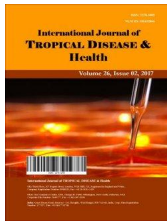

International Journal of TROPICAL DISEASE & Health

31(2): 1-10, 2018; Article no.IJTDH.42423
ISSN: 2278–1005, NLM ID: 101632866

Phenotypic and Genotypic Antimicrobial Resistance Profiles of *Escherichia coli* O157 Isolates from Cattle in Cameroon

Elvis Achondou Akomoneh[1,2], Seraphine Nkie Esemu[1,3], Gilbert Karngong Nfor[1], Roland N. Ndip[1,3] and Lucy M. Ndip[1,3,4*]

[1]Department of Microbiology and Parasitology, Faculty of Science, University of Buea, Cameroon.
[2]Department of Biological Science, Faculty of Science, University of Bamenda, Cameroon.
[3]Laboratory for Emerging Infectious Diseases, University of Buea, Cameroon.
[4]Center for Tropical Diseases, University of Texas Medical Branch, Texas, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EAA, SNE, RNN and LMN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAA and GKN collected samples and managed the analyses of the study. Authors EAA, SNE and LMN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2018/42423
Editor(s):
(1) Dr. Payala vijayalakshmi, Department of Microbiology, Gitam Institute of Medical Sciences and Research, Gitam University, India.
Reviewers:
(1) Tanvir Rahman, Bangladesh Agricultural University, Bangladesh.
(2) Raul Antunes Silva Siqueira, Federal University of Campina Grande, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/25280

Original Research Article

Received 10th April 2018
Accepted 21st June 2018
Published 26th June 2018

ABSTRACT

Background: *Escherichia coli* O157, a Shiga toxin-producing serotype of *E. coli* implicated in severe foodborne diseases, is a major public health concern worldwide. Most human infections are attributed to the consumption of infected beef and contaminated bovine products. The burden of *E. coli* O157 disease is compounded by the extensive use of antibiotics in the animal production line which might lead to the selection for antibiotic resistance. The aim of this study was to describe the antibiotic resistance patterns of *E. coli* O157 isolates from cattle in Cameroon.
Methods: Fifty-six *E. coli* O157 isolates previously obtained were subjected to antibiotic

**Corresponding author: E-mail: lndip@yahoo.com;*

susceptibility testing using the Kirby-Bauer disc diffusion technique. Polymerase chain reaction was used to screen the isolates for the presence of eight antibiotic resistance genes.

Results: Antibiotics susceptibility profiling of the 56 isolates showed ofloxacin was the most active drug (55; 98.2%), followed by gentamicin (51; 91.1%). Ampicillin was the least active drug with only 7 (12.5%) isolates susceptible. Multidrug resistance was a common phenomenon exhibited by 33 isolates comprising 17 phenotypic profiles (A8-A24). The most frequent phenotypic profile was $TET^R STR^R AMP^R TRI^R ERY^R$ (tetracycline, streptomycin, ampicillin, trimethoprim, and erythromycin resistance) which accounted for 17.0% of the resistant strains. All the 8 resistance genes investigated were observed in one or more isolates and genotypic resistance was generally consistent with the resistant phenotypes observed. The most commonly observed resistance genes were *tetA* (73.2%) and *aac(3)-IV* (57.1%). Five isolates had none of the resistance genes investigated while 25 carried at least three different resistance determinants.

Conclusions: Cattle in Cameroon are infected with multidrug-resistant *E. coli* O157 and are a potential risk to consumers. Hence adequate animal food production measures should be prescribed and implemented to minimize the development and spread of antibiotic resistant *E. coli* O157.

Keywords: E. coli O157; Cameroon; multidrug resistance; resistant gene profiles.

1. INTRODUCTION

Food borne pathogens including *E. coli* O157 are significant contributors to illness and death in developing countries and cost billions of dollars in medical care and economic loss [1,2]. Foodborne diseases have been largely attributed to changes in animal food production methods, farming habits and poor hygienic practices [3]. *E. coli* O157 was first reported as foodborne pathogen three decades ago in the USA, and since then, it remains an important public health problem, causing a disease spectrum ranging from non-bloody diarrhoea to bloody diarrhoea, haemolytic uremic syndrome, and other complications with high morbidity and fatality [4,5]. Cattle have been identified as the major reservoir of *E. coli* O157 although other animal species including sheep, goats, pigs, deer, rabbits, dogs, cats, rodents and wild birds have been implicated in the maintenance and transmission of the organism in the environment [6,7,8].

