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Molecular Characteristics of Archived Isolates of Escherichia coli from the Gut of Healthy Food Animals and Environmental Sources in Selected Counties in Kenya

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Abstract
E. coli is a reservoir of resistance genes encoding resistance enzymes including extended-spectrum beta-lactamases (ESBL) and carbapenemases. This study aims to determine the resistance pattern, the pathotypes, and the proportion of ESBL producers among archived *E. coli* isolates from healthy food animals and their immediate environments at the Center for Microbiology Research, Kenya Medical Research Institute. Of the 375 isolates, 78.4% isolates were resistant to at least one of the 13 tested antibiotics and 28.8% showed multidrug resistance. Resistance was higher to tetracycline (55.2%), co-trimoxazole (44%), trimethoprim (43.7%), and ampicillin (28.8%). The proportion of Enteroaggregative *E. coli* was 88.3% while other pathotypes were not found. The proportion of ESBL producers was 8.8% of which 100% harboured $bla_{TEM,}$ 18.1% harboured $bla_{\text{CTX-M;}}$ bla_{SHV} was not found. *E.coli* isolates from healthy food animals were multidrug resistant and harboured virulence genes and ESBL genes. Risk assessment and management is necessary to protect farmers and the public in general.
Keywords: Archived *E. coli* isolates, antimicrobial resistance, multidrug resistance, pathotypes, ESBLs.

1. Introduction

E. coli (E. coli) is a ubiquitous bacterial species commensal of humans and warm blooded animals (Kabiru *et al.* 2015).They can occasionally be isolated in association with the intestinal tract of non-mammalian animals and insects (Welch 2006). *Escherichia coli* is present in the intestinal tracts of both humans and animals and is released into the environment through fecal material; it is also a reservoir for antibiotic resistance genes (Sahoo *et al.* 2012). Humans and animals are probably main reservoir of antimicrobial resistant *E. coli* (Hoang *et al*. 2017). In the natural environment the resistant bacteria and resistance genes from animal or environmental origin might be transferred to humans (Sahoo *et al.* 2012). Although most *E. coli* strains are non-pathogenic, some of them are highly pathogenic (Barbosa *et al*. 2014).

The widespread use of agricultural antimicrobials contributes to increased clinical resistance to antimicrobials (Brower *et al* 2017). One of the current most relevant resistance mechanisms in *Enterobacteriaceae* is the production of enzymes that lead to higher generation cephalosporins and even carbapenems resistance, mainly extended-spectrum beta-lactamases (ESBLs) and carbapenemases (Zurfluh *et al*. 2013). These enzymes are predominantly found in *E. coli* and *Klebsiella* although present also in other members of the *Enterobacteriaceae* (Saedii *et al*. 2017). A worrisome aspect is the spread of ESBLs and carbapenemase producers into the environment (Abgottspon *et al.* 2014). Recently, ESBL-producing strains have also emerged in healthy human carriers (Zurfluh *et al*. 2013), in healthy food-producing animals and household pets as well as on food products like meat, fish and raw milk (Geser *et al* 2012). Almost every class of anti-microbial is used in agriculture, including many closely related to clinically relevant antimicrobials, such as penicillins, cephalosporins, fluoroquinolones, tetracyclines, sulfonamides, and amino-glycosides (Brower *et al.* 2017).

Averagely, antimicrobial use is higher in the animal industry than in human medicine (Hu *et al*. 2017). The increasing number of multiple-antibiotic resistant pathogens has become a serious threat to human health (Kappell *et al*. 2015). Since antimicrobials are routinely added to animal feeds, bacterial populations are repeatedly exposed to subtherapeutic doses ideal for the emergence and spread of antimicrobial resistance (Brower *et al.* 2017). The persistent exposure of bacterial strains to a multitude of β-lactams has induced dynamic and continuous production and mutation of β-lactamases, resulting in synthesis of enzymes known as ESBLs in these bacteria (Shaikh *et al.* 2015). The incidence of ESBL-producing organisms is difficult to resolve due to various reasons, viz difficulty in detecting ESBL production and inconsistencies in reporting (Shaikh *et al* 2015). The general objective of this study was to determine the molecular characteristics of archived *E. coli* isolates from the gut of healthy food animals and environmental sources as an important and frequent cause of diarrhea in humans. Food animals included cattle, pigs and chicken whereas environmental resources include effluents from food animal shed and food animal droppings. Selected counties were Nairobi, Kiambu, Mombasa,

Kisumu and Kwale.

