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Original article

Investigation of plasmid-mediated resistance in *E. coli* isolated from healthy and diarrheic sheep and goats

I.I. Shabana [a,*], A.T. Al-Enazi [b]

[a] Faculty of Veterinary Medicine, Department of Bacteriology, Immunology and Mycology, Suez Canal University, Egypt
[b] Biology Department, Faculty of Science, Taibah University, Al-madinah Al-munawarah, Saudi Arabia

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ABSTRACT

Escherichia coli is zoonotic bacteria and the emergence of antimicrobial-resistant strains becomes a critical issue in both human and animal health globally. This study was therefore aimed to investigate the plasmid-mediated resistance in *E. coli* strains isolated from healthy and diarrheic sheep and goats. A total of 234 fecal samples were obtained from 157 sheep (99 healthy and 58 diarrheic) and 77 goats (32 healthy and 45 diarrheic) for the isolation and identification of *E. coli*. Plasmid DNA was extracted using the alkaline lysis method. Phenotypic antibiotic susceptibility profiles were determined against the three classes of antimicrobials, which resistance is mediated by plasmids (Cephalosporins, Fluoroquinolone, and Aminoglycosides) using the disc-diffusion method. The frequency of plasmid-mediated resistance genes was investigated by PCR. A total of 159 *E. coli strains* harbored plasmids. The isolates antibiogram showed different patterns of resistance in both healthy and diarrheic animals. A total of (82; 51.5%) *E. coli* strains were multidrug-resistant. *rmtB* gene was detected in all Aminoglycoside-resistant *E. coli*, and the ESBL-producing *E. coli* possessed different *CTX-M* genes. Similarly, fluoroquinolone-resistant *E. coli* possessed different *qnr* genes. On the analysis of the *gyrB* gene sequence of fluoroquinolone-resistant *E. coli*, multiple point mutations were revealed. In conclusion, a high prevalence of *E. coli* with high resistance patterns to antimicrobials was revealed in the current study, in addition to a wide distribution of their resistance determinants. These findings highlight the importance of sheep and goats as reservoirs for the dissemination of MDR *E. coli* and resistance gene horizontal transfer.

1. Introduction

Escherichia coli is a member of the normal flora in the gastrointestinal tract of human and warm blood animals (Gerba, 2000; Katouli, 2010). *E. coli* is a Gram-negative rod, non-sporulating, a non-fastidious, motile, and facultative anaerobic bacterium which is 0.5 μm in diameter and 1.0–3.0 μm in length belonging to the family Enterobacteriaceae. *E. coli* easily grows in the laboratory on MacConkýs agar producing lactose-fermenter rose pink colonies (Roy et al., 2012). Eosin Methylene Blue medium is appropriate for the isolation of *E. coli* producing distinctive colonies with a characteristic green metallic sheen that differentiate it from any other Enterobacteria (Irfan, 2016). *E. coli* is oxidase negative, lactose, glucose, and sucrose fermenting. It grows at a temperature of 37 °C and a pH of 6.0–7.0, while some diarrheagenic *E. coli* strains can tolerate a pH of 2.0. The *uidA* encodes for β-D-glucuronidase that could be an important feature of the coliforms, therefore it is routinely used to specific identification of *E. coli* (Molina et al., 2015).

The association of *E. coli* in sheep and goats incorporates significance for human infection, as their meat can transmit infections and diseases either through handling throughout preparational procedures or as a result of ingestion by the consumer. The presence of *E. coli* in animal feces permits it to enter the food chain via fecal contamination of meat and milk with the intestinal contents during slaughtering. Animal-human contact, both direct and indirect, human-to-human contact has a key role in infection transmission (Yim et al., 2010).

Antibiotics used to cure a variety of infectious diseases affecting humans and animals; though, the continuous increase of public health worries (Van der Auwera et al., 2009). The extensive use

* Corresponding author.
E-mail address: ishabana@taibahu.edu.sa (I.I. Shabana).
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of antibiotics in medical practice and in animal food production had an adverse effect not only on the pathogens but also on the commensal bacteria and resulted in the emergence of antibiotic-resistant bacteria (Tivendale et al., 2009; Sackey et al., 2001). Microorganisms considered Multi-Drug Resistance (MDR) once they exhibit non-susceptibility to at least one agent in three or more antimicrobial categories (Santo et al., 2007). MDR strains can be transferred through food to humans (Whitworth et al., 2008; Daniels et al., 2009; Nsofor and Iroegbu, 2012). In addition, the animal's fecal materials act as a possible source of antibiotic-resistant strains. It causes the contamination of food and water sources with resistant strains and greatly affects human health (Roy et al., 2009).

Escherichia coli has developed a resistance to a variety of antibiotics mainly by both the efflux pumps interference and the resistance genes located on plasmids (Szmolka and Nagy, 2013). Plasmid considers the main vector in the acquisition and dissemination of multi-resistant either phenotypically or genotypically (Ochman et al., 2000). R-plasmids may be conjugative or non-conjugative. Plasmid-encoded antibiotic resistance encompasses most currently used clinically relevant classes of antibiotics, such as Extended-Spectrum Cephalosporins, Fluoroquinolones, and Aminoglycosides (Carattoli, 2013).

Cephalosporins and β-lactams bind a diverse of Penicillin-Binding Proteins as transpeptidases and carboxypeptidases. These Proteins implicated in the synthesis of the bacterial cell wall and cytoplasmic membrane. Consequently, lysis of the cell wall, cell shape disruption, and cessation of cell division occurred and finally cell death. β-lactam antibiotic resistance could also be because of the alterations of Penicillin-Binding Proteins (PBPs), or production of beta-lactamases enzymes inflicting hydrolysis of beta-lactam ring in the antibiotic (Livermore, 1995).