The emergence and spread of antibiotic resistant *E. coli* O157 is a matter of increasing concern globally. Although the use of antimicrobial therapy for human infections with Shiga toxin-producing *E. coli* O157 is contraindicated, antibiotics are routinely used for disease prevention and growth promotion in animal production lines. These practices lead to the selection of antimicrobial resistance through stable genetic changes and specific mechanisms including mutation, transduction, transformation and or conjugation [9,10]. Thus, antimicrobial-resistant bacteria carried by these animals may enter the human food chain through the consumption of meat or other animal products, through occupational exposure, farm runoffs, and other pathways, leading to prevention and treatment failures [11].

Since the first report of *E. coli* O157 infections in the African continent in 1992, the pathogen continues to spread in the continent with multiple cases reported in South Africa, Swaziland, Kenya, Nigeria, Ivory Coast, Gabon and the Central African Republic [12,13]. In order to understand the epidemiology of this pathogen, there is a need for continuous surveillance of *E. coli* O157 and the antimicrobial sensitivity pattern to understand the spread, geographic area-dependent characteristics and public health implications. This study, therefore, sought to investigate the phenotypic and genotypic antibiotic resistance profiles of *E. coli* O157 isolates in Cameroon.

2. MATERIALS AND METHODS

Fifty-six *E. coli* O157 isolates obtained from cattle across Cameroon at the abattoirs in Buea between October 2015 and September 2016 were analysed in this study. Isolation of the *E. coli* O157 strains was done through the pre-enrichment method for faecal specimen recommended by Wells et al. [14]. Presumptive *E. coli* O157 isolates were confirmed serologically using E. COLIPRO™ O157 latex agglutination test (Hardy Diagnostics, USA) and molecularly using PCR targeting *E. coli* rfb_{EO157} gene in the isolates (Fig. 1). The susceptibility of the isolates to antibiotic disks impregnated with streptomycin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ampicillin (10 µg),

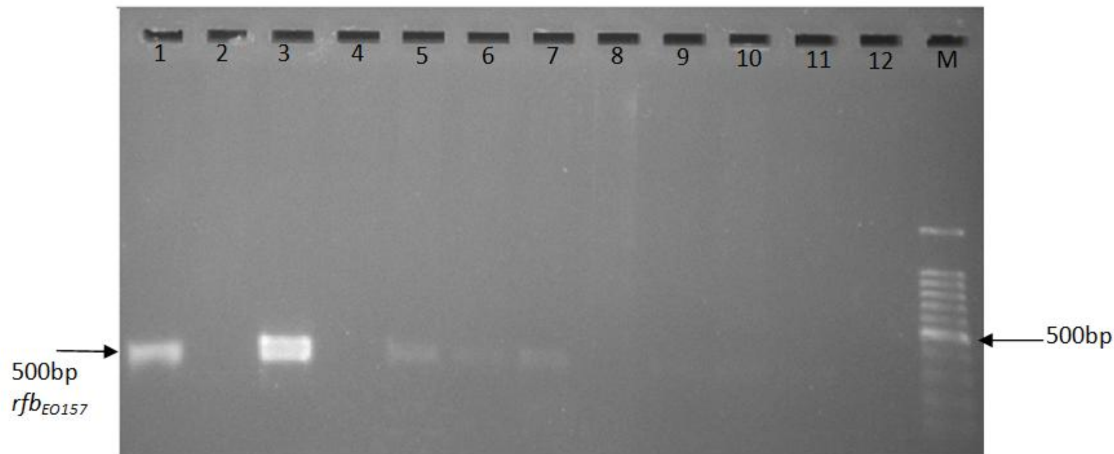

Fig. 1. Electrophoretic separation of amplified PCR products of singleplex *rfb*$_{EO157}$ PCR. Positive control (lane 1), positive samples (lanes 3,5-7), negative samples (lane 2,4,8-11), negative control (lane 12), 100 bp DNA ladder (lane M)

chloramphenicol (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), trimethoprim (5 µg) and gentamicin (10 µg) (Becton, Dickson, Fisher Scientific, USA) was done using the Kirby-Bauer disc-diffusion technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [15]. After incubation at 37°C for 16 -18 hr on Mueller-Hinton agar (Murex Biotec Ltd, UK), the antibiotic inhibition zone diameters were measured and results obtained used to classify isolates as being resistant, intermediate or susceptible to a particular antibiotic using standard reference values by the CLSI [15]. The quality control strain used was *E. coli* American Type Culture Collection (ATCC) 25922. The choice of the antibiotics was based on regular usage in human and veterinary medicine, local availability, potential public health importance and recommendations from the guideline of antimicrobial susceptibility testing from CLSI [15].