2. Material and method

2.1. Resuscitation and confirmation of archived E. coli isolates

Archived *E coli* isolates (375) at the Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI) were cultured onto MacConkey agar, without salt (Oxoid, UK) by streak plate method and incubated at 37ºC for 18-24 h for revival (Zinnah *et al* 2007). Discreet pink colonies or colorless colonies were identified as *E. coli* based on Gram stain and biochemical tests including Triple Sugar Iron, Citrate utilization, Motility Indole, Urease test (Oxoid, UK) and Ornithine (BD) (Juma *et al* 2016).

2.2 Antimicrobial susceptibility testing

Antibiotic susceptibility testing of the identified *E.coli* isolates for this study was performed using the disk diffusion technique (Bauer *et al* 1966) for commonly used antimicrobial discs including; ampicillin (AMP10µg), tetracycline (TE 30µg), trimethoprim (TMP5µg), co-trimoxazole (SXT 25µg), chloramphenicol (C 30µg), azithromycin (AZM 15µg), gentamicin (GM 10µg), cefuroxime (CXM 30µg) , cefotaxime (CTX 30µg), ceftazidime CAZ 30µg), imipenem (IMP10µg), ciprofloxacin (CIP 5µg) and nalidixic acid (NA 30µg), then tests were performed on Mueller Hinton agar (Oxoid, UK). Quality control for the microbial growth and the antimicrobial discs potency was performed using *E. coli* ATCC 25922. After incubation, the inhibition zones diameters were measured using a ruler; the antibiograms generated were used to cluster the isolates as sensitive, intermediate and resistant according to the breakpoints provided by the clinical laboratory standard institute (CLSI, 2017).

2.3 Phenotypic ESBL detection

The Double Disk Synergy test (DDST) was used whereby disks of ceftazidime (CAZ30µg), cefotaxime (CTX30µg), and ceftriaxone (CRO 30µg) were placed on either side of Augmentin (AUG 30µg), 30 mm apart, center to center, on inoculated Mueller-Hinton Agar and incubated at 35^0C for 16-18hours. The test was considered positive when a decreased susceptibility to any of the third cephalosporins used was associated to a clear-cut increase of the clear zone of the third generation cephalosporin in front of augmentin showing a characteristic shape-zone referred to as “champagne-cork”, “keyhole” (Drieux *et al* 2008). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control respectively.

2.4 PCR based detection of virulence genes and ESBL genes.

The DNA was extracted using a heat treatment protocol whereby purified colonies were suspended in an Eppendorf tube containing 1ml of sterile distilled water (DNase /RNase free) and boiled at 95^0C for 10 min in heating block. After boiling, the tubes were centrifuged at 14,000 revolutions per minute (rpm) for 5min. The supernatant containing the released DNA was transferred to new Eppendorf tubes and stored at -20 0C for further PCR use (Dashti et al 2009). Virulence genes for Enteropathogenic *E. coli* (EPEC: bfpA), Enterohemorrhagic *E. coli* (EHEC: stx1), Enteroaggregative *E.coli* (EAEC: aggR), Enteroinvasive *E. coli* (EIEC: invE), Enterotoxigenic *E. coli* (ETEC: LT, STp, STh), diffusely adherent *E. coli* (DAEC: afa) and *bla* genes including *bla*TEM, *bla*CTX-M and *bla*SHV were detected by PCR (Le Bouguenec & Servin 2006, Olsen *et al.* 2004, Monstein *et al.* 2007, Fujioka *et al.* 2009 , Gomez-Duarte *et al.* 2010, Pérez *et al*. 2010). Oligonucleotide primers, specific annealing temperatures, and sizes of products are depicted in table 1. PCR reactions were performed in 25 µl containing 12.5 µl of One Taq Master Mix with standard buffer (20 mM Tris-HCL, 22mM KCl, 22 mM NH4 Cl 0.5 pmol, 1.8 mM MgCl2, 5% glycerol, 0.05% Tween®20, 0.06% IGEPAL® CA-630, 0.2 mM dNTPs, 25 units/ml One Taq® DNA polymerase, pH 8.9 @ 250C), 0.5 µl of each primer, 1.5 µl of DNA template and 10 µl of nuclease free water. The amplification was done using a GeneAmp® PCR System 9700 Thermocycler (Applied Biosystems) as follows: initial denaturation of 95°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, an annealing step whose temperature is primer specific (table1) for 1minute, extension step at 72 °C for 2 min and final extension at 72°C for 7minutes. The amplicons were subjected to electrophoresis in 1.5% agarose gel (Oxoid, UK) with EZ-vision® In-Gel solution 10,000X for 40minutes (80V) and observed under UV light Transilluminator (Gelmax® UV imager) and image captured using a UV light software program.