Aminoglycosides exert their inhibitory effect on bacteria through binding to the 30S ribosomal subunit. Therefore, it interferes with the function of the ribosome and causes the 30S subunit to misread the genetic code. Bacterial resistance to aminoglycosides could also be because of the chromosomal mutation and/or acquiring mobile genetic elements as transposons, plasmids, and integrons carrying resistance genes (Fux et al., 2009).

Quinolones target the inhibition of bacterial type II topoisomerase (DNA gyrase), which responsible for the unwinding of DNA for the complementary base pairing and mRNA synthesis. Thus, prevents bacterial replication and protein synthesis. (Oliphant et al., 2002). Resistance to quinolones is due to point mutations in the chromosomal quinolone resistance-determining regions (*GyrA* and *GyrB*). Or by the alteration of the efflux pumps and also the *qnr* protein, produced from quinolone resistance genes located on plasmid (Ranjbar and Farahani, 2017; Sharma et al., 2009).

Hence, the current study aimed to investigate the phenotypic and genotypic plasmid-mediated resistance in *E. coli* strains isolated from both healthy and diarrheic sheep and goats in Medina.

2. Materials and methods

2.1. Bacterial strains

A total of 234 fecal samples were collected from sheep (157) and goats (77) from different farms in Al-Madinah, Saudi Arabia. Samples were collected aseptically on ice and sent to the laboratory with a minimum of delay and stored at −80 °C till use. Out of 157 sheep, 99 (63%) were healthy and 58 (36.9%) were diarrheic; while 32 (41.5%) goats were healthy and 45 (58.4%) were diarrheic. The samples were seeded on MacConkey agar plates and incubated for 24 hr at 37 °C. *E. coli* isolates were identified by using the conventional methods, including Gram's staining, biochemical activities, and the growth on EMB agar. To confirm the presumptive *E. coli* isolates, 2 oligonucleotides, *UidA-F*, CCAAAAGCCAGACAGAGT and *UidA-R* GCACAGCACATCAA AGAG (Moyo et al., 2007), were used to amplify the *uidA* gene.

2.2. Template DNA preparation

DNA templates were prepared from overnight *Escherichia coli* cultures. The colonies were pelleted, suspended in 500 μl sterile distilled water, and boiled for 15 min (Usein et al., 2009).

2.3. Antimicrobials susceptibility test

Susceptibility of the isolates to antimicrobial agents was represented by the standard disc diffusion method, using commercially available antimicrobial susceptibility discs (Kirby-Bauer SN DISC, Nissui Pharmaceuticals, Tokyo, Japan). Aminoglycosides were represented by Kanamycine (30 μg), Gentamicin (30 μg), and Amikacin (30 μg). Whereas, Cephalosporins by Cephalothin (30 μg), Ceftazidime (30 μg), Cefotaxime (30 μg), and Cefuroxim (30 μg). Finally, Fluoroquinolones were represented by Norfloxacin (10 μg), Nalidixic acid (30 μg), Ciprofloxacin (15 μg). The test was performed according to the Clinical Laboratory Standard Institute (CLSI, 2006).

2.4. Detection of the resistance determinants

Plasmid DNA was extracted using the Alkaline Lysis Method (Sambrook et al., 1989). Plasmid DNA was then tested for the presence of the resistance determinants of Cephalosporin (*CTX-M1, CTX-M2, CTX-M9, and CTX-M8/25*), Aminoglycosides (*armA, rmtB*), and Fluoroquinolones (*qnrA*, *qnrB, qnrS)* by PCR. Primers nucleotide sequences and PCR conditions were listed in Table 1. PCR performed in the Bio-Rad thermal cycler (Bio-Rad Laboratories, CA, USA). PCR products were separated in Tris–Borate–EDTA (TBE) buffer supplemented with 1.5% agarose and visualized by gel electrophoresis at 100 V. A 100 bp DNA ladder (Thermo Scientific) was included in each agarose run.

2.5. Amplification and sequencing of quinolone resistance-determining region

2.5.1. Amplification of gyrB amplicon

The plasmid-mediated quinolone-resistant *E. coli* isolates were analyzed for quinolone-resistance determining region mutations. The sequence of *gyrB* (460 bp) was amplified by PCR using gyrBF- TGATCATGACCGTTCTGCAC and gyrBR- AGGTGAGTACCGCCGTCA under the following PCR conditions, 95 °C, 30 sec; 55 °C, 30 sec; 72 °C, 30 sec (Shabana et al., 2013).

2.5.2. Purification of PCR products and sequencing

MSB® Spin PCRapace kit (Invitek GmbH, Germany) was used to purify *gyrB* PCR product accordingly the manufacturer instructions. Sequencing was made by Big Dye Terminator v3. 1®, the reaction was composed of 1 μl Big Dye terminator, 1 μl purified PCR product, 1 μl of 5× – buffer, and 1.3 μl single primer then completed to 19 μl nuclease-free water (Promega). The amplification done in a Bio-Rad thermal cycler (Bio-Rad Laboratories, CA, USA) under the following conditions, 96 °C (30 sec), 96 °C (10 sec), 50 °C (5 sec), and 60 °C (4 min). The labeled products were then analyzed by an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, USA).

Table 1
Nucleotide sequences of the resistance determinants.

