PREVALENCE OF ANTIBIOTIC RESISTANCE AND MOLECULAR CHARACTERIZATION OF *Escherichia coli* FROM FAECES OF APPARENTLY HEALTHY RAMS AND GOATS IN ILE-IFE, SOUTHWEST, NIGERIA

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ABSTRACT

The study reports isolation and identification of *Escherichia coli* from the faecal samples of goats and rams in Ile-Ife, Nigeria. It also determines the antibiotic susceptibility profile, presence of plasmid DNA, resistance (*bla_{CTX}, tetK, tetM*) and virulence (*Stx_1, eae*) genes in the isolates. Four hundred rectal swabs were collected from goats and rams at three different locations in Ile-Ife. The susceptibility of the isolates to antibiotics was carried out by disk diffusion technique. The amplification of virulence (*eae* and *stx1*) and resistance (*tetM*, *tetK* and *bla_{CTX}*(480 bp*)*genes in isolates was carried out by polymerase chain reaction. One hundred and sixty-six *E. coli* comprising rams (96) and goats (70) were recovered. Susceptibility of isolates to antibiotics varied greatly. The highest resistance was recorded against nitrofurantoin in *E. coli* from ram (97%) and goat (71%). The study revealed that *Escherichia coli* from rams(85.4%) and goats (87.1%) were multiple antibiotic resistant (MAR) types. The estimated molecular weight of plasmid DNA recovered from the MAR *E. coli*from rams and goats ranged from 9.41 to 30.84kb. Resistance genes (*tetK, tetM*) and virulence (*Stx_1, eae*) genes were not harboured by the representative *E. coli* profiled. The *bla_{CTX}*(480 bp) gene was detected in only three *E. coli* recovered from rams. The incidence of multiple antibiotic resistance in *E. coli* isolated from apparently healthy goats and rams is high in the study area, hence, indiscriminate use of antibiotics in the animal husbandry sector should be discouraged.

Keywords: *Escherichia coli,* ram, goat, antibiotic, resistant gene, virulence factors

INTRODUCTION

Escherichia coli is well recognized as a commensal inhabitant of the gastrointestinal tract (Nataro and Kaper, 1998), however, it is also associated with diarrhoea and a range of extra-intestinal diseases in both man and animals (DebRoy and Maddox, 2001). *Escherichia coli* are also the most frequently encountered microorganism in the food industry. Various disease outbreaks have been reported to be due to ingestion of food contaminated with pathogenic *E. coli* strains (Pradel *et al.,* 2000). Pathogenic strains of *E. coli* acquire virulence genes with which they cause diseases especially diarrhoea and extra-intestinal infections in humans and animals (Levine, 1987). In the debilitated or immunosuppressed host or when gastrointestinal barriers are violated, normal non-pathogenic strains of *E. coli* can cause infection.

Livestock are known to harbour not only strains pathogenic to animals but also strains which cause asymptomatic infections in animals and which can pass through the food chain to cause clinical disease in man (Arshad *et al.,* 2006; Smith *et al.,* 2009). *Escherichia coli* isolated from food animals have shown multiple drug resistance to β-lactams, including expanded-spectrum cephalosporins, aminoglycosides, sulphonamides, tetracyclines and fluoroquinolones (Bradford *et al.*, 1999). These combinations of multiple drug resistance coupled with virulence have posed an increasing threat to successful treatment of *E. coli* related veterinary diseases.

Sheep and goat meat can transmit infections and diseases either through handling during preparation procedures or as a result of ingestion by the consumer. The study seeks to provide useful information on the antibiotic resistance profile, nature of resistance and virulence genes in *E coli* from goats and rams in Ile-Ife, Southwest, Nigeria.

MATERIALS AND METHODS

Collection of Samples and Isolation of *Escherichia coli*

Rectal swabs collected from apparently healthy goats and rams with the aid of sterile swab stick were transported immediately to the laboratory for bacteriological analysis. The samples were cultured by streaking on eosin methylene blue agar (Oxoid, Ltd, UK) plates and incubated at 37°C for 24 h. Distinct colonies showing green metallic sheen were identified as *E. coli* and the identity confirmed by various biochemical tests (Chesbrough, 2000).

