans became more possible to be exposed to these organisms via food and direct and indirect transmission from animals (53).

Tripathi and Soni (54) revealed various combination of multiple antimicrobial drug resistance among the E. coli isolates during their study showing no common en bloc multiple drug resistance pattern prevalence in their report. In this study are common en bloc of multiple drug resistance pattern, the data (Table 2) are in accord with multiple previous studies suggesting use of these drugs has been a key factor in the emergence of antimicrobial-resistant *E. coli* (55,56,)

In this study, the highest rate of resistance has been detected against the antimicrobial drugs most commonly used either as feed additives or as curative agents in farm animals or for treatment in human medicine, while the *E. coli* strains isolated were susceptible to less commonly used antimicrobial agents. This warrants restriction on the use of antibiotics as feed additives and rational use of antimicrobial therapy of infections in man and animals.

While the use of antibiotics has been proven to be an effective means for the prevention and control of bacterial infection, their indiscriminate use can have adverse consequences by promoting the selection of drug resistant microbial populations (57). The observed resistance may be attributed to the natural resistance of species to certain antibiotics (58), possible transfer of antibiotic resistance among species, and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity.

This study has therefore indicated that goat meat from Huruma and Kiserian abattoirs are potential reservoir for pathogenic *E. coli* organisms, which are multiply resistant to various antimicrobials, suggesting a potential public health hazard. Improper hygienic standards in the abattoirs and the indiscriminate use of antimicrobials are the main reasons behind the emergence of antimicrobial-resistant strains. These results emphasize the need to implement proactive measures, including the implementation of Hazard Analysis and Critical Control. Point (HACCP) in the preparation and processing of foods to reduce the risk of infection.

The following recommendations should prove useful to ensure the microbiological quality of goat meat from abattoirs.

1. Proper hygiene measures in meat preparation and thorough cooking of meat to destroy any pathogenic organism.
2. Organisation of training courses for abattoir workers to improve on the humane and hygienic slaughtering of animals to avoid unnecessary suffering, improve meat quality, reduce losses and increase profitability and financial returns to the farmers;
3. Establishment of standard operating procedures to improve the occupational health of abattoir workers, meat handlers and the consumers;

4. Improvement in the methods used currently for waste disposal to prevent pollution of the environment.
5. Application of stringent hygiene practices along the food chain and prudent use of antibiotics in animal husbandry which are essential for the control of further emergency of antibiotic resistance.

REFERENCES

1 Jouve, J.L. 1990. Microbiologie alimentaire et filière viande. *Viandes et Produits Carnés*. **11**, pp. 207- 213.

2. Rosset, R. 1996. Autres viandes et produits carnés. In: Bourgeois, C.M., Mescle, J.F., Zucca, J., (Eds.), Microbiologie Alimentaire, Tome1, Aspect microbiologique de la sécurité et de la qualité des aliments. Lavoisier Tec et Doc, pp. 331- 346.

3. Brown, M.H., Gill, C.O., Hollingsworth, J., Nickelson, I.R., Seward, S., Sheridan, J.J., Stevenson, T., Sumner, J.L., Theno, D.M., Usborne, W.R., Zink, D. 2000. The role of microbiological testing systems for assuring the safety of beef. *International Journal of Food Microbiology*. **62**, pp. 7- 16.

4. Bell, R.G., Hathaway, S.C. 1996. The hygienic efficiency of conventional and inverted lamb dressing systems. *J. of Applied Microbiology*. **81**, pp. 225- 234.

5. Holmberg, S.D, Wells, J.G and Cohn, M.L, (1984). Animal to-man transmission of antimicrobial-resistant Salmonella: investigation of US out breaks, 1971–1983. *Science*; 225: – 235.

6. Duffy, E.A., Belk, K.E., Sofos, J.N., le Valley, S.B., Kain, M.L., Tahem, J.D., Smith, Duijkeren E.V, Wannet W.J.B, Houwers DJ and Pelt WV.(2003). Antimicrobial susceptibility of Salmonella strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. *J Clin Microbiol;* **41**(8): 3574– 8.

7. Brook M.O, Smith R.R, Bannister B.A, McConnel M, Chart H and Scotland S.M (1994). Prospective study of verocytotoxin producing, enteroaggregative and diffusely adherent Escherichia coli in different diarrhoeal states. *Epidemiol Infect*. **112**: 63– 67.