Primer designation	Nucleotide sequences	Specificity	PCR product size (bp)	PCR condition	Reference
CTX-M1	′5TTAGGAARTGTGCCGCTGYA′3(F) ′3CGATATCGTTGGTGGTRCCAT′5 (R)	**Cephalosporin Resistance**	688 bp	95 °C. 3 m, 95 °C. 30 sec 62 °C. 30 sec. 72 °C. 1 m	Al-Agamy et al. (2018)
CTX-M2	′5CGTTAACGGCACGATGAC′3(F) ′3CGATATCGTTGGTGGTRCCAT′5 (R)		404 bp	95 °C. 3m, 95 °C. 30 sec 50 °C. 30 sec. 72 °C. 1 m	Al-Agamy et al. (2018)
CTX-M8/25	′5AACRCRCAGACGCTCTAC′3(F) ′3TCGAGCCGGAASGTGTYAT′5(R)		326 bp	95 °C. 3 m, 95 °C. 30 sec 50 °C. 30 sec. 72 °C. 1 m	Al-Agamy et al. (2018)
CTX-M9	′5TCAAGCCTGCCGATCTGGT′3(F) ′3TGATTCTCGCCGCTGAAG′5(R)		561 bp	95 °C. 3 m, 95 °C. 30 sec 60 °C. 30 sec. 72 °C. 1 m	Al-Agamy et al. (2018)
armA	′5GGTGCGAAAACAGTCGTAGT′3 (F) ′3TCCTCAAAATATCCTCTATGT′5 (R)	**Aminoglycosides Resistance**	557 bp	95 °C. 3 m, 95 °C. 30 sec 62 °C. 30 sec. 72 °C. 1 m	González-Zorn et al. (2005)
rmtB	′5ATGAACATCAACGATGCCCT′3(F) ′3CCTTCTGATTGGCTTATCCA′5(R)		769 bp	95 °C. 3 m, 95 °C. 30 sec 62 °C. 30 sec. 72 °C. 1 m	Bogaerts et al. (2007)
qnrA	′5AGAGGATTTCTCACGCCAGG′3(F) ′3TGCCAGGCACAGATCTTGAC′5(R)	**Fluoroquinolones Resistance**	580 bp	95 °C. 3 m, 95 °C. 30 sec 60 °C. 30 sec. 72 °C. 1 m	Cattoir et al. (2007)
qnrB	′5GGMATHGAAATTCGCCACTG′3 (F) ′3TTTGCYGYYCGCCAGTCGAA′5(R)		264 bp	95 °C. 3 m, 95 °C. 30 sec 50 °C. 30 sec. 72 °C. 1 m	Cattoir et al. (2007)
qnrS	′5GCAAGTTCATTGAACAGGGT′3(F) ′3TCTAAACCGTCGAGTTCGGCG′5 (R)		428 bp	95 °C. 3 m, 95 °C. 30 sec 52 °C. 30 sec. 72 °C. 1 m	Cattoir et al. (2007)

2.5.3. Sequences analysis

gyrB sequences were aligned with BioEdit version 7.0.3. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (Hall, 2004). The phylogenetic tree was compiled with Mega 4.0 software (http://www.megasoftware.net/mega.html); using the unweighted pair group method with arithmetic mean (UPGMA).

3. Results

3.1. Prevalence of plasmid–associated E. coli

A total of 197 *E. coli* isolates were retrieved from the collected samples. *E. coli* was more prevalent in healthy (48.7%) than in diarrheic animals (35.4%). Out of 197 *E. coli* isolates, 159 were harbored plasmids. The frequency of plasmid-associated *E. coli* among healthy and diarrheic sheep was 41.3% (n = 55) and 33.8% (n = 45), respectively. In goats, plasmid-associated *E. coli* was isolated from diarrheic goats (37; 57.8%) more often than healthy ones (22; 43.3%) (Table 2).

3.2. Phenotypic antimicrobials susceptibility patterns

Phenotypic susceptibility of 159 plasmid-associated *E. coli* strains to different antimicrobial agents is summarized in Table 3. Aminoglycosides were represented by Amikacin, Gentamicin, and Kanamycin. Kanamycin resistance has been documented in healthy (4.4%) and diarrheic animals (20.7%). Gentamicin resistance rates in healthy animals were (2.5%) and (9.4%) in diarrheal animals. Resistance to Amikacin was observed at a rate of (1.2%) in diarrheic animals. Cephalothin, Ceftazidime, Cefotaxime, and Cefuroxime represented Cephalosporins. *E. coli* strains of healthy and diarrheic animals showed resistance to Cephalothin at a rate of (40.8%) and (44%), respectively. The resistance rate against Ceftazidime and Cefuroxime was (2.5%) in diarrheic animals, while in the strains of both healthy and diarrheal animals there was no resistance to Cefotaxime was reported. Fluoroquinolones represented by Nalidixic acid, Ciprofloxacin, and Norfloxacin. The rate of resistance to nalidixic acid was in healthy animals (23.8%) and in diarrheal animals (17.6%). The resistance of *E. coli* strains of diarrheic animals to Ciprofloxacin was up to (22.6%), while in healthy animals was (3.7%). For Norfloxacin, the resistance rate of the strains from healthy and diarrheic animals was (5.6%) and (11.3%), respectively.

Table 2
Prevalence of Plasmid–associated *E. coli*.

Animal	Plasmid-associated *E. coli*		Total
	Healthy	Diarrheic	
Sheep (n = 133)	55(41.3%)	45(33.8%)	100(75.1%)
Goats (n = 64)	22(34.3%)	37(57.8%)	59 (92.1%)
Total (n = 197)	77(39%)	82(41.6%)	159 (80.7%)

3.3. Multidrug resistance patterns

All of the strains included in the present study have shown resistance to at least one of the antibiotics studied. Overall (82/159; 51.5%) *E. coli* strains are multidrug-resistant. The tested strain's multiple resistance patterns ranged from two to six antimicrobial agents. Briefly, in 34 (21.3%) and 20 (12.5%) strains of healthy and diarrheic animals, a two-drug resistance pattern was observed. Three-drug resistance pattern was shown in healthy animal 3 (1.8%) and diarrheic animals 6 (3.7%). The four-drug pattern is identified in 2 (1.2%) healthy animals' strains and 9 (5.6%) diarrheic animals' strains. The five-drug pattern was more prevalent in diarrheic animals' strains (4; 2.5%) than healthy ones (1; 0.6%). The six-drug pattern was reported solely among strains of diarrheic animals (3; 1.8%) (Table 4).