Multiplex PCR analysis was performed on all the 56 isolates to detect the antibiotic resistance genes *aadA1* and *aac(3)-IV* which confers resistances to aminoglycosides, *sul1* for folate pathway inhibitors, *bla*$_{SHV}$ and *bla*$_{CMY}$ for beta-lactam resistance, *cmlA* for chloramphenicol resistance, *tetA* for tetracycline resistance and *qnrA* for resistance to the quinolones. Total DNA was extracted from pelleted cells of freshly prepared broth cultures of *E. coil* O157 isolates using the QIAamp DNA Mini Kit following the manufacturer's instructions (QIAGEN, Germany). The eluted DNA was held at -20°C until used for PCR analyses. A 25 µL total individual reaction volume was set up comprising 5 µL template DNA, 12.5 µL of 2X master mix (TopTaq™ Master Mix, Qiagen, Hilden, USA), 0.5 µL of each primer from a working solution of 20 µM (final concentration of 0.4 µM) and nuclease-free water to make up the total volume. Primer sets used for the amplification of the different genes were as previously described [*aadA1* (aadA1f and aadA1r), *aac(3)-IV* (aac(3)-IVF and aac(3)-IVR), *sul1* (sul1f and sul1r), *bla*$_{SHV}$ (bla$_{SHV}$f and bla$_{SHV}$r), *bla*$_{CMY}$ (bla$_{CMY}$f and bla$_{CMY}$r), *cmlA* (calf and colour) [16], *tetA* (tetAf and tetAr) [17] and *qnrA* (qnrAf and qnrAr) [18]]. Unless otherwise stated, initial denaturation and final extension for all PCR runs were set at 95°C for 15 min and 72°C for 10 min respectively. Multiplex PCR targeting the *aac(3)-IV, bla*$_{CMY}$*, bla*$_{SHV}$ and *tetA* genes was optimized at 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min. Multiplex PCR targeting the *aadA1*, *sul1*, *cmlA* and *qnrA* genes had the same cycling conditions as above except for the annealing temperature that was set at 55°C. PCR products were separated on 1.5% agarose gel stained with SYBR safe DNA Gel Stain (Invitrogen, Thermo Fisher Scientific, USA) and visualised under UV light using a Gel Documentation-XR (BIORAD, Hercules, CA).

3. RESULTS

The susceptibility of the 56 *E. coli* O157 isolates to nine different antibiotics was identified by measuring the antibiotic inhibition zone diameter (IZD) around impregnated discs on Mueller-Hinton agar plates (Fig. 2). The susceptibility results showed no antimicrobial had 100%

activity on all the isolates (Table 1). Three isolates were susceptible to all the antimicrobials while 53 were resistant to one or more drug. Of the nine antibiotics, ofloxacin demonstrated the best activity (55; 98.2%) against the isolates with no resistance noted, followed by gentamicin (51, 91.1%), chloramphenicol (47, 83.9%), ciprofloxacin (46, 82.1%) and erythromycin (33, 58.9%). Ampicillin was the least active drug with only 7 (12.5%) isolates susceptible.

Based on the phenotypic resistance patterns observed within the 53 isolates that were resistant to one or more antimicrobials, 24 phenotypic resistance profiles (A1-A24) were generated. The phenotypic resistance profiles showed 20 (37.7%) of the 53 resistant *E. coli* O157 were resistant to one or two antibiotics while 33 (62.3%) were multidrug resistant (resistant to three or more antibiotics) (Table 2). The isolates that were resistant to one or two antibiotics comprised seven profiles (A1-A7) with six isolates resistant to ampicillin alone while resistance to both ampicillin and tetracycline was common in five isolates. The multidrug-resistant isolates comprised 17 profiles (A8-A24) with resistance profile A20 as the most prevalent shared by nine isolates. The phenotypic resistance profile A24 was common to five isolates while the other phenotypic resistance patterns were shared by three or fewer isolates (Table 2).