Table 1. Oligonucleotide primers, specific annealing temperatures, and sizes of products

genes	primer sequence (5'-3')	product size (bp)	Annealing temperature (°C)	references
***bfp*A**	F:AATGGTGCTTGCGCTTGCTGC	324	62	(Pérez, C *et al* 2010)
	R:CCGCTTTATCCAACCTGGTA			
stx1	F:AGTTAATGTGGTGGCGAA	817	58	(Fujioka, M.*et al* 2009
	R:GACTCTTCCATCTGCCG			
***agg*R**	F:GTATACACAAAAGAAGGAAGC	254	58	(Fujioka, M.*et al* 2009)
	R:ACAGAATCGTCAGCATCAGC			
LT	F:GCACACGGAGCTCCTCAGTC	218	60	(Gomez-Duarte ,O. G *et al* 2010)
	R:TCCTTCATCCTTTCAATGGCTTT			
sth	F:CCCTCAGGATGCTAAACCAG	166	56	(Fujioka, M.*et al* 2009)
	R:TTAATAGCACCCGGTACAAGC			
***st*p**	F:TCTGTATTATCTTTCCCCTC	186	65	(Fujioka, M.*et al* 2009)
	R:ATAACATCCAGCACAGGC			
inv	F:ATATCTCTATTTCCAATCGCGT	382	58	(Fujioka, M.*et al* 2009)
	R:GATGGCGAGAAATTATATCCCG			
afa	F:GCTGGGCAGCAAACTGATAACTCT	750	66	(Le Bouguenec, C., and Servin, A. L. 2006)
	R:CATCAAGCTGTTTGTTCGTCCGCCG			
bla_{TEM}	F:ACCAATGCTTAATCAGTGAG	963	52	(Olsen, I., *et al*. 2004)
	R:GCGGAACCCCTATTTG			
bla_{SHV}	F:TTAGCGTTGCCAGTGTTC	851	58	(Olsen, I., *et al*. 2004)
	R:TTCGCCTGTGTATTATCTCCCTG			
bla_{CTX-M}	F:ATGTGCAGYACCAGTAARGTKATGGC	593	65	(Monstein, H.J*et al* 2007)
	R:TGGGTRAARTARGTSACCAGAAYCAGCG			

2.5 Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS 21.0, IBM SPSS, New York, USA). A chi square test (Fisher's exact) was used to assess the difference between ESBL producers and non ESBL producers with regard to antimicrobial resistance in *E. coli* isolates. $P < 0.05$ was regarded as statistically significant.

2. 6 Ethical approval

Ethical clearance to conduct the study was sought from KEMRI Scientific and Ethics Review Unit

3. Results

3.1 Antimicrobial susceptibility profile

Overall, 375 archived *E. coli* isolates were revived and their identity confirmed. Of the 375 isolates, 78.4% (274) isolates were resistant to at least one of the tested drugs among which 28.8% (108) were multidrug resistant. The highest resistance rate was recorded for tetracycline (55.2%), co-trimoxazole (44%), trimethoprim (43.7%) and ampicillin (28.8%). Resistance to nalidixic acid was 18.7%, azithromycin 14.9%, ciprofloxacin 10.1%, chloramphenicol 8.8%, Imipenem 0.8%, cefotaxime 5.9%, ceftazidime 3.7%, cefuroxime 2.4%, and gentamicin 1.1% (table 2).