3.4. Distribution of the resistance genes among the strains isolated from healthy animals

In all Aminoglycoside-resistant *E. coli*, the *rmtB* gene was detected, but none of the strains were positive for the *armA* gene. The ESBL-producing *E. coli* possessed different *CTX-M* genes. Briefly, the *CTX -M8/25* gene was detected in 37 (56.9%) strains. A total of 184 (52.3%) strains were harbored the *CTX -M2* gene. *CTX -M9* was detected in 13 (20%) strains, while the *CTX-M1* gene

Table 3
Phenotypic antimicrobials susceptibility pattern.

Antibiotic group	Type of antibiotic	Susceptible (S)		Intermediate (M)		Resistant (R)	
		Healthy	Diarrheic	Healthy	Diarrheic	Health	Diarrheic
Aminoglycosides	**Kanamycin (KAN)**	2 (1.2%)	2(1.2%)	53(33.3%)	40(25.1%)	7(4.4%)	33(20.7%)
	Gentamicin (GEN)	55(34.5%)	32 (20.1%)	16 (10%)	15 (9.4%)	4(2.5%)	15 (9.4%)
	Amikacin (AK)	65 (40.8%)	70 (44%)	0 (0%)	0 (0%)	0 (0%)	2 (1.2%)
Cephalosporins	**Cefuroxime (CXM)**	58(36.4%)	48(30.1%)	4(2.5%)	23(14.4%)	0 (0%)	4(2.5%)
	Cefotaxime (CTX)	65(40.8%)	68(42.7%)	0(0%)	4(2.5%)	0(0%)	0 (0%)
	Ceftazidime(CAZ)	65(40.8%)	68(42.7%)	0 (0%)	0 (0%)	0 (0%)	4(2.5%)
	Cephalothin (KF)	1(0.6%)	1(0.6%)	0(0%)	0 (0%)	65(40.8%)	70(44%)
Fluoroquinolones	**Norfloxacin (NOR)**	83(52.2%)	26(16.3%)	1(0.6%)	0(0%)	9(5.6%)	18(11.3%)
	Nalidixic acid (NA)	39(24.5%)	4(2.5%)	17(10.6%)	11(6.9%)	38(23.8%)	28(17.6%)
	Ciprofloxacin (CIP)	54(33.9%)	39(24.5%)	0(0%)	2(1.2%)	6(3.7%)	36(22.6%)

Table 4
Multidrug resistance patterns.

Patterns	Antimicrobial agents	Number (%) of resistant strains	
		Healthy animals	Diarrheic animals
Two -drug	KF-NA	22(13.8%)	9(5.6%)
	KAN -KF	1(0.6%)	6 (3.7%)
	GEN -KF	3 (1.8%)	0(0%)
	KF-NOR	4 (2.5%)	1 (0.6%)
	KF-CIP	4(2.5%)	3(1.8%)
	KAN-AK	0 (0%)	1 (0.6%)
Three - drug	KAN-KF-NA	2(1.2%)	0 (0%)
	KF-CIP-NOR	1 (0.6%)	3(1.8%)
	KF-CIP-NA	0 (0%)	3 (1.8%)
Four -drug	GEN-KF-NA-NOR	1(0.6%)	0 (0%)
	KF-CIP-NA-NOR	0 (0%)	8 (5%)
	KAN–GEN-KF-NA	0 (0%)	1 (0.6%)
	KAN-KF-NA-NOR	1(0.6%)	0(0%)
Five- drug	KAN-KF-NA-NOR-CIP	1(0.6%)	3 (1.8%)
	KAN-GEN-KF-CIP-NOR	0 (0%)	1 (0.6%)
Six -drug	KAN-GEN-KF-NA-NOR-CIP	0 (0%)	2 (1.2%)
	KAN-GEN-KF-NA-NOR-CAZ	0 (0%)	1 (0.7%)
Total		40(25.1%)	42(26.4%)

KAN: Kanamicin, **GEN:** Gentamicin, **AK:** Amikacin, **KF:** Cephalothin, **CTX:** Cefotaxime, **CXM:** Cefuroxime, **CAZ:** Ceftazidime, **NA:** Nalidixicacid, **NOR:** Norfloxacin, **CIP:** Ciprofloxacin.

was detected in 13 (20%). Fluoroquinolone-resistant *E. coli* possessed different *qnr* genes. In brief, the *qnr B* and *qnr A* were similarly possessed at a frequency of (73.5%) while qnr S genes were detected in 26 (49%) strains (Table 5).

3.5. Distribution of the resistance genes among strains isolated from diarrheic animals

Similarly, all Aminoglycoside-resistant *E. coli* were harbored *rmtB* gene, but none of the strains were harbored for the *armA* gene. The ESBL-producing *E. coli* possessed different *CTX-M* genes. Briefly, the *CTX -M8/25* gene was detected in all ESBL-producing *E. coli* strains. For the *CTX -M2* gene, 75 (96.1%) of the strains were positive. *CTX -M9* was found in 62 (79.4%) strains, while the *CTX-M1* gene was detected in 24 (30.7%) strains. In Fluoroquinolone-resistant *E. coli*, the *qnrB*, *qnr A*, and *qnr S* genes were found at a rate of 62.1%, 56%, and 52.4%, respectively (Table 6).

3.6. The analysis of gyrB sequence of fluoroquinolone-resistant E. coli isolates

Mutations have been analyzed in the quinolone-determining region (*gyrB*) in 135 *E. coli* isolates (53 of healthy and 82 of diarrheic animals) harboring the quinolone resistance determinants. There were alterations in the *gyrB* gene sequence in a total of 48 PMQR-positive strains (35.5%). At sites 27, 117, 121, 150, 174, 186, 288, 372, and 396, multiple point mutations were detected in the *gyrB* gene (Fig. 1). The generated dendogram clarify five lineages (I to V).