Antibiotic Susceptibility of Isolates

The antibiotic susceptibility testing of isolates was determined by the disc diffusion method on Müller-Hinton agar (HIMEDIA Lab. Ltd Vadhani)according to Clinical Laboratory Standard Institute(2012). The antibiotic disks (Abtek Biologicals, UK) and their concentrations (in µg) used include augmentin (30), nitrofurantoin (200), ceftriazone (30), cotrimoxazole (25), ofloxacin (5), amoxicillin (25), ciprofloxacin (10), tetracycline (30), perfloxacin (30), gentamicin (10), chloramphenicol (30), sparfloxacin (10) and streptomycin (30). The antibiotic disks were firmly placed on sterile Mueller-Hinton agar (MHA) plates previously seeded with a 24 h old culture of the isolate (10^6CFU/ml of 0.5 McFarland Standard). The plates were incubated at 37°C for 24 h and diameter of zone of inhibition was compared (Clinical and Laboratory Standards Institute, 2012). *Escherichia coli* ATCC 25922 was used as reference isolate. Multiple antibiotic resistant (MAR) isolates were defined as resistance to greater than or equal to two (≥ 2) classes of the antibiotics tested. Plates were incubated at 37°C and the diameter of zone of growth inhibition was measured to the nearest milliliter and interpreted (CLSI, 2012)

Plasmid DNA Extraction in *Escherichia coli*

Plasmid profile of representative *E. coli* that were resistant to more than five antibiotics was carried out using modified alkaline method of plasmid extraction described by Birnboim and Doly (1979). Plasmid DNA bands were separated by 0.8% agarose gel electrophoresis, visualized by ultraviolet light trans-illuminator and photographed with a Leicaflex SL-camera.

DNA Extraction in *E coli*

An overnight broth culture of *E coli* was prepared at 37°C for 24 h. This was vortexed at high speed to re-suspend the cells. One milliliter of the vortexed broth culture in an Eppendorf tube was centrifuged at 10,000rpm for 2 min. The supernatant was discarded and blotted dry on paper towel. One milliliter of sterile distilled water was then added into the tube, vortexed and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and 200µl of sterile distilled water was added and vortexed to homogenize the pellets before boiling at 100°C for 10 min after which the tube was vortexed again and centrifuged at 10,000 rpm for 5 min. The supernatant was transferred into another pre-labelled Eppendorf tube by gentle aspiration using micropipette and kept in the refrigerator at 4°C until needed for polymerase chain reaction (PCR) amplification.

Molecular Detection of Resistance (*blactx, tet K* and *tet M*) Genes in *E coli.*

Molecular detection of resistance *blactx* (480 bp) gene in thirty representatives of *E coli* from rams and goats that were resistant to beta lactam antibiotics (amoxicillin and augmentin) was carried out by polymerase chain reaction using both forward and reverse primers (Table 1).

Amplification of DNA was carried out in a volume of 25µl of a PCR mixture containing 1.5Mm MgCl 2, 1.0µl of dNTP, 0.2 µl of primer 1, 0.2 µl of primer 2, 1.5 µl of genomic DNA and 0.15 µl of Taq polymerase. The thermo cycler (Eppendorf Mastercycler, USA) was programmed for optimum conditions and was run as follows: an initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 1 min, annealing temperature of 61°C for 1 min, elongation at 72°C for 1 min and final extension at 72°C for 10 min.

Multiplex polymerase chain reaction was used for the detection of *tet K* (360 bp) and *tet M* (158 bp) genes in tetracycline resistant isolates using the oligonucleotide primers (Table 1).

The PCR amplification condition of the thermo cycler was set as follows: an initial denaturation at 95°C for 3 minutes, 30 cycles of denaturation at 95°C for 30 s, annealing temperature of 55°C for 30 s, elongation at 72°C for 30 s and final extension at 72°C for 10 min. The reaction mixture was run by 1.2% agarose gel electrophoresis alongside a 100 base pair marker and *Escherichia coli* ATCC 25922 used as negative control. The amplified products were viewed using an ultraviolet trans-illuminator.

Molecular Detection of Virulence (*eae* and *stx1*) Genes in *E coli* isolates

Multiplex polymerase chain reaction was used for the detection of virulence (*eae-482 bp* and *stx1-384 bp*) genes in the thirty representatives *E coli* using the appropriate primers (Table 1). Amplification reaction was carried out in a 25µl of a PCR reaction mixture containing 1.5mM $MgCl_2$, 2.5µl of PCR buffer 1.0mM of each dNTP, and 0.15µl of Taq polymerase 0.2pMol of each primer both reverse and forward, and 1.5µl of the DNA sample.

The PCR amplification condition of the thermocycler (Eppendorf Mastercycler, USA) programmed for optimum conditions was set as follows: an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 1.5min, annealing temperature of 60°C for 1.5 min, elongation at 72°C for 1.5 min and final extension at 72°C for 10 min. The reaction mixture was run by 1.2% agarose gel electrophoresis alongside a 100 base pair marker and *Escherichia coli* ATCC 25922 used as negative control. The amplified products were viewed using an Ultraviolet trans-illuminator.