Table 2. Antimicrobial susceptibility profile, n=375

Sensitive No (%)	**Intermediate No (%)**	**Resistant No (%)**	**Antibiotics**
226 (60.3)	41 (10.9)	108 (28.8)	Ampicillin (10 µg)
365 (97.3)	1 (0.3)	9 (2.4)	Cefuroxime (30 µg)
338 (90.1)	23 (6.1)	14 (3.7)	Ceftazidime (30 µg)
318 (84.8)	35 (9.3)	22 (5.9)	Cefotaxime (30 µg)
365 (97.3)	7 (1.9)	3 (0.8)	Imipenem (10 µg)
346 (92.3)	25 (6.7)	4 (1.1)	Gentamicin (10 µg)
319 (85.1)	0	56 (14.9)	Azithromycin (15 µg)
161 (42.9)	7 (1.9)	207 (55.2)	Tetracycline (30 µg)
271 (72.3)	34 (9.1)	70 (18.7)	Nalidixic acid (30 µg)
305 (81.3)	32 (8.5)	38 (10.1)	Ciprofloxacin (5 µg)
199 (53.1)	11 (2.9)	165 (44.0)	Co-trimoxazole (25 µg)
208 (55.5)	3 (0.8)	164 (43.3)	Trimethoprim (10 µg)
335 (89.3)	7 (1.9)	33 (8.8)	Chloramphenicol30 µg

Table3. Comparison of resistance profile between ESBL producers and non ESBL producers

	ESBL producers n=33	Non ESBL producers n=342	
Antibiotics	**No (%)**	**No (%)**	**p value**
Ampicillin 10µg	15(45.5)	93(27.2)	**0.06**
Cefotaxime 30µg	22(66.7)	0	**0.001**
Ceftazidime 30µg	13(39.4)	1(0.3)	**0.001**
Imipenem 10µg	0	3(0.9)	**0.1**
Gentamicin 10µg	1(3)	3(0.9)	**0.001**
Azithromycin 15µg	8(24.2)	48(14)	**0.1**
Tetracycline 30µg	20(60.6)	187(54.7)	**0.6**
Ciprofloxacin 5µg	7(21.2)	31(9.1)	**0.006**
co-trimoxazole 25µg	10(30.3)	155(45.3)	**0.1**
Trimethoprim 5µg	10(30.3)	154(45)	**0.2**
Chloramphenicol 30µg	5(15.2)	28(8.2)	**0.3**
Nalidixic acid 30µg	15(45.5)	55(16.1)	**0.001**
Cefuroxime30µg	6(18.2)	3(0.9)	**0.001**

3.2 Proportion of ESBL producers

Out of 375 archived *E. coli* isolates, 33 (8.80%) were ESBL-producers by DDST (figure 1). The multidrug resistance was significantly higher among ESBL producers (63.6%) than non ESBL producers (25.4%), (p equal to 0.001). Compared to non ESBL producers, ESBL producers were significantly resistant to cefuroxime, ceftazidime, cefotaxime, gentamicin, nalidixic acid and ciprofloxacin. The highest resistance rate was to cefotaxime (66.7%) and to tetracyclines (60.6 %) followed by ampicillin, nalidixic acid (45.5%) and ceftazidime (39.4%). Then co-trimoxazole, trimethoprim (30.3%), azithromycin (24.2%), ciprofloxacin (21.2 %), cefuroxime (18.2%) and chloramphenicol (15.2%). The lowest resistance rate was observed to gentamicin (3.0%). ESBL producers were sensitive to imipenem (table 3).

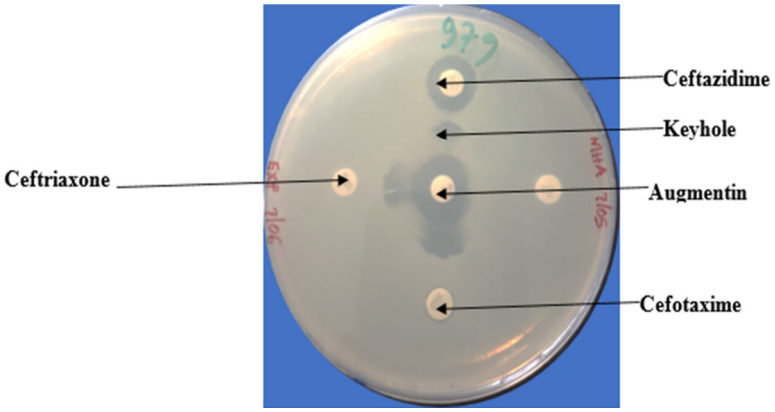

Figure1. Double disk synergy test showing "Keyhole" characteristic of ESBL production by *E. coli* strain

