

Original Research Article

Phenotypic and Genotypic Antimicrobial Resistance

**Profiles of *Escherichia coli* O157 Isolates from Cattle in
Cameroon**

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 extensive use of antibiotics in 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 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^RSTR^RAMP^RTRI^RERY^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.

INTRODUCTION

Food borne pathogens including *E. coli* O157 are significant contributors of 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].

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

57 MATERIALS AND METHODS

58 Fifty-six *E. coli* O157 isolates obtained from cattle across Cameroon at the abattoirs in Buea
59 between October 2015 and September 2016 were analysed in this study. Isolation of the *E.*
60 *coli* O157 strains was done through the pre-enrichment method for faecal specimen
61 recommended by Wells *et al.* [14]. Presumptive *E. coli* O157 isolates were confirmed
62 serologically using E. COLIPRO™ O157 latex agglutination test (Hardy diagnostics, USA)
63 and molecularly using PCR targeting *E. coli* *rfb*_{EO157} gene in the isolates. The susceptibility of
64 the isolates to antibiotic disks impregnated with streptomycin (10 µg), erythromycin (15 µg),
65 tetracycline (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), ofloxacin (5
66 µg), trimethoprim (5 µg) and gentamicin (10 µg) (Becton, Dickson, Fisher Scientific, USA) was done
67 using the Kirby-Bauer disc-diffusion technique as recommended by the Clinical and Laboratory
68 Standard Institute (CLSI) [15]. After incubation at 37 °C for 16 -18 hr on Mueller-Hinton agar (Murex
69 Biotec ltd, UK), the antibiotic inhibition zone diameters were measured and results obtained used to
70 classify isolates as being resistant, intermediate or susceptible to a particular antibiotic using standard
71 reference values by the CLSI [15]. The quality control strain used was *E. coli* American Type Culture
72 Collection (ATCC) 25922. The choice of the antibiotics was based on regular usage in human and
73 veterinary medicine, local availability, potential public health importance and recommendations from
74 the guideline of antimicrobial susceptibility testing from CLSI [15].

75 Multiplex PCR analysis was performed on all the 56 isolates to detect the antibiotic resistance genes
76 *aadA1* and *aac(3)-IV* which confers resistances to aminoglycosides, *sulI* for folate pathway inhibitors,
77 *bla_{SHV}* and *bla_{CMY}* for beta-lactam resistance, *cmlA* for chloramphenicol resistance, *tetA* for
78 tetracycline resistance and *qnrA* for resistance to the quinolones. Total DNA was extracted from
79 pelleted cells of freshly prepared broth cultures of *E. coli* O157 isolates using the QIAamp
80 DNA Mini Kit following the manufacturer's instructions (QIAGEN, Germany). The eluted
81 DNA was held at -20 °C until used for PCR analyses. A 25 µL total individual reaction volume
82 was set up comprising 5 µL template DNA, 12.5 µL of 2X master mix (TopTaq™ Master Mix,
83 Qiagen, Hilden, USA), 0.5 µL of each primer from a working solution of 20 µM (final concentration
84 of 0.4 µM) and nuclease-free water to make up the total volume. Primer sets used for the
85 amplification of the different genes were as previously described [*aadA1* (*aadA1f* and *aadA1r*),
86 *aac(3)-IV* (*aac(3)-IVf* and *aac(3)-IVr*), *sulI* (*sul1f* and *sul1r*), *bla_{SHV}* (*bla_{SHVf}* and *bla_{SHVr}*), *bla_{CMY}*
87 (*bla_{CMYf}* and *bla_{CMYr}*), *cmlA* (*cmlAf* and *cmlAr*) [16], *tetA* (*tetAf* and *tetAr*) [17] and *qnrA* (*qnrAf* and
88 *qnrAr*) [18]]. Unless otherwise stated, initial denaturation and final extension for all PCR runs were
89 set at 95 °C for 15 min and 72 °C for 10 min respectively. Multiplex PCR targeting the *aac(3)-IV*,
90 *bla_{CMY}*, *cmlA* and *tetA* genes was optimized at 30 cycles of 94 °C for 1 min, 56 °C for 1 min and 72
91 °C for 1 min. Multiplex PCR targeting the *aadA1*, *sulI*, *bla_{SHV}* and *qnrA* genes had the same cycling
92 conditions as above except for the annealing temperature that was set at 55 °C. PCR products were
93 separated on 1.5% agarose gel stained with SYBR safe DNA Gel Stain (Invitrogen, Thermo Fisher
94 Scientific, USA) and visualised under UV light using a Gel Documentation-XR (BIORAD, Hercules,
95 CA).

