Genomic characterization of foodborne *Salmonella enterica* and *Escherichia coli* isolates from Saboba district and Bolgatanga Municipality Ghana

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Abstract

Salmonella enterica and *Escherichia coli* are well-known bacteria commonly associated with foodborne illnesses in humans and animals. Genomic characterization of these pathogens provides valuable insights into their evolution, virulence factors and resistance determinants. This study aimed to characterized previously isolated *Salmonella* (n = 14) and *E. coli* (n = 19) from milk, meat and its associated utensils in Ghana using whole-genome sequencing. Most of the *Salmonella* serovars (Fresno, Plymouth, Infantis, Give and Orleans) identified in this study are yet to be reported in Ghana. Most *Salmonella* isolates were pan-sensitive, but genes conferring resistance to fosfomycin (*fosA7.2*) and tetracycline (*tet(A)*) were detected in one and three isolates, respectively. Seven of the *Salmonella* isolates carry the IncI1-I(Gamma) plasmid replicon. Although antimicrobial resistance was not common among *Salmonella* strains, most (11/19) of the *E. coli* strains had at least one resistance gene, with nearly half (8/19) being multidrug resistant and carrying mobile elements. Three of the 19 *E. coli* strains belong to serovars commonly associated with enteroaggregative *E. coli* (EAEC) pathotype. While strains belonging to virulence-associated lineages lacked key plasmid-encoded virulence plasmids, several plasmid replicons were detected in most of the *E. coli* (14/19) strains. Food contaminated with these pathogens can serve as a vehicle for disease transmission, posing a significant public health risk and necessitating stringent food safety and hygiene practices to prevent outbreaks. Hence, there is need for continuous surveillance and preventive measures to stop the spread of foodborne pathogens and reduce the risk of associated illnesses in Ghana.

INTRODUCTION

Milk and meat are essential protein sources that constitute a significant and nutrient-rich component of human diets. However, their consumption is often associated with foodborne infections (1), particularly those caused by *Salmonella* and *Escherichia coli* (2-5). Salmonellosis is under-reported in Ghana, and only a few studies have investigated the plausible role of contaminated milk (6), meat, meat products, handlers' hands and associated surfaces such as knives, tables and aprons (7-9) in facilitating their transmission. Knowledge of food safety practices by key food handlers in Ghana has recently been reported to be suboptimal, as have food safety infrastructure and regulatory enforcement (10, 11). There have been a few reports of meat samples contaminated with *E. coli* in Ghana (11-14). Similarly, *E. coli* has been recovered from milk, milking utensils, faeces of lactating cow and milkers' hands (15). Some of

these *E. coli* strains harbour both virulence and antimicrobial resistance genes (ARGs), raising public health concerns. However, few of these strains have been thoroughly characterized.

There are public health and food safety implications of finding *Salmonella* and *E. coli* in food because they are invariably of faecal origin. Globally, in addition to contamination risks, *Salmonella* and *E. coli* sourced from milk and meat increasingly exhibit resistance to different classes of antibiotics that is commonly mediated by mobile elements (13, 16-18). The prevalence of multidrug-resistant (MDR) *Salmonella* and *E. coli* is also on the rise in clinical infections (19, 20). Notably, resistance to extended spectrum beta-lactams, trimethoprim/sulfamethoxazole, chloramphenicol and ciprofloxacin has been reported, often associated with plasmids that could mediate their spread, in both *Salmonella* and *E. coli* isolates from milk and retail meats in Ghana (6, 12, 14).

Various methods, including serotyping, antibiotic profiling, pulsed-field gel electrophoresis and whole genome sequencing, have been employed to elucidate the phenotypic and genotypic attributes of foodborne pathogens and to determine their interrelationships and connections to pandemic clones of interest (13, 21). Next-generation sequencing (NGS) technology, the most versatile and informative approach, has gained recent prominence (22, 23). NGS is now used by PulseNet to categorize foodborne diseases, enabling nuanced epidemiological investigations (24). Identification of virulence factors, antibiotic resistance genes, and serotypes are all possible by genomic analysis, which can also provide enhanced information on strain inter-relatedness, and therefore enable source attribution. Data on the genomic characterization of *Salmonella* and *E. coli* from milk and meat are few from low- and middle-income countries, including Ghana (7, 25). In light of this gap, this study aims to characterize the resistance, virulence and plasmid profile of previously isolated *Salmonella* and *E. coli* isolated from fresh different retail meats, milk, and associated samples (handler's hand swab, table, knife and faecal samples) in Saboba district and Bolgatanga Municipality of Ghana.