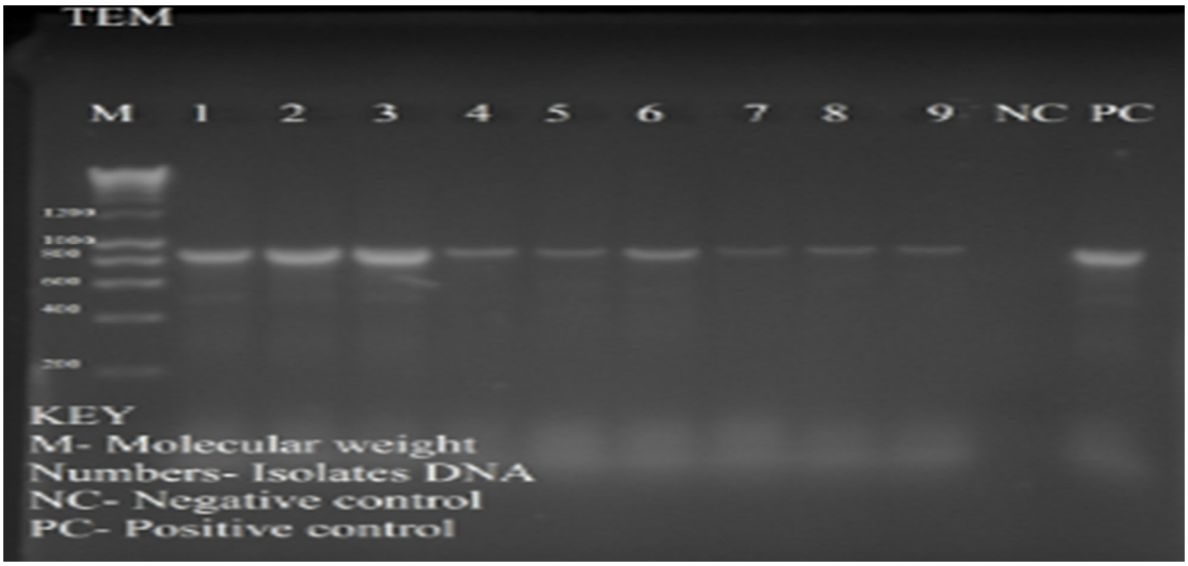

Figure 2. Agarose gel electrophoresis showing PCR amplification of bla_{TEM} with a size of 963bp.For isolate numbers, 1=842, 2=979, 3=1732, 4=1749, 5=1814, 6=23, 7=108, 8=114, 9=769