96 RESULTS

97 The susceptibility of the 56 *E. coli* O157 isolates to the nine antibiotics showed no antimicrobial had
98 100% activity on all the isolates (Table 1). Three isolates were susceptible to all the antimicrobials
99 while 53 were resistant to one or more drug. Of the nine antibiotics, ofloxacin demonstrated the best
100 activity (55; 98.2%) against the isolates with no resistance noted, followed by gentamicin (51, 91.1%),

101 chloramphenicol (47, 83.9%), ciprofloxacin (46, 82.1%) and erythromycin (33, 58.9%). Ampicillin
102 was the least active drug with only 7 (12.5%) isolates susceptible.

103 **Table 1: Susceptibility of isolates to different antibiotics**

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

104
105 Based on the phenotypic resistance patterns observed within the 53 isolates that were resistant to one
106 or more antimicrobials, 24 phenotypic resistance profiles (A1-A24) were generated. The phenotypic
107 resistance profiles showed 20 (37.7%) of the 53 resistant *E. coli* O157 were resistant to one or two
108 antibiotics while 33 (62.3%) were multidrug resistant (resistant to three or more antibiotics) (Table 2).
109 The isolates that were resistant to one or two antibiotics comprised seven profiles (A1-A7) with six
110 isolates resistant to ampicillin alone while resistance to both ampicillin and tetracycline was common
111 in five isolates. The multidrug resistant isolates comprised 17 profiles (A8-A24) with resistance
112 profile A20 as the most prevalent shared by nine isolates. The phenotypic resistance profile A24 was
113 common to five isolates while the other phenotypic resistance patterns were shared by three or less
114 isolates (Table 2).

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

Phenotypic pattern	Antimicrobial resistance profiles	Isolates (%) (n = 53)
<i>Resistant to one or two antibiotics</i>		
A1	TRI ^R	1 (1.9)
A2	TET ^R	3 (5.7)
A3	AMP ^R	6 (11.3)
A4	AMP ^R TRI ^R	3 (5.7)
A5	TET ^R AMP ^R	5 (9.4)
A6	TET ^R GEN ^R	1 (1.9)
A7	CIP ^R AMP ^R	1 (1.9)
<i>Multidrug resistant</i>		
A8	STR ^R AMP ^R TRI ^R	1 (1.9)
A9	TET ^R STR ^R AMP ^R	1 (1.9)
A10	TET ^R CHL ^R AMP ^R	1 (1.9)
A11	TET ^R AMP ^R TRI ^R	2 (3.8)
A12	AMP ^R TRI ^R ERY ^R	1 (1.9)
A13	TET ^R GEN ^R AMP ^R	1 (1.9)
A14	TET ^R STR ^R AMP ^R ERY ^R	2 (3.8)
A15	TET ^R STR ^R AMP ^R TRI ^R	2 (3.8)
A16	TET ^R AMP ^R TRI ^R ERY ^R	2 (3.8)
A17	TET ^R GEN ^R CIP ^R AMP ^R	1 (1.9)
A18	TET ^R STR ^R TRI ^R ERY ^R	1 (1.9)
A19	TET ^R GEN ^R STR ^R AMP ^R ERY ^R	1 (1.9)
A20	TET ^R STR ^R AMP ^R TRI ^R ERY ^R	9 (17.0)
A21	TET ^R CHL ^R CIP ^R STR ^R AMP ^R TR ^R	1 (1.9)
A22	TET ^R CHL ^R CIP ^R STR ^R AMP ^R ERY ^R	1 (1.9)
A23	GEN ^R CHL ^R CIP ^R STR ^R AMP ^R TRI ^R ERY ^R	1 (1.9)
A24	TET ^R CHL ^R CIP ^R STR ^R AMP ^R TRI ^R ERY ^R	5 (9.4)
Total		53

116 TET^R, Tetracycline resistance; GEN^R, Gentamicin resistance; CHL^R, Chloramphenicol resistance;
 117 CIP^R, Ciprofloxacin resistance; STR^R, Streptomycin resistance; AMP^R, Ampicillin resistance; OFL^R,
 118 Ofloxacin resistance; TRI^R, Trimethoprim resistance and ERY^R, Erythromycin resistance.