3.7. Profiles of the multidrug- resistant E. coli strains isolated from healthy and diarrheic animals

Out of 82 strains, 13 strains were confirmed to be resistant to the three groups of antimicrobials, which resistance is mediated by plasmids (Cephalosporins, Fluoroquinolone, and Aminoglycosides). And they harbored their resistance determinants (*CTX-Ms*, *rmtB*, *qnrA-*, *qnrB-*, and *qnrS*). Briefly, 6 were isolated from diarrheic goats, 4 were from healthy goats, 2 were from diarrheic sheep, and 1 was from healthy sheep (Table 7).

4. Discussion

Sheep and goats are well known to harbor pathogenic *E. coli* strain through the food chain not only to animals but also to humans (Shabana et al., 2013; Shabana, 2014; Arshad et al., 2006). Sheep and goats have been identified as major reservoirs, and food contaminated its fecal material is a common source for human infections (Kiranmayi et al., 2010). *Escherichia coli* infections are a challenge especially for the nomadic peoples as they closely live with sheep and goats, and have no knowledge about the pathogenicity of *E. coli* and how it can be transmitted (Irfan, 2016).

Table 5
Distribution of the resistance genes in the resistant *E. coli* strains isolated from healthy animals.

Antimicrobial group	Aminoglycosides (n = 11)		Cephalosporin (n = 65)				Fluoroquinolones (n = 53)		
Gene	*arm A*	*rmt B*	*CTX-M1*	*CTX -M2*	*CTX -M9*	*CTX -M8/25*	*qnr A*	*qnr B*	*qnr S*
No. of strains	0	11	13	34	27	37	39	39	26
%	0%	100%	20%	52.3%	41.5%	56.9%	73.5%	73.5%	49%

Table 6
Distribution of the resistance genes in the resistant *E. coli* strains isolated from diarrheic animals.

Antimicrobial group	Aminoglycosides (n = 50)		Cephalosporin (n = 78)				Fluoroquinolones (n = 82)		
Gene	*arm A*	*rmt B*	*CTX-M1*	*CTX-M2*	*CTX-M9*	*CTX-M8/25*	*qnr A*	*qnr B*	*qnr S*
No. of strains	0	50	24	75	62	78	46	51	43
%	0%	100%	30.7%	96.1%	79.4%	100%	56%	62.1%	52.4%

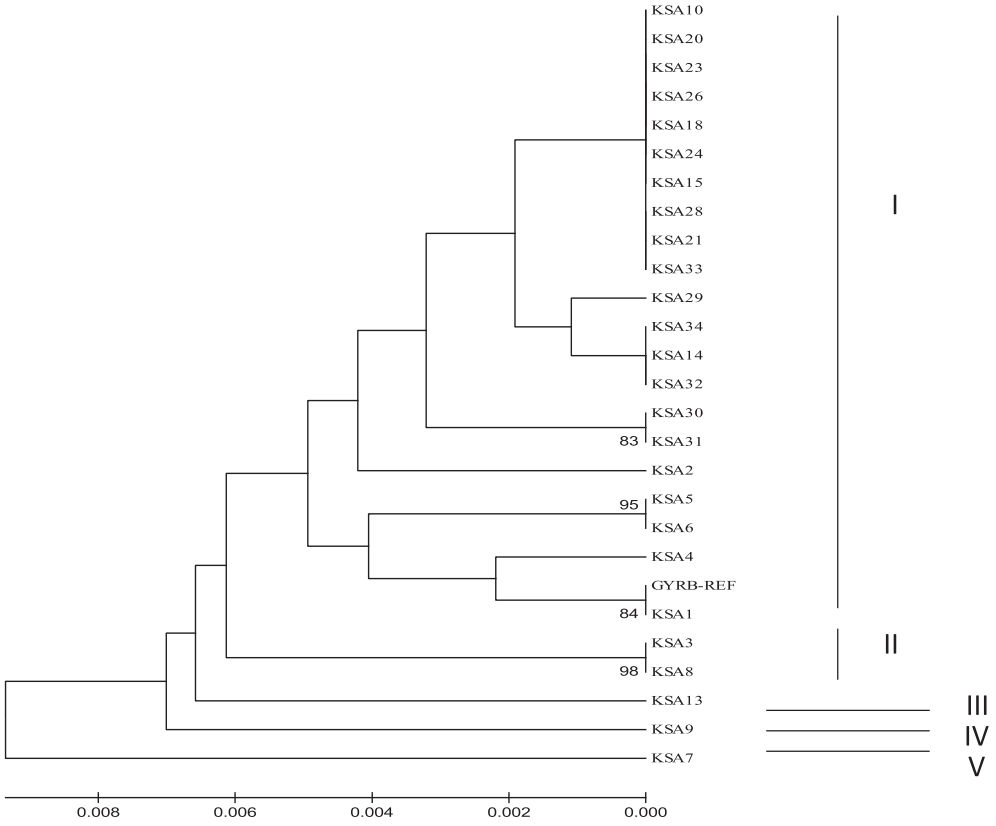

Fig. 1. Phylogenetic tree for *gyrB* sequences in *E. coli* strains from healthy and diarrheic sheep and goats built with MEGA 4.0 software using the unweighted pair group method with arithmetic mean. Bootstrap values ≥70% are shown.

In the current study, the prevalence of E. coli among healthy sheep (58.5%) was higher than diarrheic one (26.1%). These results were higher than those reported by Purkayastha et al. (2010), that the prevalence of E. coli in healthy sheep fecal samples was 41.67%, however, Bhat et al. (2007) isolated E. coli in non-diarrheic lambs at a rate of 18.28% and 30% in diarrheic lambs. The occurrence of plasmid-associated E. coli was 41.3% and 33.8% in healthy and diarrheic sheep, respectively. In goats, plasmid-associated E. coli has also prevailed among diarrheic (57.8%) and healthy ones (34.3%). Similarly, isolates harboring plasmids reported at a rate of 64% (Uma, 2009). As well as Smith et al. (2004) reported that 47% of E. coli isolates of healthy sheep and goats had plasmids ranged from 0.564 kb to >23 kb. Also, Kalantar et al. (2011) isolated a total of 35(35.4%) out of 99 E. coli isolates harboring plasmids ranged from 1.7 kb to 4.5 kb. Also, 68 out of 100 E. coli strains reported by Elsayed et al. (2017) were harbored R-plasmids from 1 to 5 per isolates, ranging from 1 to 33 kb in size.

