Short Communication

Whole-genome sequencing of *Escherichia coli* isolated from contaminated meat samples collected from the Northern Region of Ghana reveals the presence of multiple antimicrobial resistance genes

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ABSTRACT

Objectives: This study reports the draft genomes of 14 *Escherichia coli* isolated from contaminated meat samples collected from the Northern Region of Ghana in order to determine the presence of antimicrobial resistance genes (ARGs) and genetic relatedness of the isolates.
Methods: The 14 *E. coli* isolates were of beef ($n = 3$), mutton ($n = 2$), chevon ($n = 3$), local chicken ($n = 3$) and guinea fowl ($n = 3$) origin. Whole-genome sequencing (WGS) was performed using an Illumina MiSeq sequencer. Double-disk synergy test (DDST) was also used to confirm the production of extended-spectrum β-lactamase (ESBL).
Results: WGS confirmed the identity of all of the *E. coli* isolates. All of the isolates contained at least one ARG and 57.1% (8/14) of them were multidrug-resistant (MDR). The *mdf(A)* gene was most common ARG, found in all 14 isolates. DDST confirmed the production of an ESBL in a MDR *E. coli* of guinea fowl origin. The sequence types (STs) varied among the *E. coli* isolates, with the exception of three isolates of ST155. Similarly, the serotypes of the *E. coli* isolates from meat sample were genetically diverse. Eleven different plasmid sequences were detected in ten of the isolates.
Conclusion: *E. coli* from contaminated meat sources in Ghana possessed multiple ARGs and were genetically diverse. To the best of our knowledge, this is the first work on WGS of *E. coli* isolated from various meat samples in the study area. The sequence data add to data base for epidemiological studies.
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1. Introduction

Escherichia coli is a Gram-negative, facultative, anaerobe bacterium that is globally distributed in the environment, food and intestines of animals [1,2]. Pathogenic strains can cause intestinal and extraintestinal infections. Human foodborne illnesses resulting from consumption of meat and meat products contaminated by *E. coli* have been reported. For instance, an outbreak of *E. coli* O157:H7 associated with the consumption of beef and veal tartares occurred among seven individuals, two of whom were hospitalised and one suffered severe haemolytic–uraemic syndrome, but none died [3]. *E. coli* are important foodborne pathogens in Ghana owing to their wide dissemination and association with foodborne illness. However, almost all cases of foodborne illness go unreported.

Resistance of foodborne pathogens to multiple antibiotics is a worldwide concern and a serious threat to public health. The occurrence of multidrug-resistant (MDR) bacteria has been linked to the use of antimicrobials in animal production as well as the treatment of animals and humans. The development and use of appropriate techniques for detecting antimicrobial resistance genes (ARGs) as well as characterisation of foodborne pathogens contribute to combating MDR foodborne pathogens. Molecular techniques, including PCR, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) etc., have evolved in their use to characterise and/or detect ARGs in foodborne pathogens [4,5]. However, the limitations of previous techniques required the development and use of a more robust technique such as whole-genome sequencing (WGS).

The Northern Region of Ghana dominates animal production in the country [6]. These animals, and to a lesser extent meats, are distributed throughout the country for human consumption and serve as an important protein source for Ghanaians and tourists. The processes involved in handling and slaughtering of animals in this region have been reported not to be of standard and thus

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Table 1
Whole-genome sequencing characterisation of 14 *Escherichia coli* isolated from various meat types in the Northern Region of Ghana.