119

120 The 56 isolates were also screened for the presence of genes coding for eight antimicrobial resistance
 121 determinants. 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 3: Genotypic antimicrobial resistance profiles

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

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 *sulI* was detected in 5 (8.6%) of the isolates as opposed to the 29 (51.8%) that showed phenotypic resistance.

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)	<i>aadA1</i>	21 (37.5)
Gentamicin resistance	5 (8.9)	<i>aac(3)-IV</i>	32 (57.1)
Folate pathway inhibitor	29 (51.8)	<i>sulI</i>	5 (8.6)
Ampicillin (Beta- lactams) resistance	47 (83.9)	<i>bla_{SHV}</i>	3 (5.4)
		<i>bla_{CMY}</i>	17 (30.4)
Chloramphenicol resistance	9 (16.1)	<i>cmlA</i>	10 (17.9)
Tetracycline resistance	40 (71.4)	<i>tetA</i>	41 (73.2)

Fluoroquinolones	10 (17.9)	<i>qnrA</i>	4 (7.1)
resistance			

143

144 DISCUSSION

145 According to Scott *et al.* [19], antimicrobial resistance can emerge through indiscriminate use of
 146 antibiotics in animals, with the subsequent spread of resistance among the bacteria. This has
 147 influenced the frequency of multidrug resistant bacteria [11]. These drug resistant pathogenic bacteria
 148 can spread through the food chain to humans leading to treatment failure. This might explain why
 149 despite the limited use of antibiotics in the treatment of human *E. coli* O157 infections, mark
 150 resistance to commonly used drugs in human and veterinary medicine is still being reported. This has
 151 been recognized by the WHO [20] as a serious global human and animal health problem. Our
 152 susceptibility results showed no antimicrobial had 100% activity and only three isolates were
 153 susceptible to all the antibiotics. The development of resistance to these drugs by *E. coli* poses a major
 154 challenge in both human and veterinary medicine as they are broadly used in the treatment of patients
 155 and in veterinary practice. In agreement with van den Bogaard and Stobberingh [21], this is
 156 particularly worrisome as *E. coli* is a reservoir of antimicrobial resistance genes which could be
 157 spread horizontally to other pathogenic bacteria including other members of the family
 158 Enterobacteriaceae. Investigating the prevalence of antimicrobial resistant *E. coli* isolates therefore
 159 can facilitate risk assessment of infection and the choice of effective antimicrobial agents in clinical
 160 settings.

161 Ampicillin, tetracycline and trimethoprim each showed less than 50% activity. These findings are in
 162 line with numerous reports [22, 23, 24] on high antimicrobial resistance to these drugs with most
 163 reports citing their use as growth promoter and in routine chemoprophylaxis among livestock as well
 164 as self-medication in humans [25] as the major causes. Tetracycline and penicillin (ampicillin) are
 165 first line drugs which are routinely prescribed or readily purchased over the counter for self-
 166 medication [26]. The development of antimicrobial resistance might limit their use leading to
 167 treatment failure and onset of complications. In agreement with Reuben and Owuna [27],

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 [28]. The quinolones ciprofloxacin and ofloxacin, the aminoglycoside gentamicin and the phenicol chloramphenicol all showed great activity, agreeing with the suggestions of Scheld [29] 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 [11, 19]. Multidrug resistance, the resistance of an isolate to more than two antimicrobials [30] 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, 31] and is linked to various aspects including the indiscriminate use of antibiotics in food producing animals [11]. These multidrug resistant bacteria are either disseminated to humans through food [32] or are shed into the environment by cattle, leading to a widespread dissemination of antibiotic resistant genes to the resident bacteria in the environment [13, 33].

According to Goodman *et al.* [9] and Metlay *et al.* [34], 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 Mooljunttee *et al.* [35]. Of the 25 streptomycin-resistant isolates, the aminoglycoside resistant gene *aadA1* was found in 21 (84%) isolates, and was similar to other previous studies [36, 37] 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* [37] 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 [38] in which the *aac(3)-II* was detected in 12 *E. coli* O157 isolates with none showing resistance to gentamicin. Based on similar findings [39, 40], 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*, *sull*, *qnrA* and *bla_{SHV}* genes as previously reported [38, 40], highlighting the high proliferation and dissemination of resistant genes in this region.

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 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.

Declarations

Consent for publication

Not applicable.

221 Availability of data and materials

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

224 Ethics approval and consent

225 Not applicable

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