Statistical Analysis

Significant differences in the incidence of resistance to antibiotics were compared using

Table 1. Primer sets used for the detection of resistance and virulence genes

Primer	Target gene	Amplicon (bp)	Reference
Forward-*Bla-ctx*5'ATGTGCAGYACCAGTAARGTKATGGC3' Reverse-*Bla-ctx*5'TGGGTRAARTARGTSACCAGAATCAGCGG 3'	*Blactx*	480	Mulvey *et al.* (2003)
Forward-*Tet-K*5'GTAGCGACAATAGGTAATAGT3' Reverse-*Tet-K*5'GTAGTGACAATAAACATCCTA3'	*Tet K*	360	Strommeger *et al.* (2003)
Forward-*Tet-M*5'AGTGGAGCGATTACAGAA3' Reverse-*Tet-M* 5'CATATGTCCTGGCGT TA3'	*Tet M*	158	Strommeger *et al.* (2003)
Forward-*Eae*-5'TCAATGCAGTTCCGTTATCAGTT3' Reverse-*Eae*-5'GTAAAGTCCGTTACCCCAACCTG3'	*Eae*	482	Vida *et al.*(2005)
Forward-*Stx-1*5'CAGTTAATGTGGTGGCGAAGG3' Reverse-*Stx 1* 5'CACCAGACAATGTAACCGCTG3'	*Stx 1*	384	Cebula *et al.* (1995)

Results

One hundred and sixty-six *E. coli* comprising 96 (57.8 %) from rams and 70 (42.2 %) from goats were recovered from 400 stool samples analyzed. The susceptibility of the isolates from rams and goats to various antibiotics is shown in Table 2. The highest resistance was recorded against nitrofurantoinin isolates from ram (97%) and goat (71%). The resistance to ceftriazone, amoxicillin, ofloxacin, streptomycin and ceftriazone was more pronounced in *E. coli* from rams than in goats. However, *E. coli* from goats were frequently resistant to ciprofloxacin, augmentin, gentamicin, pefloxacin and cotrimoxazole. Resistance to fluoroquinolone by the isolates was generally low. Isolates from goats were least resistant to ofloxacin. There was no significant statistical difference between the resistance of the *E. coli* strains from the two animals (P=0.9404, t = 0.07635).

Table 2: Antibiotic resistance profile of *Escherichia coli* from rams and goats

Antibiotics (μg)	**Number of Resistant Isolates n (%)**	
	Rams	**Goats**
Chloramphenicol (30)	13 (13.54)	11 (15.71)
Sparfloxacin(10)	19 (19.79)	11 (15.71)
Ciprofloxacin (10)	10 (10.42)	11 (15.71)
Amoxicillin (30)	67 (69.79)	9 (12.86)
Augmentin (30)	28 (29.17)	49 (70.0)
Gentamicin (10)	21 (21.88)	59 (84.28)
Pefloxacin(30)	6 (6.25)	19 (27.14)
Ofloxacin(10)	15 (15.63)	1 (1.43)
Streptomycin (30)	16 (16.67)	2 (2.86)
Ceftriazone (30)	95 (98.96)	11 (15.71)
Nitrofurantoin(200)	95 (97.0)	67 (95.71)
Cotrimoxazole (25)	62 (64.58)	66 (94.29)
Tetracycline (30)	87 (90.63)	64 (91.43)

Tables 3 and 4 show the multiple antibiotic resistance patterns of *E. coli* from rams and goats. Multiple antibiotic resistance was defined as resistance to at least two or more different classes of antibiotics. In the study, 82 (85.4%) of *E. coli* from rams and 61 (87.1%) from goats were resistant to multiple antibiotics which ranged from 2 to 6 different classes of antibiotics.