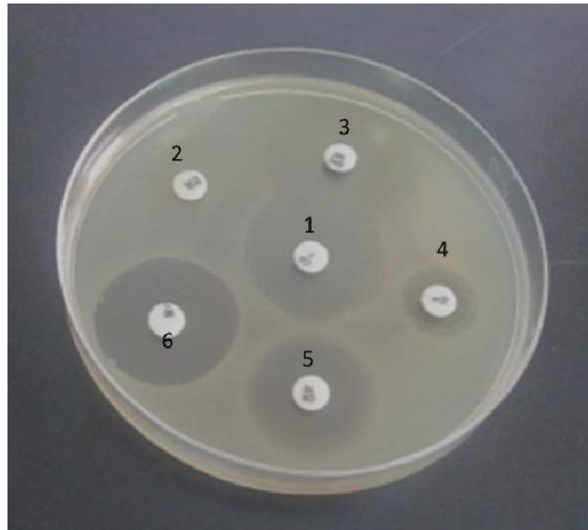

Fig. 2. Susceptibility test of *E. coli* O157:H7 on Muller Hinton agar impregnated with antibiotic disks with IZD indicating susceptibility of isolates: 1-chloramphenicol; 2- streptomycin; 3-ampicillin; 4-gentamicin; 5- trimethoprim; 6-ciprofloxacin

Table 1. Susceptibility of isolates to different antibiotics

Drug class	Antibiotic	Susceptibility patterns (n = 56)		
		Susceptible (%)	Intermediate (%)	Resistant (%)
Beta-Lactams	Ampicillin (10 μg)	7 (12.5)	2 (3.6)	47 (83.9)
Aminoglycosides	Gentamicin (10 μg)	51 (91.1)	0 (00)	5 (8.9)
	Streptomycin (10 μg)	25 (44.6)	6 (10.7)	25 (46.6)
Tetracyclines	Tetracycline (30 μg)	15 (26.8)	1 (1.8)	40 (71.4)
Fluoroquinolones	Ciprofloxacin (5 μg)	46 (82.1)	0 (00)	10 (17.9)
	Ofloxacin (5 μg)	55 (98.2)	1 (1.8)	0 (00)
Folate pathway inhibitor	Trimethoprim (5 μg)	27 (48.2)	0 (00)	29 (51.8)
Phenicols	Chloramphenicol(30 μg)	47 (83.9)	0 (00)	9 (16.1)
Macrolides	Erythromycin (15 μg)	33 (58.9)	0 (00)	23 (41.1)

Table 2. Phenotypic antimicrobial resistance profiles of *E. coli* O157

Phenotypic pattern	Antimicrobial resistance profiles	Isolates (%) (n = 53)
Resistant to one or two antibiotics		
A1	TRI^R	1 (1.9)
A2	TET^R	3 (5.7)
A3	AMP^R	6 (11.3)
A4	AMP^RTRI^R	3 (5.7)
A5	TET^RAMP^R	5 (9.4)
A6	TET^RGEN^R	1 (1.9)
A7	CIP^RAMP^R	1 (1.9)
Multidrug resistant		
A8	$STR^RAMP^RTRI^R$	1 (1.9)
A9	$TET^RSTR^RAMP^R$	1 (1.9)
A10	$TET^RCHL^RAMP^R$	1 (1.9)
A11	$TET^RAMP^RTRI^R$	2 (3.8)
A12	$AMP^RTRI^RERY^R$	1 (1.9)
A13	$TET^RGEN^RAMP^R$	1 (1.9)
A14	$TET^RSTR^RAMP^RERY^R$	2 (3.8)
A15	$TET^RSTR^RAMP^RTRI^R$	2 (3.8)
A16	$TET^RAMP^RTRI^RERY^R$	2 (3.8)
A17	$TET^RGEN^RCIP^RAMP^R$	1 (1.9)
A18	$TET^RSTR^RTRI^RERY^R$	1 (1.9)
A19	$TET^RGEN^RSTR^RAMP^RERY^R$	1 (1.9)
A20	$TET^RSTR^RAMP^RTRI^RERY^R$	9 (17.0)
A21	$TET^RCHL^RCIP^RSTR^RAMP^RTR^{IR}$	1 (1.9)
A22	$TET^RCHL^RCIP^RSTR^RAMP^RERY^R$	1 (1.9)
A23	$GEN^RCHL^RCIP^RSTR^RAMP^RTRI^RERY^R$	1 (1.9)
A24	$TET^RCHL^RCIP^RSTR^RAMP^RTRI^RERY^R$	5 (9.4)
Total		**53**

TET^R, Tetracycline resistance; GEN^R, Gentamicin resistance; $CHL^{R,}$ Chloramphenicol resistance; CIP^R, Ciprofloxacin resistance; STR^R, Streptomycin resistance; AMP^R, Ampicillin resistance; OFL^R, Ofloxacin resistance; TRI^R, Trimethoprim resistance and ERY^R, Erythromycin resistance

The 56 isolates were also screened for the presence of genes coding for eight antimicrobial resistance determinants (Fig. 3). Five isolates had none of the resistance genes investigated. Fifteen isolates had at least one, 11 isolates had a combination of two different genes and 25 carried at least three different resistance determinants (Table 3). All the eight genes were detected in one or more isolates.