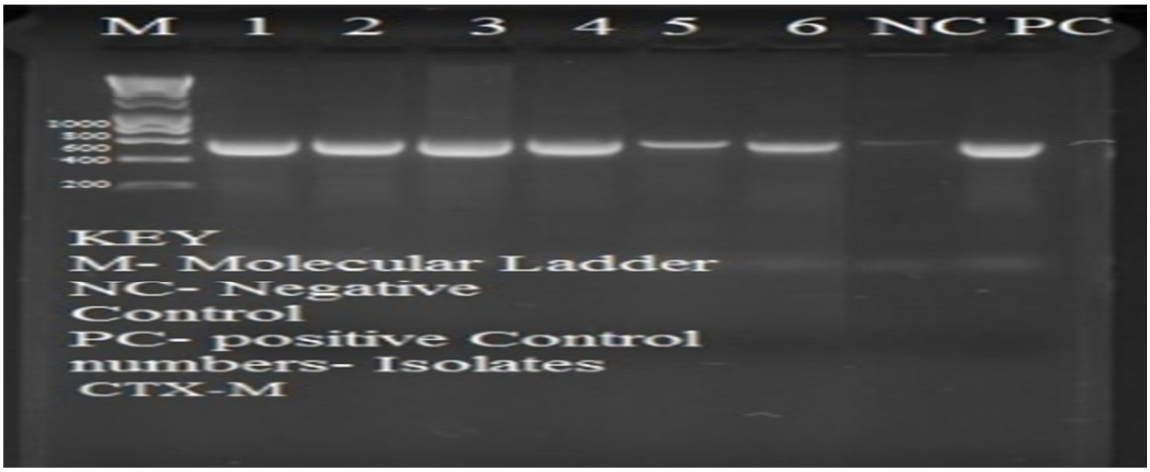

Figure 3. Agarose gel electrophoresis showing PCR amplification of bla_{CTX-M} with a size of 593bp. For isolates numbers 1=1777, 2=1778, 3=1814, 4=1004, 5=817, 6=1023

3.3 Carriage of virulence genes among E. coli pathotypes

Overall, 120 multidrug resistant *E. coli* isolates and/or phenotypic ESBL producers were tested for the carriage of virulence genes. 106 out of 120 (88.3%) isolates were found to harbour *agg*R (figure 4), a virulence gene for Enteroaggregative *E. coli*, 21.7% of EAEC carried *bla* genes. None of the isolates carried virulence determinants such as *inv*E for Enteroinvasive *E.coli*, stx1 for Enterohemorrhagic *E. coli*, bfpA for Enteropathogenic *E. coli*,

LT, STh and STp for Enterotoxigenic *E. coli*, *afa* for Diffusely adherent *E. coli.*

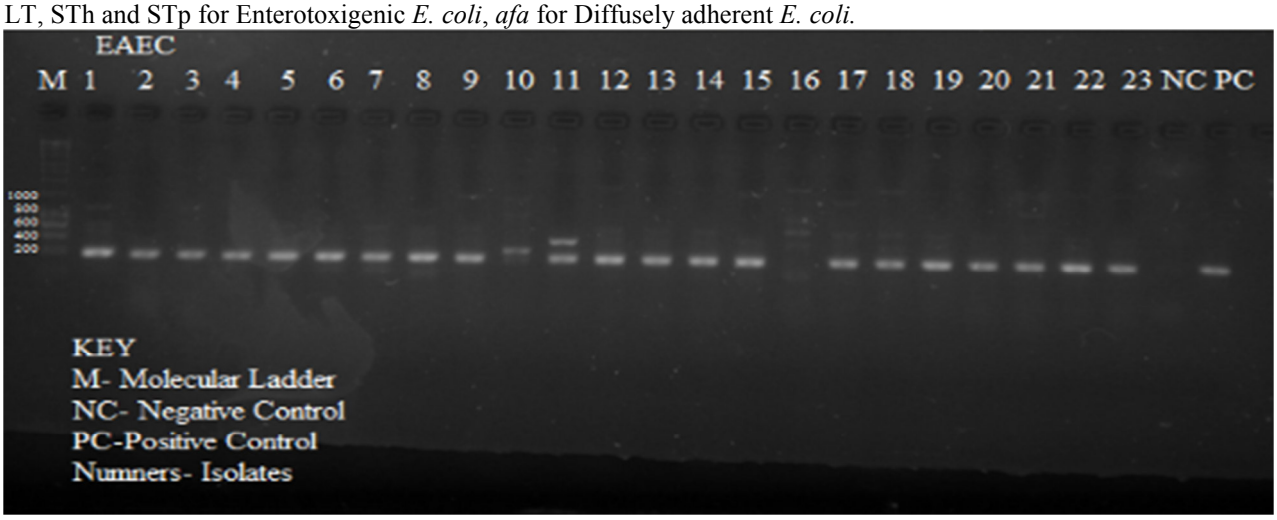

Figure4. Agarose gel electrophoresis showing PCR amplification of aggR gene characteristic for EAEC with a size of 254 bp For isolates numbers 1=824, 2=740, 3=1057, 4=1544, 5=422, 6=2010, 7=1769, 8=1723, 9=1600, 10=1587, 11=1593, 12=819, 13=815, 14=815, 15=1838, 16=1610, 17=1834, 18=1829, 19=1927, 20=1562, 21=1559, 22=1771, 23=16