Table 3. Multiple antibiotic resistant pattern of *E. coli* isolates from rams

Number of antibiotic classes	MAR patterns	Number of Isolates (%)
2	STR, TET	1(1.2)
	OFX, TET	2(2.4)
	AMX, NIT	1(1.2)
	AUG, NIT	3(3.6)
3	GEN, PEF, TET	1(1.2)
	AMX, SPA, TET	1(1.2)
	AMX, OFX, TET	4(4.9)
	AMX, NIT, TET	1(1.2)
	AMX, COT, TET	1(1.2)
	AMX, NIT, TET	15(18.3)
	AUG, NIT, TET	3(3.65)
	AMX, GEN, NIT	1(1.2)
4	AMX,OFX, STR, TET	1(1.2)
	AMX, CH, OFX, TET	4(4.9)
	CPX, SPA, STR, TET	1(1.2)
	AMX, NIT, COT, TET	12(14.6)
	AMX, GEN, NIT, TET	1(1.2)
	AUG, NIT, COT, TET	4(4.9)
	AMX,CPX, NIT, COT	1(1.2)
5	AMX, CH, OFX, STR, TET	1(1.2)
	AMX, COT, GEN, NIT, TET	11(13.4)
	AMX, COT, CPX, NIT, TET	5(6.1)
	AMX, GEN, OFX, NIT, TET	1(1.2)
	AUG, COT, GEN, NIT, TET	2(2.4)
6	AMX, COT, GEN, PEF, NIT, TET	1(1.2)
	AMX,CPX, CRO, GEN, NIT, TET	3(3.6)

AUG-augmentin, NIT-nitrofurantoin, COT-cotrimoxazole,OFX-ofloxacin, AMX-amoxicillin, CPX-ciprofloxacin, TET-tetracycline, PEF-perfloxacin, GEN-gentamicin, CH-chloramphenicol, SPA-sparfloxacin, STR- streptomycin.

Table 4. Multiple Antibiotic Resistance Patterns of *E. coli* Isolates in Goat

Number of antibiotic classes	MAR patterns	Number of isolates (%)
2	OFX, TET	1(1.63)
	CPX, SXT	1(1.63)
	COT, SPA	1(1.63)
	AMX, NIT	3(4.91)
	AMX, TET	3(4.91)
3	AMX, NIT, TET	15(24.6)
	AUG, NIT, TET	6 (9.83)
	AMX, COT, TET	1 (1.63)
	AMX, COT, NIT	1 (1.63)
4	AMX,CPX, NIT, TET	1(1.63)
	AMX, COT, GEN, NIT	1 (1.63)
	AUG, GEN, NIT, TET	1(1.63)
	AMX, COT, NIT, TET	7(11.5)
	AMX, GEN, NIT, TET	3(4.91)
	CPX, NIT, SPA, TET	1 (1.63)
	AUG, COT, NIT, TET	1(1.63)
5	AMX,CPX, COT,NIT, TET	1(1.63)
	AMX, COT, GEN, NIT, TET	4(6.55)
	AMX, COT, GEN, NIT, TET	1(1.63)
	AMX, GEN, PEF, NIT, TET	1(1.63)
6	AMX,COT,CPX, GEN, NIT, TET	6(9.83)
	AMX, COT,GEN, OFX, NIT, TET	1(1.63)

AUG-augmentin, NIT-nitrofurantoin, COT-cotrimoxazole, OFX-ofloxacin, AMX-amoxicillin, CPX-ciprofloxacin, TET-tetracycline, PEF-perfloxacin, GEN-gentamicin, CH-chloramphenicol, SPA-sparfloxacin, STR- streptomycin

A single large molecular weight plasmid of 23.13 kb was harboured by *E. coli* from rams (11) and goats (8). However, multiple plasmids of large molecular weight were recovered from only one isolate of goat (Table 5). Plates 1a and b show agarose gel electrophoresis of plasmids in multiple antibiotic resistant *Escherichia coli*.

Table 5. Plasmid Profile of the *Escherichia coli* Isolates in Rams and Goats

	Escherichia coli **isolates**	**Number of Plasmid observed**	**Estimated molecular sizes of plasmid (kb) observed**
Ram	L1-R1	1	23.13
	L2-R2	1	23.13
	L3-R3	1	23.13
	L4-R4	-	-
	L5-R5	1	23.13
	L6-R6	-	-
	L7-R7	1	23.13
	L8-R8	-	-
	L9-R9	1	23.13
	L10-R10	1	23.13
	L11-R11	-	-
	L12-R12	1	23.13
	L13-R13	-	-
	L14-R14	-	-
	L15-R15	1	23.13
	L16-R16	-	-
	L17-R17	-	-
	L18-R18	-	-
	L19-R19	1	23.13
	L20-R20	1	23.13
Goat	L21-G1	1	23.13
	L22-G2	1	23.13
	L23-G3	1	23.13
	L24-G4	1	23.13
	L25-G5	1	23.13
	L26-G6	1	23.13
	L27-G7	1	23.13
	L28-G8	3	30.84, 23.13, 9.41
	L29-G9	1	23.13
	L30-G10	-	-
	L31-*E.coli* ATCC 25922	-	-