Table 4 details the resistance of the isolates to each antibiotic class against the resistance genes detected. The detection of resistance genes was generally consistent with expressed resistance to antibiotic although some discrepancies were observed. For example, the *tetA* gene responsible for tetracycline resistance was the most common genotypic determinant, present in 41(73.2%) isolates and consistent with phenotypic resistance by 40 (71.4%) of the isolates. With regards to aminoglycoside resistance, the *aadA1* streptomycin resistance gene was also common in 21 of the 25 isolates that showed phenotypic resistance to streptomycin. On the other hand, the high frequency (32; 57.1%) of detection of the *aac(3)-IV* gene depicting resistance to gentamicin, however, contradicted the low phenotypic resistance to the antibiotic (5; 8.9%). Of the 9 chloramphenicol-resistant strains detected, 10 carried the *cmlA* gene while among the 10 isolates that showed resistance to the quinolone ciprofloxacin, the *qnrA* gene was detected in only four. A marked discrepancy in phenotypic resistance to the presence/detection of the genetic determinant was observed in resistance to the beta-lactam antibiotic ampicillin in which, out of the 47 isolates resistant to ampicillin, the bla_{CMY} gene was detected only in 17 isolates and the bla_{SHV} gene in 3 isolates. With regards to the Folate pathway inhibitor, the sulphonamide efflux resistance gene *sul1* was detected in 5 (8.6%) of the isolates as opposed to the 29 (51.8%) that showed phenotypic resistance.

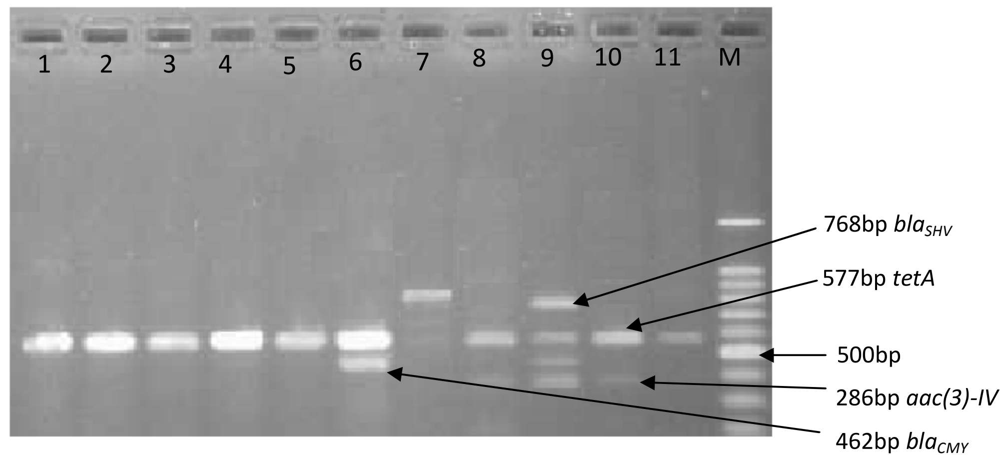

Fig. 3. Electrophoretic separation of amplified PCR products of multiplex PCR: *tetA* (lanes 1-6, 8-11), *bla_{CMY}* (lanes 6, 9), *bla_{SHV}* (lanes 7-9) *and aac(3)-IV* (lanes 8-10)

Table 3. Genotypic antimicrobial resistance profiles

Number of genes detected	Genes detected	Genotypic pattern	Number of isolates (%)
0	-	G1	5 (8.9)
1	*tetA*	G2	7 (12.5)
	aadA1	G3	5 (8.9)
	aac(3)-IV	G4	3 (5.4)
2	*aadA1+tetA*	G5	2 (3.6)
	aac(3)-IV+tetA	G6	5 (8.9)
	Sul1+tetA	G7	2 (3.6)
	qnrA+ aac(3)-IV	G8	1 (1.8)
	qnrA+bla_{CMY}	G9	1 (1.8)
3	*aadA1+ aac(3)-IV+tetA*	G10	5 (8.9)
	aadA1+sul1+tetA	G11	1 (1.8)
	Bla_{SHV}+ aac(3)-IV+tetA	G12	1 (1.8)
	aac(3)-IV+ bla_{CMY}+tetA	G13	3 (5.4)
	Sul1+aac(3)-IV+tetA	G14	2 (3.6)
4	*aac(3)-IV+ bla_{CMY}+cmlA+tetA*	G15	4 (7.1)
	qnrA+aac(3)-IV+ bla_{CMY}+tetA	G16	1 (1.8)
	aadA1+aac(3)-IV+ bla_{CMY}+tetA	G17	1 (1.8)
	aadA1+ Bla_{SHV}+aac(3)-IV+tetA	G18	1 (1.8)
	aadA1+bla_{CMY}+cmlA+tetA	G19	1 (1.8)
5	*Bla_{SHV}+aac(3)-IV+bla_{CMY}+cmlA+tetA*	G20	1 (1.8)
	aadA1+aac(3)-IV+ bla_{CMY}+cmlA+tetA	G21	3 (5.4)
6	*aadA1+qnrA+aac(3)-IV+ bla_{CMY}+cmlA+tetA*	G22	1 (1.8)
Total			56 (100)