METHODS

Strains

A total of 33 bacterial isolates (14 *Salmonella* and 19 *E. coli* species) previously isolated from various fresh and ready-to-eat meats, meat sellers' tables, milk, milk-collecting utensils, milkers' hands and faeces of lactating cows were characterized for this study (Table 1) (8, 15, 26, 27). The isolates originated from markets and farms in Bolgatanga Municipality and Saboba District in Northern Ghana and were cryopreserved in 50% glycerol in Luria broth at -80°C.

Salmonella and *E. coli* identification

Salmonella isolates were initially confirmed using a latex agglutination kit for *Salmonella* (Oxoid Limited, Basingstoke, UK) and by PCR targeting the *invA* gene as described by Rahn et al. (1992) (28), using PCR oligonucleotides *invA139f* GTGAAATTATCGCCACGTTCGGGCAA and *invA141r* TCATCGCACCGTCAAGGAACC. PCR was performed using PuRe Taq Ready-To-Go PCR Beads (illustra^{TM}). The PCR cycle used an initial denaturation temperature of 95^{0}C for two minutes, followed by 35 cycles of denaturation at 95^{0}C for 30 seconds, annealing at 55^{0}C for 30 seconds and extension at 72^{0}C for two minutes, then a terminal extension at 72^{0}C for five minutes.

Visualization of the 284 bp amplicon was accomplished after electrophoresis on 1.5% (w/v) agarose gels stained with Gel red (biotium), using a UVP GelMax transilluminator and imager. *Salmonella* isolates positive for *invA* and *E. coli* isolates were biotyped with the Gram-negative (GN) test kit (Ref: 21341) on VITEK 2 systems (version 2.0, Marcy-l'Etoile, France, Biomérieux) according to manufacturer's instructions

Table 1: *E. coli* and *Salmonella* isolates characterized in this study

Id	Code	Species	Sample	Reference
GH-FA-M23_S31	M2 *3*	*Salmonella enterica*	Milk	(27)
GH-FA-M87_S6	M8 *1*	*Salmonella enterica*	Milk	(27)
GH-FA-FS24_S23	FS24	*Salmonella enterica*	Faecal Sample (Milking cow)	(27)
GH-FA-M25_S2	M25	*Salmonella enterica*	Milk	(27)
GH-FA-US15_S32	US15-2	*Salmonella enterica*	Utensil sample	(27)
GH-FA-FK4_S10	FK4	*Salmonella enterica*	Knife (Fresh meat)	(8)
GH-FA-FK4d_S7	FK4d	*Salmonella enterica*	Knife (Fresh meat)	(8)
GH-FA-RCH2_S11	Rch2 extra	*Salmonella enterica*	RTE Chicken	(8)
GH-FA-RG2_S29	RG2	*Salmonella enterica*	RTE Guinea fowl	(8)
GH-FA-RK4_S19	RK4	*Salmonella enterica*	Knife (RTE utensil)	(8)
GH-FA-RM4_S20	RM4	*Salmonella enterica*	RTE Mutton	(8)
GH-FA-RP5_S5	RP5	*Salmonella enterica*	RTE Pork	(8)
GH-FA-RP5D_S11	RP5D	*Salmonella enterica*	RTE Pork	(8)
GH-FA-RPSD_S30	RPSD	*Salmonella enterica*	RTE Pork	(8)
GH-FA-FS23_S22	FS23	*Escherichia coli*	Faecal Sample (milking cow)	(27)
GH-FA-US24_S14	US24	*Escherichia coli*	Utensil Sample	(15)
GH-FA-HS11_S26	HS11	*Escherichia coli*	Hand swab	(15)
GH-FA-HS3_S27	HS3	*Escherichia coli*	Hand swab	(15)
GH-FA-M9_S16	M9	*Escherichia coli*	Milk	(15)
GH-FA-US3_S21	US3	*Escherichia coli*	Utensil sample	(15)
GH-FA-HS12_S21	HS12	*Escherichia coli*	Hand Swab	(15)
GH-FA-M25D_S22	M25	*Escherichia coli*	Milk	(15)
GH-FA-FB4_S24	FB4	*Escherichia coli*	Fresh Beef	(26)
GH-FA-FC1_S23	FC1	*Escherichia coli*	Fresh Chicken	(26)
GH-FA-FH2_S15	FH2	*Escherichia coli*	Hand Swab (Fresh meat vendor)	(26)
GH-FA-FGS_S16	FG5	*Escherichia coli*	Fresh Guinea fowl	(26)
GH-FA-FM4_S34	FM4	*Escherichia coli*	Fresh Mutton	(26)
GH-FA-FT1_S17	FT1	*Escherichia coli*	Table swab (Fresh meat)	(26)
GH-FA-RB4_S24	RB4	*Escherichia coli*	RTE Beef	(26)
GH-FA-RH4_S3	RH4	*Escherichia coli*	Hand swab (RTE meat Vendor)	(26)
GH-FA-RT3_S6	RT3	*Escherichia coli*	Table swab (RTE meat)	(26)