8. Griffith, P.M. & Tauxe, .R.V. (1991). The epidemiology of infection caused by and the associated hemolytic uremic syndrome. *Epidemiologic Reviews* **13**: 60-98.

9. Jackson, M.P.; Newland, J.W.; Holmes, R.K. and O'Brien, A.D. (1987) Nucleotide sequence analysis of the structural genes for Shiga-like toxin I encoded by bacteriophage 933J from *Escherichia coli*. *Microbial Pathogenesis*. **2**: 147–153.

10. Paton, A. W., and Paton, J. C. (2002). Direct detection and characterization of Shiga toxigenic Escherichia coli by multiplex PCR for stx1, stx2, eae, ehxA, and saa. *J. Clin. Microbiol*. **40**: 271–274.

11. Oswald, E.; Schmidt, H.; Morabito,S.; Karch, H.; Marche`s, O.; and Caprioli, A. (2000). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic Escherichia coli: characterization of a new intiminvariant. *Infect. Immun*. **68**: 64–71.

12. Hornes, E.; Wasteson, Y. ; Olsvik, Ø. (1991). Detection of Escherichia coli heat-stable enterotoxin genes in pig stool specimens by an immobilized, colorimetric, nested polymerase chain reaction. *J. Clin. Microbiol.* **29**: 2375– 2379.

13. Tamanai-Shacoori, Z.; Jolivet-Gougeon, A. (1994). Detection of enterotoxigenic Escherichia coli in water by polymerase chain reaction amplification and hybridization. *Can. J. Microbiol*. **40**: 243–249.

14. Toma, C.; Lu, Y.; Higa, N.; Nakasone, N.(2003) Multiplex PCR Assay for Identification of Human Diarrheagenic *Escherichia coli*. *J. Clin. Micro*, 41: 2669–2671.

15. Ratchtrachenchai, O. A.; Subpasu, S.; and Ito, K. (1997). Investigation on enteroaggregative Escherichia coli infection by multiplex PCR. Bull. Dep. Med. Sci. **39**:211–220.

16. Sethabutr, O.; Venkatesan, M.; Murphy G. S.; Eampokalap,B.; Hoge, C. W.; Echeverria, P.(1993). Detection of Shigellae and Enteroinvasive Escherichia coli by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. *J. Infect. Dis*. **167**:458–461.

17. ICMSF, (2006). Microorganisms in Foods. Their Significance and Methods of Enumeration, 2nd Edn., University of Toronto, Press, Toronto and Buffalo, Canada.

18. Amin, A. and P. Borah (2002). Bacteriological quality of goat meat marketed in Guwahati city. *Indian Vet. J*. **79**: 944-947.

19. Edwards, R. and Ewing, W.N. (1972). Identification of *Enterobacteriaceae*. 3rd Edn, Burgess Publishing Co., Minnesota Pg. 475-479.

20. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A. and Struhl, K., (1987). Current Protocols in Molecular Biology. Pg 23-25 Greene Publishing Associates and Wiley-Interscience, John Wiley and Sons, NY, USA.

21. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*. CLSI document M100-S20; Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

22. Gill, C.O., Jones, T. (2000). Microbiological sampling of carcasses by excision or swabbing. *Journal of Food Protection*. **63**, pp. 167-173.

23. Rahman, H. (2002). Prevalence of verotoxin genes among non-enterohaemorrhagic *Escherichia coli*. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*. **23**: 189-190.

24. McEvoy, J.M., Doherty, A.M., Finnerty, M., Sheridan, J.J., Mc Guire, L., Blair, I.S., McDowell, D.A., Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*. **30**, pp. 390-395.

25. Murray, K.A., Gilmour, A. and Madden, R.H. (2001). Microbiological quality of chilled. *J Food Prot*. **64**(4):498-502.

26. Khan, A.; Yamasaki, S.; Sato, T.; Ramamurthy, T.; Pal, A.; Datta, S.; Chowdhury, N.R.; Das, S.C.; Sikdar, A.; Tsukamoto, T.; Bhattacharya, S.K.; Takeda, Y. and Nair, G.B. (2002). Prevalence and genetic profiling of virulence determinants of non-O157 Shiga toxin-producing Escherichia coli isolated from cattle, beef and humans, Calcutta, *India. Emerg. Infect. Dis*. **8**: 54-62.