4. Discussion

The overall prevalence of antibiotic resistance among archived *E. coli* isolates from this study was 78.4%; this is close to the prevalence observed in a similar study done in Reunion on poultry (Gay *et al* 2018). The multidrug resistance observed is nearly the same as the findings from a study conducted in Kenya which was 26% (Mapenay *et al.* 2006). The level of antimicrobial resistance in *E. coli* is a useful indicator of resistance dissemination in bacterial populations, and of selective pressure imposed by antimicrobials used in food animals (Zhao *et al.* 2012). In early 1952, antimicrobials were introduced to commercial feed for cattle, pigs and poultry (Mazurek *et al* 2015). Since antimicrobials used in animal production and in human are similar and some are even the same (Teuber 2001; Nhung *et al.* 2016), dissemination of antimicrobial resistance determinant to humans could lead to limited therapy options and associated consequences including treatment failure, economic loss and high rate of morbidity and mortality. The highest resistance rate was recorded for tetracycline, co-trimoxazole, trimethoprim and ampicillin (table 2). Similar studies conducted in Tanzania, Nigeria, Belgium exhibited higher resistance to commonly used antimicrobials as demonstrated in the current study (Nsofor & Iroegbu 2012; Ogunleye *et al.* 2013; Adelowo *et al*. 2014; Chantziaras *et al* 2014; Hamisi et 2014; Adenipekun, *et al* 2015). Low resistance to gentamicin, chloramphenicol and cefuroxime was observed (table 2) which concur with findings from Nigeria and Rwanda (Adenipekun *et al*. 2015; Manishimwe *et al* 2017). In Kenya as well as in other countries such as Japan, tetracycline is used at high extent followed by sulfonamides (Mitema *et al.* 2002; GARP 2011; Harada & Asai 2010); data from surveys conducted in Nigeria, Zambia and South Africa indicated that tetracyclines and beta-lactam were among the first four antibiotics used in food animals, sulfonamides and macrolides, quinolones and cephalosporins are also used in livestock production (Alonso *et al.* 2017). This may explain the reason why *E. coli* isolates are highly resistant to such antibiotics. The use of antimicrobial agents in the veterinary field influence the emergence, prevalence, and spread of antimicrobial resistance in bacteria isolated from food-producing animals and the resulting resistant bacteria reduce the efficacy of the antimicrobial agents in humans (Harada & Asai 2010). Multidrug resistant *E. coli* from food animals and their immediate environment represents a great risk particularly to farmers who are permanently exposed through direct contact with the animals and their waste and to the public in general given that effluents and animals feces containing resistant organisms can infect people through food chain. When multidrug resistance is conveyed to pathogenic bacteria, it becomes a public health challenge since resistance will leads to treatment failure and implies resort to second line antibiotics for therapy which is very costly and contributes to the development of antimicrobial resistant strains (Chantziaras *et al* 2014).

From this study, 88 .3% Enteroaggregative *E.coli* (figure 4) were recovered. All EAEC were multidrug resistant and 21.7% were carrying ESBL genes; EIEC, EHEC, ETEC, EPEC and DAEC were not found. A worrying level of multidrug resistance in EAEC strains was reported in several studies and ESBL production as well as an enhanced resistance to quinolones was reported (Jensen *et al* 2014). The findings from this study concur with those from a recent study conducted in Kenya which demonstrated 80% of EAEC in fecal samples from healthy cows and whereby EPEC, EHEC, EIEC were not detected in animal samples (Ochi *et al* 2017). In a study conducted in Vietnam, 88.2% of EAEC exhibited a high multidrug resistance, 64.7 % were resistant to 3rd

generation cephalosporins and 50% were ESBL positive (Trung *et al* 2016). Different results were obtained in Burkina Faso where *E. coli* pathotypes were isolated from slaughtered food animals at varying rates, EAEC (32% in pigs, 6% in cattle and 7% in chickens), EIEC (1% in chickens), STEC (37% in cattle, 6% in Chicken, 30% in pigs), ETEC (4% in cattle,5% in chicken and 18% in pigs) and EPEC (8% in cattle, 37% in chicken and 32% in pigs) (Kagambèga *et al* 2012) while in China 9% EAEC were recovered from fecal samples from non-clinical settings including healthy animals and healthy humans (Zhang *et al* 2016). The high occurrence of EAEC is a public health threat since some strains possess additional virulence factors which have been linked with the ability to cause diarrhea and other symptoms which can be life-threatening for susceptible people (EFSA BIOHAZ Panel 2015).