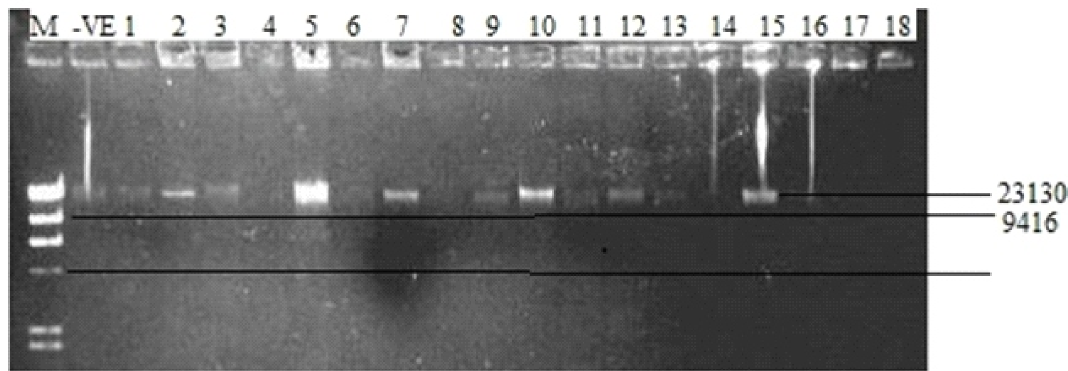

Plate1a.Agarose gel electrophoresis of plasmids in multiple antibiotic resistant *Escherichia coli* isolated from faeces of apparently healthy rams and goats.

Lane M=DNA marker (HIND III digest) Lane 1=R1, Lane 2=R2, Lane 3=R3, Lane 4 =R4, Lane 5=R5, Lane 6=R6, Lane 7 =R7 Lane 8=R8, Lane 9=R9, Lane 10=R10, Lane 11= R11, Lane 12 =R12, Lane 13=R13 Lane 14=R14, Lane 15=R15, Lane 16=R16, Lane 17=R17, Lane 18=R18,

M 19 20 21 22 23 24 25 26 27 28 29 30 31

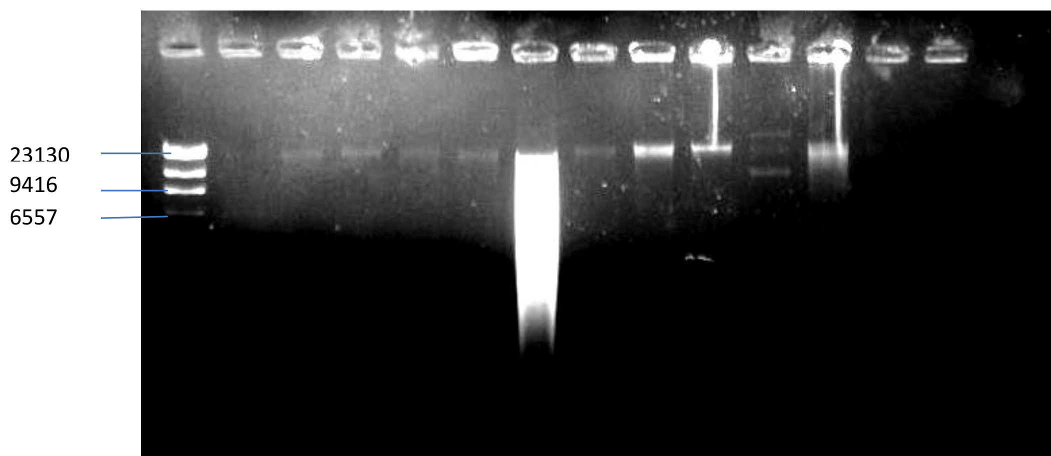

Plate 1b. Agarose gel electrophoresis of plasmids in multiple antibiotic resistant *Escherichia coli* from faeces of apparently healthy rams and goats

Lane M=DNA marker (HIND III digest), Lane19 = R19 Lane 20 = R20, Lane 1=G1, Lane 2 = G2, Lane 3 =G3, Lane 4 = G4, Lane 5=G5 Lane 6= G6, Lane 7=G7, Lane 8= G8, Lane 9=G9, Lane 10=G10, Lane 31=*E. coli* ATCCC25922, R-isolate from ram, G-isolate from goat

Plates 2-5 show the results of PCR gel electrophoreses of resistance and virulence genes in representative *E. coli* from rams and goats. The resistance (*bla_{CTX}* (480 bp)) gene was found in only three *E. coli* isolates from rams (Plate 2). However,all the 30 representative isolates profiled did not contain *tet M (158 bp)* (Plate 3), *tet K (360 bp)* (Plate 4), and *eae (482 bp)* and *stx 1 (384 bp)* (Plate 5).