GH-FA-RT3_S29	RT3d	*Escherichia coli*	Table swab (RTE meat)	(26)
GH-FA-RCLI_S18	Rch1	*Escherichia coli*	RTE Chevon	(26)

Key: RTE: Ready-to-eat – meats sampled in prepared form

DNA Extraction, Library Preparation and Whole Genome Sequencing

Genomic DNA of the isolates was extracted using Wizard DNA extraction kit (Promega; Wisconsin, USA) in accordance with manufacturer's protocol. Using a dsDNA Broad Range quantification assay, the extracted DNA was quantified on a Qubit fluorometer (Invitrogen; California, USA). dsDNA libraries were prepared using NEBNext Ultra II FS DNA library kit for Illumina with 96-unique indexes (New England Biolabs, Massachusetts, USA; Cat. No: E6609L). DNA libraries was quantified using dsDNA High Sensitivity quantification assay on a Quibit fluorometer (Invitrogen; California, USA) and fragment length analysed with the Bioanalyzer (Agilent). Denatured libraries were sequenced on an Illumina MiSeq (Illumina, California, USA). The raw sequence reads were *de novo* assembled using SPAdes v3.15.3 (29) according to GHRU protocols (https://gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/assembly).

Sequence Typing of *Salmonella* and *E. coli* Genomes

Sequence reads were deposited in the *Salmonella* and *E. coli* database for *Salmonella* and *E. coli* respectively on EnteroBase (30) and analyzed using publicly available tools that we have previously validated (31, 32). Multi-locus sequence types (MLST) for the isolates were determined using ARIBA (33). Novel ST strains were assigned ST using EnteroBase (30). The *Salmonella* genome assemblies were analysed using the *Salmonella* In-Silico Typing Resource (SISTR) for the prediction of serovars and serogroups (34) (https://github.com/phac-nml/sistr_cmd), while the serotyping of the *E. coli* genome was done using ECtyper (35).

Identification of AMR, Plasmids and Virulence genes

PlasmidFinder (36) was utilized to identify plasmid replicons that were present in the assembled genomes. AMRFinderPlus v3.10.24 (37) and its associated database (version 2022-04-04.1) were used to predict the antimicrobial resistance genes carried by the isolates and the drug classes to which they probably conferred resistance. Using ARIBA (33) and the virulence factor database (VFDB), we were also able to identify the virulence genes that were present in the isolates.

Single Nucleotide Polymorphism (SNP) Calling and Phylogenetic analysis

For phylogenetic analysis, reference sequences for the *Salmonella* and *E. coli* genomes were objectively selected from the National Center for Biotechnology Information Reference Sequence (RefSeq) database (https://www.ncbi.nlm.nih.gov/refseq/) using BactinspectorMax v0.1.3 (https://gitlab.com/antunderwood/bactinspector). The selected references were the *S. enterica* subsp. enterica serovar Fresno strain (assembly accession: GCF_003590695.1) and the *E. coli* O25b:H4-ST131 strain (assembly accession: GCF_000285655.3). The sequence reads for each species were then mapped to the chromosome of the reference using BWA (v0.7.17) (38) and variants were called and filtered using bcftools (v1.9) (39) as implemented in the GHRU SNP

138 phylogeny pipeline (https://gitlab.com/cgps/ghru/pipelines/snp_phylogeny). Variant positions
139 were concatenated into a pseudoalignment and used to generate a maximum likelihood tree
140 using iqtree (v1.6.8) (40). SNP distances between the genome pairs were calculated using snp-
141 dists v.0.8.2 (https://github.com/tseemann/snp-dists).
142
143 **Data availability**
144 The sequence reads were deposited in the European Nucleotide Archive with the study
145 accession PRJEB58695 (Supplementary table 1).
146
147 **RESULTS**
148 ***Salmonella* Serotypes, Sequence Types (STs), Virulence Factors and Phylogeny**
149 We used SISTR software to predict the serovars of the 14 *Salmonella* strains characterized in
150 this study from whole genome sequence. The most common serotype was Fresno (n=6)
151 followed by Give (n=3), Orleans (n=2), Plymouth (n=1), Agona (n=1) and Infantis (n=1). The *S.*
152 Fresno and *S.* Orleans isolates were from previously unreported sequence types, now
153 designated ST10742 and ST10465 respectively (Supplementary table 2). As shown in Figure 1,
154 all the isolates from ready-to-eat pork, mutton and chicken belong to serovar Fresno. *S.* Fresno
155 isolates were also isolated from a meat vendor's knife, as was *S.* Orleans. The three milk
156 isolates, which were all from Saboba, belong to the serovars Plymouth (ST565), Give (ST516)
157 and Agona (ST13) (Figure 1). Two more ST516 *S.* Give isolates were recovered from the faeces
158 of milking cow and from a milking utensil.
159
160 Phylogenetic analysis of the 14 *Salmonella* isolates from this study showed that all *S.* Fresno
161 isolates, irrespective of source, clustered together and differed by < 3 SNPs. The two *S.* Orleans
162 isolates were identical (0 SNPs) and the three *S.* Give isolates were also identical, with the
163 isolates originating from milk, faeces of milking cow and from a milking utensil, also in Saboba.
164