4. DISCUSSION

Antimicrobial resistance can emerge through indiscriminate use of antibiotics in animals, with the subsequent spread of resistance among the bacteria [19]. This has influenced the frequency of multidrug-resistant bacteria [11]. These drug-resistant pathogenic bacteria can spread through the food chain to humans leading to treatment failure. This might explain why despite the limited use of antibiotics in the treatment of human *E. coli* O157 infections, mark resistance to commonly used drugs in human and veterinary medicine is still being reported. This has been recognized by the WHO [20] as a serious global human and animal health problem. Our susceptibility results showed no antimicrobial had 100% activity and only three isolates were

Table 4. Detection of the antimicrobial resistance genes in the *E. coli* O157:H7 isolates

Resistant phenotype	Number of isolates x/56 (%)	Genes detected	
		Gene	Frequency in isolates (n=56) (%)
Streptomycin resistance	25 (46.6)	*aadA1*	21 (37.5)
Gentamicin resistance	5 (8.9)	*aac(3)-IV*	32 (57.1)
Folate pathway inhibitor	29 (51.8)	*sul1*	5 (8.6)
Ampicillin (Beta-lactams) resistance	47 (83.9)	bla_{SHV}	3 (5.4)
		bla_{CMY}	17 (30.4)
Chloramphenicol resistance	9 (16.1)	*cmlA*	10 (17.9)
Tetracycline resistance	40 (71.4)	*tetA*	41 (73.2)
Fluoroquinolones resistance	10 (17.9)	*qnrA*	4 (7.1)

susceptible to all the antibiotics. The development of resistance to these drugs by *E. coli* poses a major challenge in both human and veterinary medicine as they are broadly used in the treatment of patients and in veterinary practice. In agreement with van den Bogaard and Stobberingh [21], this is particularly worrisome as *E. coli* is a reservoir of antimicrobial resistance genes which could be spread horizontally to other pathogenic bacteria including other members of the family Enterobacteriaceae. Investigating the prevalence of antimicrobial resistant *E. coli* isolates, therefore, can facilitate risk assessment of infection and the choice of effective antimicrobial agents in clinical settings.

Ampicillin, tetracycline and trimethoprim each showed less than 50% activity. These findings are in line with numerous reports [22,23,24] on high antimicrobial resistance to these drugs with most reports citing their use as a growth promoter and in routine chemoprophylaxis among livestock as well as self-medication in humans [25] as the major causes. Tetracycline and penicillin (ampicillin) are first-line drugs which are routinely prescribed or readily purchased over the counter for self-medication [26]. The development of antimicrobial resistance might limit their use leading to treatment failure and onset of complications. In agreement with Reuben and Owuna [24], streptomycin resistance was below average, and this could be probably due to the relatively less exposure to the antibiotic as a result of its discouraged usage and the fact that it is usually administered intravenously, which restricts its indiscriminate use [27]. The quinolones ciprofloxacin and ofloxacin, the aminoglycoside gentamicin and the phenicol chloramphenicol all showed great activity, agreeing with the findings of Iwu et al. [28] that they are the drug of choice for *E. coli* O157 infections.

Multiple antimicrobial resistance can develop as a result of antimicrobial selection pressure in livestock or humans [29]. Multidrug resistance, the resistance of an isolate to more than two antimicrobials was a common phenomenon in this study. This was observed in 33 of the 56 isolates with 17 different phenotypic multidrug resistance profiles noted. Tetracycline and ampicillin resistance was detected in almost all the phenotypic resistance patterns observed (Table 2). These results are consistent with other findings [3,27] and are linked to various aspects including the indiscriminate use of antibiotics in food-producing animals [30]. These multidrug-resistant bacteria are either disseminated to humans through food [31] or are shed into the environment by cattle, leading to a widespread dissemination of antibiotic-resistant genes to the resident bacteria in the environment [32].