Antibiotic's misuse has resulted in an increased incidence of antibiotic resistance among Enterobacteriaceae strains (French, 2010). Because of the lack of published data on the prevalence of antimicrobial resistance among the enteric bacteria of sheep and goats (Carl et al., 2002), the current study was aimed to investigate the rate of antimicrobial resistance of *E. coli* strains isolated from sheep and goats. Based on the animaĺs health, *E. coli* isolates from healthy and diarrheic sheep and goats were tested against ten antibiotics representing the three classes of antibiotics that their resistance mediated by the plasmid.

In the present study, the phenotypic resistance profiles of *E. coli* of healthy animals to aminoglycosides were as follows: Kanamycin (20.7%:4.4%), Gentamicin (2.5%:9.4%), and Amikacin (0.0%:1.2%). The isolates from healthy and diarrheic animals were resistant to Gentamicin at a rate of (2.5%) and (9.4%), respectively. Resistance to Amikacin was detected at a rate of (1.2%) among the isolates of diarrheic animals. This was agreed with Amadi et al. (2015) who reported a very low incidence of Gentamicin resistance (1%), however, Mahanti et al. (2015) recorded levels of resistance contrary to findings of the current study results, where the resistance to Amikacin, Kanamycin, Gentamicin, and Neomycin was 56%, 44%, 40%, and 36%, respectively. Regarding the Aminoglycoside resistance determinants distribution, all Aminoglycoside-resistant *E. coli* isolates (100%) harbored the *rmtB* gene, but none of the strains harbored the *armA* gene. Similarly, high resistance to Amikacin was reported by Deng et al. (2013) and *E. coli* isolates possessed mainly *rmtB* gene (12.6%) while *armA* was detected at a very low incidence (0.1%). Liu et al., (2013) also recorded an occurrence of 1.27% for *armA* and 11.5% for *rmtB* in *Escherichia coli* isolates from various food-producing animals.

Table 7
Profiles of the Multidrug- Resistant *E. coli* Strains Isolated from Healthy and Diarrheic Animals.