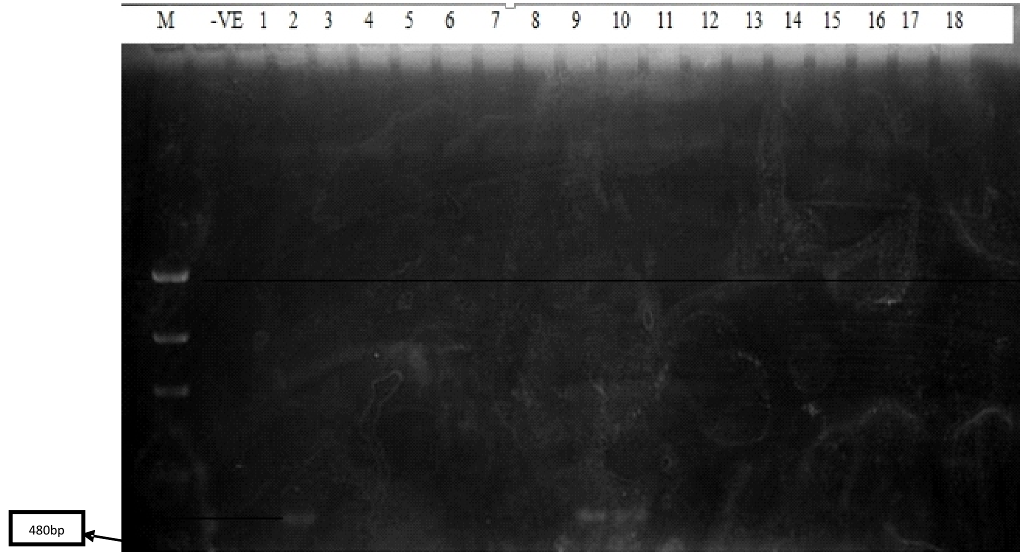

Plate 2. PCR amplification of *BlaCTX* gene in *Escherichia coli* isolated from feaces of apparently healthy rams
Lane M=100bp marker, Lane =-ve, Lane 1=R1, Lane 2=R2, Lane 3=R3, Lane 4 =R4,Lane 5=R5, Lane 6=R6, Lane 7 =R7 Lane 8=R8, Lane 9=R9, Lane 10=R10, Lane 11= R11, Lane 12 =R12, Lane 13=R13 Lane 14=R14, Lane 15=R15, Lane 16=R16, Lane 17=R17, Lane 18=R18,R-isolate from ram