According to Munita et al. [9] and Schroeder et al. [33], antimicrobial resistance can occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation, and or conjugation. In this study, we also identified the genetic determinants implicated in drug resistance, and these provided important clues in explaining the phenotypic resistance trends observed. Eight different resistance genes were searched in the 56 isolates and the results were generally consistent with the resistant phenotypes observed. To begin with, the high phenotypic resistance seen in tetracycline (40; 71.4%) could be associated to the detection of *tetA* gene (41; 73.2%) among the isolates and is in agreement with the findings of Messele et al. [34]. Of the 25 streptomycin-resistant isolates, the aminoglycoside resistant gene *aadA1* was found in 21 (84%) isolates, and was similar to other previous studies [35,36] where it was hypothesised that, in cases where the *aadA1* gene was not identified, streptomycin

resistance may be due to other resistance genes (*strA* or *strB* for instance) which were not screened in this study. On the other hand, one of the four classes of the aac(3) acetyltransferases, *aac(3)-IV* associated with gentamicin resistance in *E. coli* [36] was detected in 32 (57.1%) of the 56 isolates while only 5 of these showed phenotypic resistance to gentamicin. Similar contrasting findings have been reported by Yue and Xiu-Ying [37] in which the *aac(3)-II* was detected in 12 *E. coli* O157 isolates with none showing resistance to gentamicin. Based on similar findings [38,39], it is possible that resistance genes may not be expressed or expressed in a low level in isolates and the search for antimicrobial resistance genes should not only be limited to phenotypically resistant isolates. The marked discrepancy observed in phenotypic resistance to the presence/detection of the genetic determinant to the beta-lactam antibiotic ampicillin could be due to the fact that the isolates carry other or even novel genetic resistance determinants. This, considering the fact that ampicillin is a commonly used antibiotic in animal production and is easily accessible over the counter. The over use of this drug may create several modes of resistance. We also detected the *cmlA*, *sul1, qnrA* and bla_{SHV} genes as previously reported [37,39], highlighting the high proliferation and dissemination of resistant genes in this region.

5. CONCLUSIONS

Escherichia coli O157 strains circulating in Cameroon have developed strategies for resistance to currently used drugs. These strains harbour a number of resistance genes and demonstrate a high prevalence of multidrug resistance to most of the antibiotics tested. These observations suggest that the indiscriminate use of antibiotic in the animal production line whether as therapeutics or prophylaxis imposes a selection pressure and the circulation of this pathogen in cattle poses an environmental and human risk.

CONSENT AND ETHICS APPROVAL

It is not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Buzby JC, Roberts T. The economics of enteric infections: Human foodborne disease costs. Gastroenterology. 2009;136:1851-1862.
2. Centres for Disease Control and Prevention. FoodNet surveillance report for 2008. Centers for Disease Control and Prevention, Atlanta; 2010.
3. Bekele T, Zewde G, Tefera G, Feleke A, Zerom Z. *Escherichia coli* O157:H7 in raw meat in Addis Ababa, Ethiopia: Prevalence at an abattoir and retailers and antimicrobial susceptibility. Int J of Food Contamination. 2014;1:4.
4. Griffin PM, Tauxe RV. The epidemiology of infections caused by and the associated haemolytic uremic syndrome. Epidemiology Review. 1991;13:60-98.
5. Gould LH, Demma L, Jones TF, Hurd S, Vugia DJ, Smith K, et al. Hemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection. Clin Infect Dis. 2009;49:1480–1485.
6. Nielsen EM, Skov MN, Madsen JJ, Lodal J, Jespersen JB, Baggesen DL. Verocytotoxin-producing *Escherichia coli* in wild birds and rodents in close proximity to farms. Applied and Environmental Microbiology. 2004;70:6944-6947.
7. Hogg RA, Holmes JP, Ghebrehewet S, Elders K, Hart J, Whiteside C, et al. Probable zoonotic transmission of verocytotoxigenic *Escherichia coli* O157 by dogs. Veterinary Record. 2009;164:304-305.
8. La Ragione RM, Best A, Woodward MJ, Wales AD. *Escherichia coli* O157:H7 colonization in small domestic ruminants. FEMS Microbiology Reviews. 2009;33: 394-410.
9. Munita JS, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2):481-511.
10. WHO guidelines on use of medically important antimicrobials in food-producing animals; 2017. Available:http://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia_guidelines/en/ (Accessed on 29 April 2018)

11. Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: A developing country-perspective. Front Microbiol. 2016;7:1881.
DOI: 10.3389/fmicb.2016.01881
12. Effler P, Isaäcson M, Arntzen L. First outbreak of *Escherichia coli* O157 in Africa in 1992. Emerg Infect Dis. 2001;7:812.
13. Mashood A, Minga U, Machugun R. Current epidemiologic status of enterohaemorrhagic *Escherichia coli* 0157:H7 in Africa. Chin Med J. 2006;119:217.
14. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, et al. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-like toxin-producing *E. coli* from dairy cattle. Journal of Clinical Microbiology. 1991;29:985–989.
15. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI, Wayne, PA 19087 USA. M100-S24; 2014.
16. Van TT, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. International Journal of Food Microbiology. 2008;124:217–223.
17. Randall LP, Cooles SW, Osborn MK, Piddock LJ, Wood-ward MJ. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. Journal of Antimicrobial Chemotherapy. 2004;53: 208–216.
18. Mammeri H, van de Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrobial Agents and Chemotherapy. 2005;49:71–76.
19. Vegad JL. Antimicrobial resistance: A threat to livestock production and a potential risk for public health. LOHMANN Information. 2016;50(2):18-23.
20. World Health Organisation. High levels of antibiotic resistance found worldwide, new data shows.
Available:http://www.who.int/news-room/detail/29-01-2018-high-levels-of-antibiotic-resistance-found-worldwide-new-data-shows
(Accessed 15 June 2018)
21. van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents. 2000;14:327–335.
22. Shitandi A, Sternesjö A. Detection of antimicrobial residues in Kenyan milk. J Food Saf. 2001;21:205–215.
23. Olatoye IO. The incidence and antibiotics susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Nigeria. African Journal of Biotechnology. 2010;9(8):1196-1199.
24. Reuben RC, Owuna G. Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. International Journal of Pharmaceutical Science Invention. 2013;2:38-44.
25. Chigor VN, Umoh JV, Smith IS, Igbinosa OE, Okoh IA. Multidrug resistance and plasmid patterns of *Escherichia coli* O157 and other *E. coli* isolated from diarrhoeal stools and surface waters from some selected sources in Zaria, Nigeria. Int J Environ Res Public Health. 2010;7:3831–3841.
26. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrobial Resistance and Infection Control. 2017;6:47.
27. Cheesbrough M. District laboratory practice in tropical countries, 2nd edn. Cambridge University Press, United Kingdom. 2000;151–220.
28. Iwu CJ, Jaja IF, Iweriebor BC, Obi LC, Okoh AI. Antibiotic resistance profiles of *Escherichia coli* O26, O145, and O157:H7 isolated from swine in the Eastern Cape Province, South Africa. Asian Pac J Trop Dis. 2017;7(9):553-559.
29. WHO. Antimicrobial Resistance Global Report on Surveillance. Geneva. Switzerland; 2014.
30. Chantziaras I, Boyen F, Callens B, Dewulf J. Correlation between veterinary antimicrobial use and antimicrobial resistance in food producing animals: A report on seven countries. J Antimicrob Chemother. 2014;69(3):827-34.
31. Lekshmi M, Ammini P, Kumar S, Varela MF. The food production environment and the development of antimicrobial resistance in human pathogens of animal origin. Microorganisms. 2017;1-15.

32. Thanner S, Drissner D, Walsh F. Antimicrobial resistance in agriculture. mBio. 2016;7(2):e02227-15.
33. Schroeder M, Brooks BD, Brooks AE. The complex relationship between virulence and antibiotic resistance. Genes. 2017;8: 39.
DOI: 10.3390/genes8010039
34. Messele YE, Abdi RD, Yalew ST, Tegegne DT, Emeru BA, Werid GM. Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. Ann Clin Microbiol Antimicrob. 2017;16:55.
DOI: 10.1186/s12941-017-0233-x
35. Chang LL, Chang TM, Chang CY. Variable gene cassette patterns of class 1 integron-associated drug-resistant *Escherichia coli* in Taiwan. Kaohsiung J Med Sci. 2007;23:273–280.
36. Costa D, Poeta P, Sáenz Y, Vinué L, Coelho AC, Matos M. Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. Microb Drug Resist. 2008;14:71–77.
37. Yue J, Xiu-Ying Z. Resistance patterns and detection of resistance genes in *Escherichia coli* isolated from diarrheic calves in Northeastern China. African Journal of Microbiology Research. 2013;7:389-397.
38. Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF. Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J Clin Microbiol. 2003;41:5224-5226.
39. Ryu SH, Lee JH, Park SH, Song MO, Park SH, Jung HW, et al. Antimicrobial resistance profiles among *Escherichia coli* strains isolated from commercial and cooked foods. Int J Food Microbiol. 2012;159(3):263-266.

© 2018 Akomoneh et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25280