Isolate ID	Sample type	MLST[a]	Predicted serotype[b]	ARG(s)[c]	Plasmid replicons[d]	pMLST[e] IncF	IncI1	No. of contigs (≥1000 bp)[f]	BioSample accession no.	Total length (≥1000 bp)[f]	N_{50}[f]	GC (%)[f]	Coverage
AB1	Beef	540	O11	*mdf(A)*				75	**SAMN09763741**	4 696 592	153 020	50.94	71.3
CB1	Beef	6646	H39	*mdf(A)*	IncFIB, IncFIC(FII), IncX1	[F89:?:B43]		59	**SAMN09763743**	5 422 503	382 930	50.47	68.2
NB12	Beef	7483	O8	*aph(3″)-Ib, aph(6)-Id, mdf(A), sul2, tet(B)*				77	**SAMN09763746**	4 906 611	268 931	50.75	60.8
AC1	Chevon	44	O162	*aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1B}}$*, qepA4, mdf(A), catA1, catA2, sul2, tet(B)*	Col156, IncFIA, IncFIB, IncFII	[F36:A6:B49]		111	**SAMN09763742**	4 895 238	131 969	50.8	70.1
CC6	Chevon	469	O8	*aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1B}}$*, mdf(A), sul2, tet(A)*	Col156, IncQ1			78	**SAMN09763744**	5 021 356	175 186	50.55	63.8
NC3	Chevon	1727	O88	*fosA7, mdf(A)*	IncFIA, IncFIB, IncFII	[F57:?:?]		62	**SAMN09763747**	4 892 649	242 084	50.76	67.1
SG6	Guinea fowl	69	O15	*aph(3″)-Ib, aph(6)-Id, bla*$_{\text{TEM-1B}}$*, mdf(A), sul2, sul1, tet(A), dfrA17*	Col440I, IncFIB, IncFII, IncQ1	[F10:?:B10]		116	**SAMN09763749**	5 369 103	223 226	50.57	48.1
TG1[g]	Guinea fowl	540	O9	*aph(6)-Id, aph(3″)-Ib, bla*$_{\text{CTX-M-15}}$*, bla*$_{\text{TEM-1B}}$*, qnrS1, mdf(A), sul2, tet(A), dfrA14*	IncY			86	**SAMN09763751**	4 709 891	202 963	50.98	70.9
TG5	Guinea fowl	7473	O61	*mdf(A)*	Col156, IncFII, IncI1			99	**SAMN09763752**	5 002 851	121 622	50.68	59.0
SLC2	Local chicken	155	H9	*aadA5, mdf(A), sul2, tet(A), dfrA17*	Col440I, p0111			80	**SAMN09763750**	4 640 562	145 148	50.73	56.3
TLC1	Local chicken	297	H9	*mdf(A)*	IncFII			90	**SAMN09763753**	5 256 202	163 744	50.61	53.2
TLC13	Local chicken	155	O132	*aadA5, mdf(A), sul2, tet(A), dfrA17*	p0111			82	**SAMN09763754**	4 638 724	145 148	50.73	56.6
CM4	Mutton	155	H40	*aadA5, mdf(A), sul2, tet(A), dfrA17*				68	**SAMN09763745**	4 662 045	182 574	50.61	59.0
NM11	Mutton	1141	O113	*qnrS1, mdf(A)*				78	**SAMN09763748**	4 799 745	136 711	50.4	56.7

MLST, multilocus sequence typing; ARG, antimicrobial resistance gene; pMLST, plasmid multilocus sequence typing.

[a] Using MLST v.2.0.
[b] Using SeroTypeFinder v.2.0.
[c] Using ResFinder v.3.0 (minimum percentage identity of 90% and minimum length of 60%).
[d] Using PlasmidFinder v.1.3 (minimum percentage identity of 95% and minimum length of 60%).
[e] Using pMLST v.2.0.
[f] Using Quast v.4.6.3.
[g] Confirmed to be extended-spectrum β-lactamase (ESBL)-producing bacteria by the double-disk synergy test using amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg), ceftazidime (30 μg) and cefotaxime (30 μg).

predispose meats to various bacterial contaminations [7,8]. However, it has not been clearly established whether these bacteria are harmful, genetically diverse, or possess resistance and virulence genes, among others. This study was therefore performed to determine the presence of ARGs and the genetic relatedness of *E. coli* isolated from various contaminated meat types in the Northern Region of Ghana.

2. Methods

A total of 14 *E. coli* isolated from beef (*n* = 3), mutton (*n* = 2), chevon (*n* = 3), local chicken (*n* = 3) and guinea fowl (*n* = 3) in 2016 from five different locations within the Tamale Metropolis (Ghana) were studied. The isolates were confirmed phenotypically using *E. coli* Latex Agglutination Test (Oxoid Ltd., Basingstoke, UK) and growth/gas production in Brilliant Green Bile (Oxoid Ltd.) with Durham tubes. *E. coli* were grown in Luria–Bertani broth overnight for DNA extraction.

DNA was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). DNA quality was checked using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and the concentration was determined using a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA). WGS was performed using an Illumina MiSeq sequencer (Illumina Inc., San Diego, CA) at a read length of 300-bp paired-end reads as previously described [9].

Raw reads were de novo assembled using the Shovill pipeline v.0.9.0 (https://github.com/tseemann/shovill) that uses SPAdes v.3.11.0 available in the GalaxyTrakr pipeline (https://www.galaxytrakr.org) [10]. Assembled sequence data were analysed using tools from the Center for Genomic Epidemiology (http://cge.cbs.dtu.dk/). The bacterial species identity of all 14 sequences was confirmed by KmerFinder v.3.0 [11]. PlasmidFinder v.1.3 [12] and ResFinder v.3.0 [13] were used to identify plasmids and ARGs, respectively. MLST v.2.0 [11] and pMLST v.2.0 [13] were used to determine the MLST profiles of the genome and plasmids, respectively.

A double-disk synergy test (DDST) was performed to confirm the production of extended-spectrum β-lactamases (ESBLs) using amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg), ceftazidime (30 μg) and cefotaxime (30 μg) [14].