	Isolate ID	Animal	Status	Phenotypic Resistance profile	Resistance determinants
1	G 1	Goat	Healthy	KF-NA	*CTX-M2- CTX-M8/25,qnrA-qnrB-nrS*
2	G 2	Goat	Healthy	KF-NA	*CTX-M2-CTX-M8/25,qnrA-qnrB*
3	G 5	Goat	Healthy	KF-NA	*CTX-M2- CTX-M8/25,qnrA-qnrB-qnrS*
4	G 6	Goat	Healthy	GEN-KF	*rmtB-CTX-M2-CTX-M9- CTX-M8/25*
5	G 8	Goat	Healthy	KAN-KF-NA	*rmtB- CTX-M2-CTX-M9- CTX-M8/25,qnrA-qnrB-qnrS*
6	G 9	Goat	Healthy	GEN-KF-NA-NOR	*rmtB- CTX-M2- CTX-M8/25,qnrA-qnrB-qnrS*
7	G 10	Goat	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25,qnrA-qnrB-qnrS*
8	G 11	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M9- CTX-M825,/CTX-M1- qnrA-qnrB-qnrS*
9	G 12	Goat	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25,CTX-M1- qnrA-qnrB-qnrS*
10	G 13	Goat	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25,CTX-M1-qnrA*
11	G 14	Goat	Diarrheic	NA-KF	*qnrA-qnrB-qnrS-CTX-M1-CTX-M9*
12	G 15	Goat	Diarrheic	KAN-GEN-KF-NA	*rmtB- CTX-M2- CTX-M9- CTX-M8/ 25,qnrB-qnrS*
13	G 16	Goat	Diarrheic	KAN-GEN-KF-CAZ-NA-NOR	*rmtB- CTX-M2- CTX-M9- CTX-M8/25,CTX-M1- qnrA*
14	G 17	Goat	Healthy	KAN-KF-NA-NOR	*rmtB- CTX-M2- CTX-M8/25,qnrA-qnrB-qnrS*
15	G 18	Goat	Healthy	KF-CIP	*CTX-M2- CTX-M8/25,qnrA-qnrB-qnrS*
16	G 26	Goat	Healthy	KF-CIP	*CTX-M2- CTX-M8/25-qnrA-qnrB-qnrS*
17	G 27	Goat	Diarrheic	KAN-KF-NA-NOR-CIP	*rmtB- CTX-M9- CTX-M8/25-CTX1- qnrA-qnrB-qnrS*
18	G 28	Goat	Healthy	KAN-KF-NA	*rmtB- CTX-M9- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
19	G 29	Goat	Healthy	KAN-KF	*rmtB- CTX-M9- CTX-M8/25- CTX-M1*
20	G 30	Goat	Healthy	GEN-KF	*rmtB-CTX-M2- CTX-M8/25*
21	G 33	Goat	Healthy	GEN-KF	*rmtB-CTX-M8/25*
22	G 34	Goat	Healthy	KF-NOR	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA*
23	G 35	Goat	Healthy	KF-NOR	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA-qnrB*
24	G 37	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA-qnrB-qnrS*
25	G 38	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M8/25-qnrA-qnrB-qnrS*
26	G 39	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
27	G 40	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
28	G 41	Goat	Diarrheic	KAN-KF	*rmtB- CTX-M2- CTX-M8/25*
29	G 42	Goat	Diarrheic	KAN-KF	*rmtB- CTX-M2- CTX-M8/25*
30	G 43	Goat	Diarrheic	KF-NA	*CTX-M9- CTX-M8/25-qnrA*
31	G 44	Goat	Diarrheic	KF-NA-CIP-NOR	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB*
32	G 45	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25-qnrB*
33	G 46	Goat	Diarrheic	KAN-KF	*rmtB- CTX-M2- CTX-M8/25- CTX-M1*
34	G 50	Goat	Diarrheic	KF-NA	*CTX-M2- CTX-M8/25-qnrA-qnrB*
35	G 51	Goat	Diarrheic	KF-CIP	*CTX-M2- CTX-M9- CTX-M CTX-M8/25-qnrS*
36	G 53	Goat	Diarrheic	KF-NA-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB*
37	G 56	Goat	Diarrheic	KF-CIP	*CTX-M2- CTX-M8/25-qnrA-qnrB*
38	G 59	Goat	Diarrheic	KAN-KF-NA-NOR-CIP	*rmtB- CTX-M2- CTX-M8/25- qnrB*
39	G 60	Goat	Diarrheic	KAN-KF-NA-NOR-CIP	*rmtB- CTX-M2- CTX-M8/25- qnrA-qnrB-qnrS*
40	G 61	Goat	Diarrheic	KAN-GEN-KF-NOR-CIP	*rmtB- CTX-M2- CTX-M8/25-qnrB-qnrS*
41	S 1	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M8/25-qnrA-qnrB*
42	S 2	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M8/25- qnrA-qnrB-qnrS*
43	S 3	Sheep	Healthy	KF-NOR-CIP	*CTX-M2- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
44	S 4	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25-qnrA-qnrB*
45	S 5	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
46	S 6	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB*
47	S 7	Sheep	Healthy	KF-CIP	*CTX-M2- CTX-M9- CTX-M8/25-qnrA*
48	S 8	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25-qnrB*
49	S 9	Sheep	Healthy	KAN-KF-NA-CIP-NOR	*rmtB- CTX-M2- CTX-M9- CTX-M8/25- qnrB-qnrS*
50	S 10	Sheep	Healthy	KF-NOR	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA*
51	S 11	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
52	S 12	Sheep	Healthy	KF-NA	*CTX-M9- qnrA-qnrB-qnrS*
53	S 13	Sheep	Healthy	KF-CIP	*CTX-M2- CTX-M9- CTX-M8/25-qnrS*
54	S 14	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
55	S 15	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25-qnrA*
56	S 16	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA*
57	S 20	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
58	S 22	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrB-qnrS*
59	S 23	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1-qnrA*
60	S 24	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
61	S 26	Sheep	Diarrheic	KF-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25qnrA-qnrB*
62	S 27	Sheep	Healthy	KF-NOR	*CTX-M2- CTX-M8/25- qnrA-qnrB-qnrS*
63	S 28	Sheep	Healthy	KF-NA	*CTX-M2- CTX-M9- qnrA-qnrB-qnrS*
64	S 30	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
65	S 31	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
66	S 32	Sheep	Diarrheic	KF-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
67	S 33	Sheep	Diarrheic	KF-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
68	S 34	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
69	S 35	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- qnrB-qnrS*
70	S 36	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
71	S 37	Sheep	Diarrheic	KF-NA-CIP	*CTX-M2- CTX-M9- CTX-M8/25- CTX-M1- qnrA-qnrB-qnrS*
72	S 38	Sheep	Diarrheic	KF-NA-CIP	*CTX-M2- CTX-M9- qnrA-qnrB-qnrS*
73	S 39	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
74	S 40	Sheep	Diarrheic	KF-CIP	*CTX-M2- CTX-M9- CTX-M1- qnrA-qnrB-qnrS*

(*continued on next page*)

Table 7 (*continued*)

	Isolate ID	Animal	Status	Phenotypic Resistance profile	Resistance determinants
75	S 41	Sheep	Diarrheic	KAN-GEN-KF-NA-NOR-CIP	*rmtB- CTX-M2- CTX-M9- CTX-M 8/25- qnrA-qnrB-qnrS*
76	S 42	Sheep	Diarrheic	KAN-GEN-KF-NA-NOR-CIP	*rmtB- CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
77	S 43	Sheep	Diarrheic	KF-NA-NOR-CIP	*CTX-M2- CTX-M9- CTX-M8/25- qnrA-qnrB-qnrS*
78	S 44	Sheep	Diarrheic	KF-NOR	*CTX-M2- CTX-M9- CTX-M8/25-qnrA-qnrB*
79	S 45	Sheep	Diarrheic	KAN-KF	*rmtB- CTX-M9- CTX-M8/25*
80	S 46	Sheep	Diarrheic	KAN-KF	*rmtB- CTX-M2- CTX-M9- CTX-M8/25*
81	S 48	Sheep	Diarrheic	KAN-KF	*rmtB- CTX-M2- CTX-M9- CTX-M8/25- CTX-M1*
82	S 49	Sheep	Diarrheic	KAN-AK	*rmtB*