The proportion of ESBL producers was 8.8 % *E. coli* isolates, 63.6% of the ESBL producers were significantly multidrug resistant; this is alarming given that multidrug resistant bacteria are currently acknowledged as one of the most important public health problem while antimicrobial resistance is regarded as one of the greatest threats to human health worldwide (van Duin & Paterson 2016). The highest resistance rates among ESBL producers were noted for cefotaxime and tetracyclines followed by ampicillin and nalidixic acid, ceftazidime, co-trimoxazole and trimethoprim, azithromycin, ciprofloxacin, cefuroxime and chloramphenicol. None of the isolates was resistant to imipenem (table 3). Similar studies showed many different ESBL production rates in diverse food animals ranging from 2 % to 88.8% in India, China and Zambia (Bandyopadhyay *et al* 2014; Chishimba *et al.* 2016; Li. *et al.* 2016; Xu *et al.* 2018); ESBL producers exhibited high resistance rate to non β-lactam drugs and the multidrug resistance was significantly higher comparing to non ESBL producers (Xu *et al* 2018; Wu *et al.* 2018). The high susceptibility to imipenem in ESBL-producing *E coli* from this study may be explained not only by the fact that ESBLs do not hydrolyse carbapenems (Walkty *et al.* 2016), but also by the lack of direct selection pressure since carbapenem are not approved to be used in food producing animals (Michael *et al*. 2015). The most negative aspect of *E. coli* as an ESBL-producing organism is the fact that they frequently carry genes encoding for resistance to other classes of antibiotics for example aminoglycosides, quinolones and sulfonamides (Thenmozhi *et al*. 2014). All the ESBL producers were found to harbour *bla* genes; bla_{TEM} was detected in all the ESBL producing *E. coli* while bla_{CTX-M} was detected in 18.1%. There was coexistence of bla_{TEM} and bla_{CTX-M} in ESBL producing *E. coli*; bla_{SHV} was not found in these isolates. In China and Spain various level of bla_{TEM} and bla_{CTX-M} were found from healthy chickens; bla_{SHV} was not detected (Wu *et al.* 2018; Costa *et al*. 2009; Yuan *et al.* 2009); in Madagascar, Mayotte, Japan, and Reunion high prevalence of bla_{CTX-M} in poultry, pigs and cattle was exhibited while bla_{TEM} and bla_{SHV} were either low or absent (Gay *et al*.2018; Nahar *et al*. 2018). Coexistence of bla_{TEM} and bla_{CTX-M} in *E. coli* recovered from pigs and chickens was reported in China and Portugal (Goncalves *et al*. 2010; Li *et al.* 2014). These findings showed a great variation in the distribution of these resistance determinants across different geographical region and among food animals. The raise of ESBL genes in *E. coli* from food animals could be due to the selective pressure resulting from the increased veterinary use of antibiotics including penicillins and cephalosporins as it was demonstrated by other researchers (Yuan *et al*. 2009; Van Boeckel *et al*. 2015).

5. Conclusion

The archived *E.coli* isolates from healthy food animals and environmental sources in selected counties in Kenya were found to be resistant to commonly used antibiotics. A great proportion of *E. coli* isolates revealed to be multidrug resistant; cross-resistance and co-resistance were observed. The isolates were highly resistant to tetracyclines, ampicillin, co-trimoxazole and trimethoprim. Resistance to cephalosporins, quinolones and fluoroquinolones, macrolides and chloramphenicol was noted. Archived *E. coli* isolates from healthy food animals and environmental sources were found to be reservoirs for resistance determinants and virulence genes. This is risky for public health since infection by resistant pathogens could result in treatment failure ending in human lives loss. Farmers should be sensitized on the safe handling and disposal of animal wastes in order to protect themselves as well as the population from contracting diarrheal diseases due to pathogenic and antimicrobial resistant *E. coli.*

6. Conflict of interest declaration

The authors declare there is no conflict of interest.

7. Acknowledgement

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