27. Salvadori, M.R.; Valadares, G.F.; Leite, D.S.; Blanco,J. and Yano, T. (2003). Virulence factors of Escherichia

coli isolated from calves with diarrhea in Brazil. *Brazilian Journal of Microbiology*. **34**:230-235.

28. Irino, K.; Kato, M.A.M.F.; Vaz, T. M. I.; Ramo, I.I.; Souza, M.A.C., Cruz, A.S.; Gomes, T.A.T.; Vieira, M.A.M. and Gath, B.E.C. (2005). Serotypes and virulence markers of shiga toxin producing E. coli (STEC) isolated from dairy cattle in Sãu Paulo State, *Brazil. Vet. Microbiol.* **105**: 29-36.
29. Zweifel, C.; Baltzer, D. and Stephan, R. (2005). Microbiology contamination of cattle and pigs carcasses at five abattoir determined by swab sampling in accordance with E.U Decision 2001/471/E.C. *Meat Science*. **69**(3): 559-566.
30. Chattopadhyay, U.K.; Gupta, S. and Dutta, S. (2003). Search for Shiga toxin producing Escherichia coli (STEC) including O157:H7 strains in and around Kolkata. *Indian J. Med. Microbiol*. **21**: 17-20.
31. Beutin, L., Krause, G., Zimmermann, S., Kaulfuss, S. & Gleier, K. (2004). Characterization of Shiga toxin-producing Escherichia coli strains isolated from human patients in Germany over a 3-year period. *J. Clin Microbiol* **42**, 1099–1108.
32. Beutin, L., Tao, J., Feng, L., Krause, G., Zimmermann, S., Gleier, K., Xia, Q. & Wang, L. (2005). Sequence analysis of the Escherichia coli O15 antigen gene cluster and development of a PCR assay for rapid detection of intestinal and extraintestinal pathogenic E. coli O15 strains. *J. Clin Microbiol* **43**, 703–710.
33. Butler, D. (1996). Novel pathogens beat food safety checks. Nature 384:397.
34. Parma, A. E; Sanz, M. E; Blanco, J. E; Blanco, J; Vinas, M. R; Blanco, M., et al. (2000). Virulence genotypes and serotypes of verotoxigenic Escherichia coli isolated from cattle and foods in Argentina. *Eur. J. Epidem*. **16**:757-762.
35. Hedberg, C. W.; Savarino, S. J; Besser, J. M; Paulus, C. J; Thelen, V. M et al., (1997). An outbreak of foodborne illness caused by Escherichia coli O39:NM: an agent that does not fit into the existing scheme for classifying diarrheagenic E. coli. J. Infect. Dis. **176**:1625–1628.
36. Matthews L, McKendrick IJ, Ternent H, Gunn GJ, Synge B, Woolhouse MEJ: Super-shedding cattle and the transmission dynamics of Escherichia coli O157. *Epidemiol Infect* 2006, **134**:131-142.
37. Kudva, I. T., P. G. Haffield and C. J. Hodve (1997): Characterization of Escherichia coli O157: H7 and other shigatoxin-producing E. coli serotypes isolated from sheep. *J. Clin. Microbiol*. **35**, 892-899.
38. Moseley, S. L; Echeverria, P; Seriwatana, J; Tirapat, C; Chaicumpa, W; Sakuldaipeara, T. and S Falkow. (1982). Identification of enterotoxigenic Escherichia coli by colony hybridization using three enterotoxin gene probes. *J. Infect. Dis*. **146**:863-869
39. Levine, M.M. (1987). Escherichia coli that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohaemorrhagic and enteroadherent. *J. Infect. Dis*. **155**: 377-89.
40. Levine, M. M; Ferreccio, C; Prado, V; Cayazzo, M; Aprego, P; J Martinez, et al., (1993). Epidemiological studies of Esherichia coli diarrheal infections in a low socioeconomic lever periurban community in Santiago, Chile. *Am. J. Epidemiol*. **30**:849-869.
41. Baudry B, Savarino, S. J; Vial, P; Kaper, J. B; and M. M. Levine. (1990). A sensitive and specific DNA probe to identify enteroaggregative Escherichia coli, a recently discovered diarrhoeal pathogen. *J. Infect. Dis*. **161**:1249-1251.