In the current study, Fluoroquinolone resistance profiles revealed a high resistance to Nalidixic acid in the healthy (23.8%) and diarrheic animals (20.4%). These results were in agreement with those of Cid et al. (1996), who reported resistance to Nalidixic acid at a rate of 27% in *E. coli* isolates from healthy sheep. Ciprofloxacin resistance rate reached up to 22.6% in diarrheic animals and 3.7% in healthy animals. A very low occurrence (less than 1%) of Ciprofloxacin resistance was detected in parallel with Orden (2001). For Norfloxacin, the resistance rate was 5.6% and 11.3% in healthy and diarrheic animals, respectively. These findings were consistent with Shakya et al. (2013), where the Norfloxacin resistance rate was 11% among diarrheic animals. Quinolone resistance resulted from changes in the expression of the efflux pumps and protection of DNA gyrase, the target of quinolones by the qnr proteins (mediated by plasmids), or from chromosomal point mutations in the Quinolone Resistance-Determining Regions of DNA gyrase (Ranjbar and Farahani, 2017; Sharma et al., 2009). The current study showed that out of 135 PMQR-positive strains, 48 had alterations in the *gyrB* gene sequence; this was in agreement with other studies that documented the responsibility of *gyrB* gene mutation for the quinolone resistance (Röderova et al., 2016). Plasmid-mediated resistance traits of Quinolone are omnipresent and common in commensal strains of healthy animals, and their distribution is typically due to plasmid transfer (Fortini et al., 2011). The present study showed that *qnrB* and *qnrA* were the most frequently encountered PMQR genes (73.5%) followed by *qnrS* gene (49%). Similarly, Diwan et al. (2012) identified *qnr B* as the most prevalent *qnr* genes among *E. coli* isolates. Ranjbar and Farahani (2017) reported only *qnrB* and *qnrS* genes in a total of 24 out of 51 quinolone-resistant strains; none of them had the *qnrA* gene. Four *E. coli* isolates (3.3%) were reported by Cengiz et al. (2012) harbored *qnr A* and *qnr S.* while, *E. coli* isolates of diarrheic animals possessed *qnr B* at a higher rate (62.1%) followed by the *qnrA* (56%) and *qnr S* gene (52.4%). Mansouri-Jamshidi et al. (2013) reported that *qnrA, qnrB,* and *qnrS* were carried by 31.8%, 56.5% and 28.9% of the resistant isolates, respectively.

ESBL producing Enterobacteriaceae is an emerging public-health concern, that the advent of ESBLs reduces the possible therapeutic options (Farzana et al., 2013). In the current study, the Cephalothin resistance rate was 40.8% and 44% in healthy and diarrheic animals, respectively. Cefuroxime and Ceftazidime resistance rate was (2.5%) in diarrheic animals. Nevertheless, no resistance was recorded to Cefotaxime among both healthy and diarrheic animals. Such findings were agreed with Elsayed et al. (2017), who reported similar resistance levels to Cephalothin. Amadi et al. (2015) reported similar findings of zero resistance to Cefotaxime. Such results are nearly identical to those reported by Scott et al. (2012), Orden (2001), and Mora et al. (2005) with a very low resistance to Cefotaxime and Ceftriaxone (less than 1%). The results showed that the ESBLs exist more frequently in diarrhea-associated *E. coli* isolates than commensal ones. The high prevalence of ESBL-producing *E. coli* in diarrheic animals may be due to the common use of cephalosporins for diarrhea treatment (Lei et al., 2010; Barton, 2014). Therefore, the recovered animals carrying ESBL resistant strains entering the food chain raises a public health concern. Cavaco et al. (2008) the overuse of third-generation cephalosporins were correlated with the spread and development of ESBL *E. coli*, and therefore their uses were prevented in animal\'s production and consequently, the occurrence of ESBL *E. coli* was decreased significantly (Agersø and Aarestrup, 2013). Resistance of *E. coli* isolates of healthy animals to the third-generation cephalosporins was frequently associated by the possession of the *CTX -M8/25* gene (56.9%), followed by the *CTX-M2* gene (52.3%), *CTX-M9* and *CTX-M1* genes at a rate of (20%), these findings were consistent with Hoang et al. (2017) who reported ten *E. coli* strains of diarrheic animals were ESBL-producers and had *CTX -M8/25* (100%), *CTX-M2* (96.1%), *CTX-M9* (79.4%), and *CTX-M1* (30.7%).

Microorganisms considered Multi-Drug Resistance (MDR) once they exhibit non-susceptibility to at least one antimicrobial agent. MDR strains can be transmitted through food to humans (Whitworth et al., 2008; Daniels et al., 2009; Nsofor and Iroegbu, 2012). This study showed that the overall prevalence of multidrug-resistant *E. coli* strains was (82/159; 51.5%). The resistance to a single antibiotic is less common than that of multiple antibiotics. Similarly, there is a high percentage of multi-resistant *E. coli* in sheep (88.2%) (Nsofor and Iroegbu, 2012).

The profiles of the highly resistant strains of *E. coli* from diarrheic and healthy animals are described in the current study. Of the 82 strains, 13 are found to be phenotypically resistant to the three classes of antibiotics (Aminoglycosides, Cephalosporin, and Fluoroquinolones) which their resistance is mediated by plasmids and harbored their resistance traits (*rmtB, CTX-Ms, qnrA- qnrB-qnrS*). This was consistent with what Paterson and Bonomo (2006) stated; ESBL-encoding strains often show resistance to Aminoglycoside and Fluoroquinolones. Carattoli (2013) studied 1000 plasmids obtained from resistant Enterobacteriaceae, and found that they harbored aminoglycoside, quinolones or ESBL resistance traits. The occurrence of multiple antibiotics resistance traits on plasmids is accompanied by the increased occurrence of virulence traits (Pitout, 2012). Certainly, exposure to antibiotics by commensal bacteria raises the possibility of the development of resistant strains that can transfer the resistance genes to a virulent strain (Tenover and McGowan, 1996). The horizontal transfer of antibiotic resistance traits can occur between bacteria from different sources, or from resistant bacteria to susceptible ones of the same source (Johnson et al., 2008). One of the most important public health issues is the presence of virulent strains harboring antibiotics resistance traits together with commensals, as these traits can be passed to them.

5. Conclusion

The high prevalence of the plasmid-mediated resistance in *E. coli* of both healthy and diarrheic animals, together with the multidrug resistance and the wide distribution of the resistance traits highlights the importance of sheep and goats as reservoirs

for the dissemination of the plasmid-mediated resistance to the human populations through food. Consequently, appropriate management practices should be implemented.

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