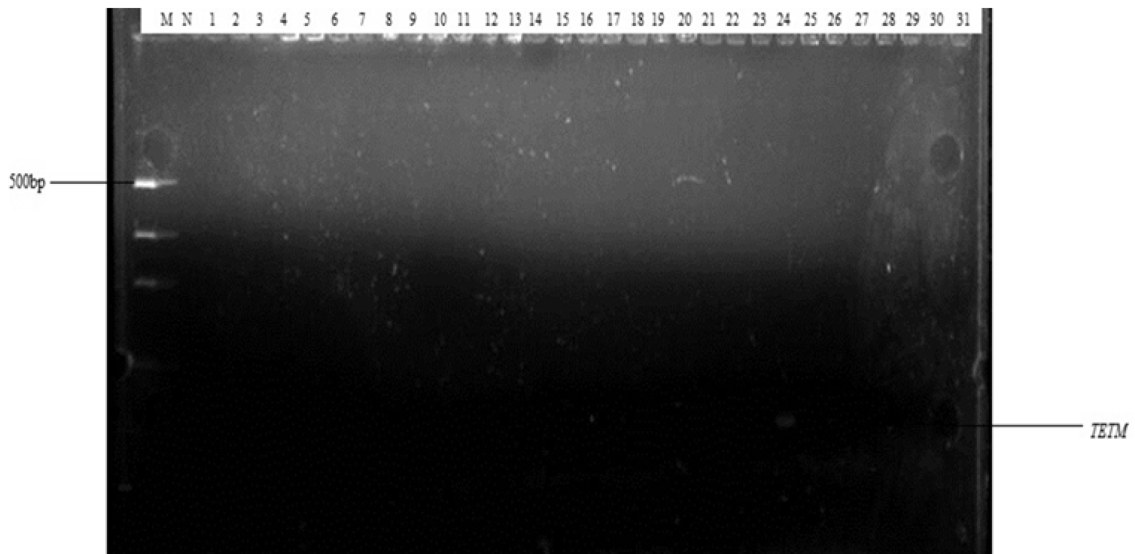

Plate 3. PCR amplification of *Tet M* (158 bp) gene in *Escherichia coli* isolated from faeces of apparently healthy rams and goats
Lane M=100bp marker, Lane N =-ve Lane 1=R1, Lane 2=R2, Lane 3=R3, Lane 4 =R4, Lane 5=R5, Lane 6=R6, Lane 7 =R7 Lane 8=R8, Lane 9=R9, Lane 10=R10, Lane 11= R11, Lane 12 =R12, Lane 13=R13 Lane 14=R14, Lane 15=R15, Lane 16=R16, Lane 17=R17, Lane 18=R18, Lane19= R19 Lane 20= R20, Lane 21=G1, Lane 22= G2, Lane 23 =G3, Lane 24= G4, Lane 25=G Lane 26= G6, Lane 27=G7, Lane 28= G8, Lane2 9=G9, Lane 30=G10, Lane 31=*E. coli* ATCCC25922, R-isolate from ram, G-isolate from goat

M-ve12 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

Plate 4. PCR amplification of *tet K* (360 bp) gene in *Escherichia coli* isolated from faeces of apparently healthy rams and goats

Lane M=100bp marker, Lane =ve, Lane 1=R1, Lane 2=R2, Lane 3=R3, Lane 4 =R4, Lane 5=R5, Lane 6=R6, Lane 7 =R7 Lane 8=R8, Lane 9=R9, Lane 10=R10, Lane 11= R11, Lane 12 =R12, Lane 13=R13 Lane 14=R14, Lane 15=R15, Lane 16=R16, Lane 17R17, Lane 18=R18, Lane19= R19 Lane 20= R20, Lane 21=G1, Lane22= G2, Lane 23 =G3, Lane24= G4, Lane 25=G Lane 26= G6, Lane 27=G7, Lane 28= G8, Lane29=G9, Lane 30=G10, Lane 31=*E. coli* ATCCC25922, R-isolate from ram, G-isolate from goat

M -ve 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

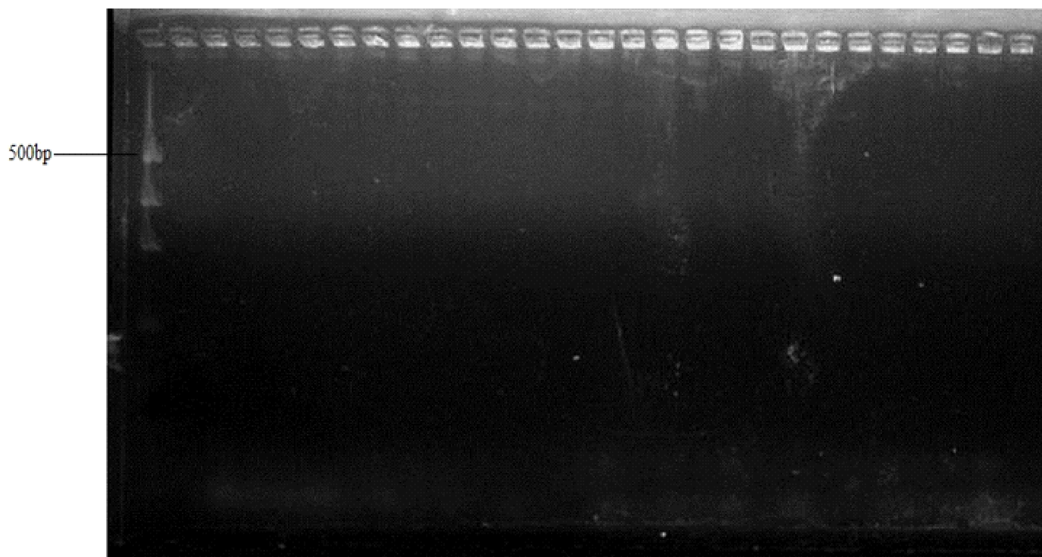

Plate 5. PCR amplification of *eae and stx1* genes in *Escherichia coli* isolated from faeces of apparently healthy rams and goats

Lane M=100bp marker, Lane = ve Lane 1=R1, Lane 2=R2, Lane 3=R3, Lane 4 =R4, Lane 5=R5, Lane 6=R6, Lane 7 =R7 Lane 8=R8, Lane 9=R9, Lane 10=R10, Lane 11= R11, Lane 12 =R12, Lane 13=R13 Lane 14=R14, Lane 15=R15, Lane 16=R16, Lane 17=R17, Lane 18=R18, Lane19= R19 Lane 20 = R20, Lane 21=G1, Lane 22= G2, Lane 23 =G3, Lane2 4= G4, Lane 25=G5, Lane 26= G6, Lane 27= G7, Lane 28=*E. coli* ATCCC25922, R-isolate from ram, G-isolate from goat

DISCUSSION

The recovery of *E. coli* from faecal samples of rams and goats in the study agrees with the reports of earlier researchers. *Escherichia coli* shedding has been demonstrated in small ruminants in a research carried out by Zschock *et al.* (2000).In this study, 69.79% of *E. coli* from goats were resistant to amoxicillin which is similar to the prevalence rate recorded by Allen *et al.* (1999) in children and Karlowsky *et al.* (2002) in urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States.