3. Results

Results of WGS analysis for the *E. coli* isolates are shown in Table 1. The draft genomes ranged in size from 4 638 724 bp and 5 422 503 bp (mean 4 922 434 bp), in contigs that ranged in number between 59 and 116 (mean 82.93). The GC content also ranged between 50.4% and 50.98% (mean 50.68%). The serotypes of the *E. coli* isolates varied widely, as follows: O11 (*n* = 1); H39 (*n* = 1); O8 (*n* = 2); O162 (*n* = 1); O88 (*n* = 1); O15 (*n* = 1); O9 (*n* = 1); O61 (*n* = 1); H9 (*n* = 2); O132 (*n* = 1); H40 (*n* = 1); and O113 (*n* = 1). MLST types also varied remarkably and belonged to 11 different types, namely ST540 (*n* = 2), ST6646 (*n* = 1), ST7483 (*n* = 1), ST44 (*n* = 1), ST469 (*n* = 1), ST1727 (*n* = 1), ST69 (*n* = 1), ST7473 (*n* = 1), ST155 (*n* = 3), ST297 (*n* = 1) and ST1141 (*n* = 1).

ARGs were detected in all of the *E. coli* isolates. Of the 14 isolates, 8 (57.1%) were MDR (defined as resistance to three or more different classes of antibiotics). The most prevalent ARG was *mdf (A)* conferring resistance to macrolide–lincosamide–streptogramin B antibiotics, found in all 14 *E. coli* isolates. Other commonly found ARGs were those conferring resistance to sulfonamides (*n* = 9; 8 *sul2* and 1 *sul1*) in eight isolates, aminoglycosides (*n* = 13; 5 *aph(3′′)-Ib*, 5 *aph(6)-Id* and 3 *aadA5*) in eight isolates, tetracycline [*n* = 8; 6 *tet(A)* and 2 *tet(B)*] in eight isolates, β-lactams (*n* = 5; 4 *bla*$_{TEM-1B}$ and 1 *bla*$_{CTX-M-15}$) in four isolates and trimethoprim (*n* = 5; 4 *dfrA17* and 1 *dfrA14*) in five isolates. Resistance genes for

fluroquinolones (*qepA4* and *qnrS1*), fosfomycin (*fosA7*) and phenicols (*catA1* and *catA2*) were found in three, one and one *E. coli* isolates, respectively. *E. coli* TG1 of guinea fowl origin harboured the highest number of ARGs from different antimicrobial classes (seven different classes) with as many as nine ARGs. Moreover, the DDST confirmed the production of an ESBL by this isolate, which were predicted as *bla*$_{CTX-M-15}$ and *bla*$_{TEM-1B}$ by WGS. *E. coli* AC1 of chevon origin and *E. coli* SG6 of guinea fowl origin also harboured nine (from seven antimicrobial classes) and eight (from six antimicrobial classes) different ARGs, respectively.

Of the 14 *E. coli* isolates, 10 (71.4%) were found to contain one or more plasmid replicons, whereas 4 (28.6%) isolates did not find a match to any plasmid in the database. The four isolates without plasmid replicons included both of mutton origin. The plasmid replicon IncFII was the most common. *E. coli* AC1 (Col156, IncFIA, IncFIB, IncFII) of chevon origin and *E. coli* SG6 (Col440I, IncFIB, IncFII, IncQ1) of guinea fowl origin harboured four plasmid replicons.

4. Discussion

This study revealed that *E. coli* isolated from various contaminated meat types in the same geographical area were genetically diverse. The majority of the *E. coli* isolates also possessed multiple ARGs. Successful antibiotic therapy has been hampered immensely by the emergence of MDR bacterial isolates. This is worrying because meat consumers in the Tamale Metropolis are at risk of acquiring and/or harbouring MDR isolates. ARGs can also be transferred from one bacterium to another and/or cross-contaminate other food samples when exposed. The presence of plasmid replicons in some of the *E. coli* isolates suggests that they have the potential to pass on ARGs to other bacteria. The *mdf(A)* gene was most common, and bacteria expressing Mdf(A) from a multicopy plasmid demonstrate resistance to cationic or zwitterionic lipophilic compounds such as ethidium bromide, tetraphenylphosphonium, rhodamine, daunomycin, rifampicin, tetracycline and puromycin [15]. The *mdf(A)* gene also confers resistance to chemically unrelated, clinically important antibiotics such as chloramphenicol, erythromycin and certain aminoglycosides and fluoroquinolones [15]. The *bla*$_{CTX-M-15}$ ESBL gene was identified in *E. coli* strain TG1. ESBL-producing *E. coli* are highly resistant to numerous antibiotics, and infections by them are associated with higher mortality, longer length of hospital stay and increased health costs compared with infections with susceptible *E. coli* [16]. ESBL-producing *E. coli* can also persist in the farm environment for prolonged periods.

WGS correctly confirmed the identity of the *E. coli* isolates from the various meat types. The *E. coli* isolates were genetically diverse in their serotypes and MLST and also possessed multiple ARGs.

Nucleotide sequence accession number(s)

The sequence data of the 14 *E. coli* isolates have been submitted to GenBank with the BioProject no. **PRJNA484345**. The BioSample accession numbers are presented in Table 1.

Competing interests

None declared.

Ethical approval

Not required.

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