42. Smith, H.W. and Hall, S. (1966). Observations on infective drug resistance in Britain. *Vet. Rec.* **78**:415-424.
43. Sharma, V.D.; Thapliyal, D.C.; Singh, S.P. and Malik, P. (1992). Cytotonic and cytotoxic enterotoxin of enterobacteria. *Indian J. Microbiol*. **32**:327-356.
44. Mukhopadhyay, H,K., Puvarajan, B. and Dorairajan, N. (1998). Detection of microbial load in fresh mutton and its impact to public health. *Indian J.Animal Hlth*. **37**: 81-83.
45. Kruse H, Sbrum H. Transfer of multi-drug resistance plasmids between bacteria of diverse origins in the natural environment. *Appl Environ Microbiol* 1994;**60** (11):4015–21.
46. Bauer, A.W.; Kirly, W.M.M.; Sherris, J.C. and Turck, M.(1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path*. **45**: 493-496.
47. Indu Sharma and B. Bist. (2010). Antibiotic Resistance in *Escherichia coli* Isolated from Raw Goat, Pig and Poultry Meat in Mathura city of Northern India, Assam University Journal of Science & Technology **6**: 89-92
48. Hariharan H., Coles, M., Poole D., Page, R. (2004). Antibiotic resistance among enterotoxigenic Escherichia coli from piglets and calves from piglets and calves with diarrhea, *Can. Vet. J*.; **45**:605–606.
49. Speer, B. S.; Shoemaker, N. V. and Salyers, A. A. (1992). Bacterial resistance to tetracycline: Mechanisms, transfer, and clinical significance. *Clin. Microbiol. Rev*., **51**: 387-399.
50. Kapusnik-uner, J. E.; Sande, M. A. and Chambers, H. F. (1996). Antimicrobials agents-Tetracyclines, Chloramphenicol, Erythromycin and miscellaneous antibacterial agents. In: Goodman and Gillman's, The Pharmacological Basis of Therapeutics. Hardman, J. G., Limbrid, L. E., Molinoff, P. B., Rudden, R. W. and Gillman, A. G. (eds.), 9th edn., pp. 1123-1153, McGraw Hill, New York.
51. Babila, A. and Akcadag, B. (1992). Drug sensitivity and biochemical and serological characteristics of E. coli strains isolated in the Harmaora region. *Pendik Hayvan Markez Arastirma Enstitusii Dergisi*. **23**(1):37-50.
52. Van den Bogaard, A.E; Stobberingh, E.E, (2000) Epidemiology of resistance to antibiotics. Links between animals and humans. *Int. J. of Antimicrobial Agents* **14**: 327–335.
53. Mainil, J. (1999). Shiga/verocytotoxin and Shiga/verotoxigenic Esch¬erichia coli in animals. *Vet Res;* **30**: 235-257.
54. Tripathi, R.D. and Soni, J.L. (1982). Antibiotic sensitivity test with E.coli isolated from cases of neonatal calf diarrhea. *Indian Vet. J*. **59**:413-416.
55. Meng J, Zhao S, Doyle MP, Joseph SW. Antibiotic resistance of Escherichia coli O157:H7 and O157:NM isolated from animals, food, and humans. *J. Food Prot* 1998; **61**:1511–4.

56. Zhao S, White D.G, Ge B, Ayers S, Friedman S, English L, et al. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin–producing Escherichia coli isolates. *Appl Environ Microbiol* 2001; **67**:1558–64.
57. Threlfall E.J; Ward L.R; Frost J.A, and Willshaw G.A. (2000). The emergence and spread of antibiotic resistance in food-borne bacteria. *Int J Food Microbiol.*; **62**:1–5.
58. Allison, D.G; Gilbert, P, An evaluation of the potentialof the multiple antibiotic resistance operon (mar) and the multidrug efflux pump acrAB to moderate resistance towards ciprofloxacin in Escherichia coli biofilms. *J Antimicrob Chemother* 2000; **45**: 789–95.