Resistance to tetracycline was equally high in this study. This finding agrees with the reports by Hariharan *et al.* (2004) which recorded high resistance to tetracycline by *E. coli* from different animals. Although tetracycline initially was useful for treatment of infections with aerobic Gram negative organisms, many *Enterobacteriaceae* are now becoming relatively resistant to them. Resistance to tetracycline in *E. coli* and related species is principally plasmid-mediated and an inducible trait as seen in the present study. The resistance to tetracycline observed may also be as a result of decreased accumulation of tetracycline due to either acquisition of an energy-dependent 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 (Speer *et al.*, 1992). It has been reported that commensal *E. coli* strains from humans and animals express high resistance to common antimicrobial agents (Kang *et al.*, 2005).

In the present study, moderately high percent of isolates were resistant to ciprofloxacin. However, Bradford *et al.* (1999) and Chattopadhyay *et al.* (2003) reported that 6.25% and 15.38% of *E. coli*, respectively were resistant to ciprofloxacin in their studies on animals. The recovery of multiple antibiotic resistant *E. coli* in the study may probably be plasmid-mediated. Generally, multiple resistance to antibiotics observed in the study may be due to abuse of antibiotics by man and frequent application in animal feeds as supplement and in veterinary treatment. Studies on antimicrobial resistance of *E. coli* from different animal species showed an increase in the incidence of resistance over the years as a result of the wide-spread use of antimicrobial drugs in animals (Cid *et al.,* 1996). The problem is further aggravated by the transfer of *E. coli* from livestock to poultry to human (Kapoor *et al.,* 1994). Hinton *et al.* (1986) reported that the use of drugs does not induce resistance, but rather provides an intense selection pressure which eliminates the susceptible normal flora in the host and spares the resistant ones. In the study, highest rate of resistance was 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, and were least resistant 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.

Large molecular weight plasmids were detected in some of the representative multiple antibiotic resistant isolates; this may account for the multi-resistance observed in the study.

None of the isolates showed the presence of virulent genes. Though, reports by Wani *et al.* (2003) from India reported a prevalence rate of (6%), Suheyla *et al.* (2013) reported more prevalence of *stx*2 gene than *stx*1 gene in *E. coli* from lambs and goat kids, and Bandyopadhyay *et al.* (2011) also reported a predominance of the *stx*2 gene in STEC strains isolated from small ruminant with diarrhea in India.

Similarly, the tetracycline resistance (*tet M, tet K*) determinant genes were not detected in the isolates in this study. However, studies by Andrew *et al.* (2004) which investigated the presence of 14 types of tetracycline resistance determinants genes in a non-selected and natural populations of *Escherichia coli* from 12 animal sources, reported the presence of *tet M* which represents the first report of *tet M* in *E. coli*. The non- detection of *tet K* in the present study also corroborates the absence of *tet K* determinant gene in their own reports.

It is noteworthy that the variance or dissimilarity in the finding of this study and earlier studies on animals may be explained by the differences in sample size and health status, the type of sample, and method of sample collection. A number of

reports have attempted to establish an association between resistance to antibacterial agents (ABA) and the presence of a virulence gene (Garcia 2002; Boerlin 2005; Diarrassouba *et al.*, 2007). It is possible that an antibacterial resistance and a virulence gene are located on the same transferable plasmid. However, the results of this research do not indicate any association between virulence genes of *Escherichia coli* and the antibiotic resistance.

Moreover the results of this research do not provide any significant proven link of association between antibiotic resistance in commensal *E coli* and antibiotic resistance genes with respect to isolates resistant to β–lactams and the tetracycline antibiotics as only very few of the isolates haboured the resistance (bla_{CTX}) genes.

Conclusion

Escherichia coli isolated in this study are multiple antibiotic-resistant and lack virulence (*stx 1*,*eae)* genes. The highest rate of resistance was detected against the antibiotics used as either additives or curative purposes, hence, irrational use of antibiotics in man and animals should be discouraged.

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