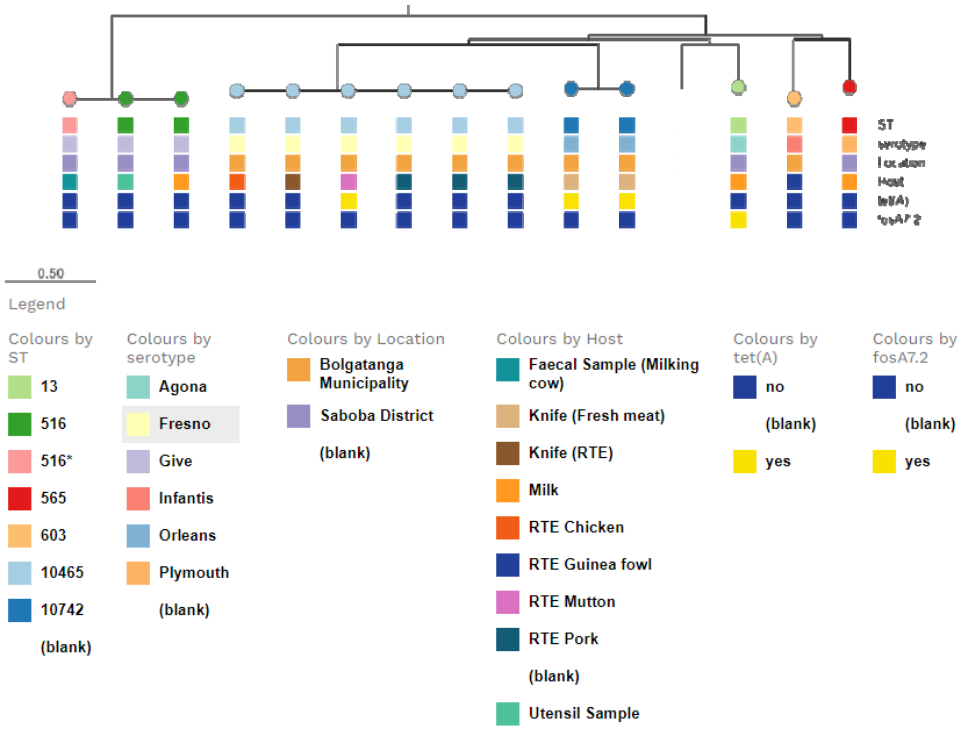


Figure 1: Core genome SNP-based Maximum likelihood tree showing phylogenetic relationships among strains sequenced in this study. The matrix below the tree shows the sequence types, serotypes, location of isolation (Bolgatanga municipality or Saboba district) as well as the source/host sample and presence or absence of the *tetA* and *fosA* resistance genes.

All *Salmonella* isolates harboured curli (*csg*) genes as well as *bcf*, *fim* and *ste* fimbrial operons and ten of them, representing all serovars except *S.* Give and Plymouth, carried long polar fimbriae (*lpf)* genes. The *S.* Infantis and *S.* Agona strains carried *ratB* and *shdA*. Type III secretion system effector genes, such as: *inv*, *org*, *prg*, *sif*, *spa*, *sse*, *ssa* and *sop* were detected in all the isolates while *avr* was present in 57.1% (8/14) of the isolates and only one isolate harbours *gogB*. Four of the isolates also encode the cytholethal distending toxin gene, *cdtB* (Supplementary table 3).

Plasmid Replicons and ARG Profiles of *Salmonella*

The *Salmonella* isolates were largely pan-sensitive but genes conferring resistance to fosfomycin (*fosA7.2*) and tetracycline (*tet(A)*) were detected in one and three isolates respectively. Both *S.* Orleans isolates and one of the *S.* Fresno, from ready-to-eat mutton carried *tetA*, along with an IncI1-I(Gamma) plasmid replicon, which was also seen in six other, *tetA*-negative strains. Interestingly, the IncI1-I(Gamma) plasmid replicon was detected in all isolates from Bolgatanga municipality, irrespective of serovar, and no isolate from Saboba district harboured this plasmid. The fosfomycin resistance gene was found in the *S.* Agona genome, in which no plasmid replicons were detected (Figure 1).

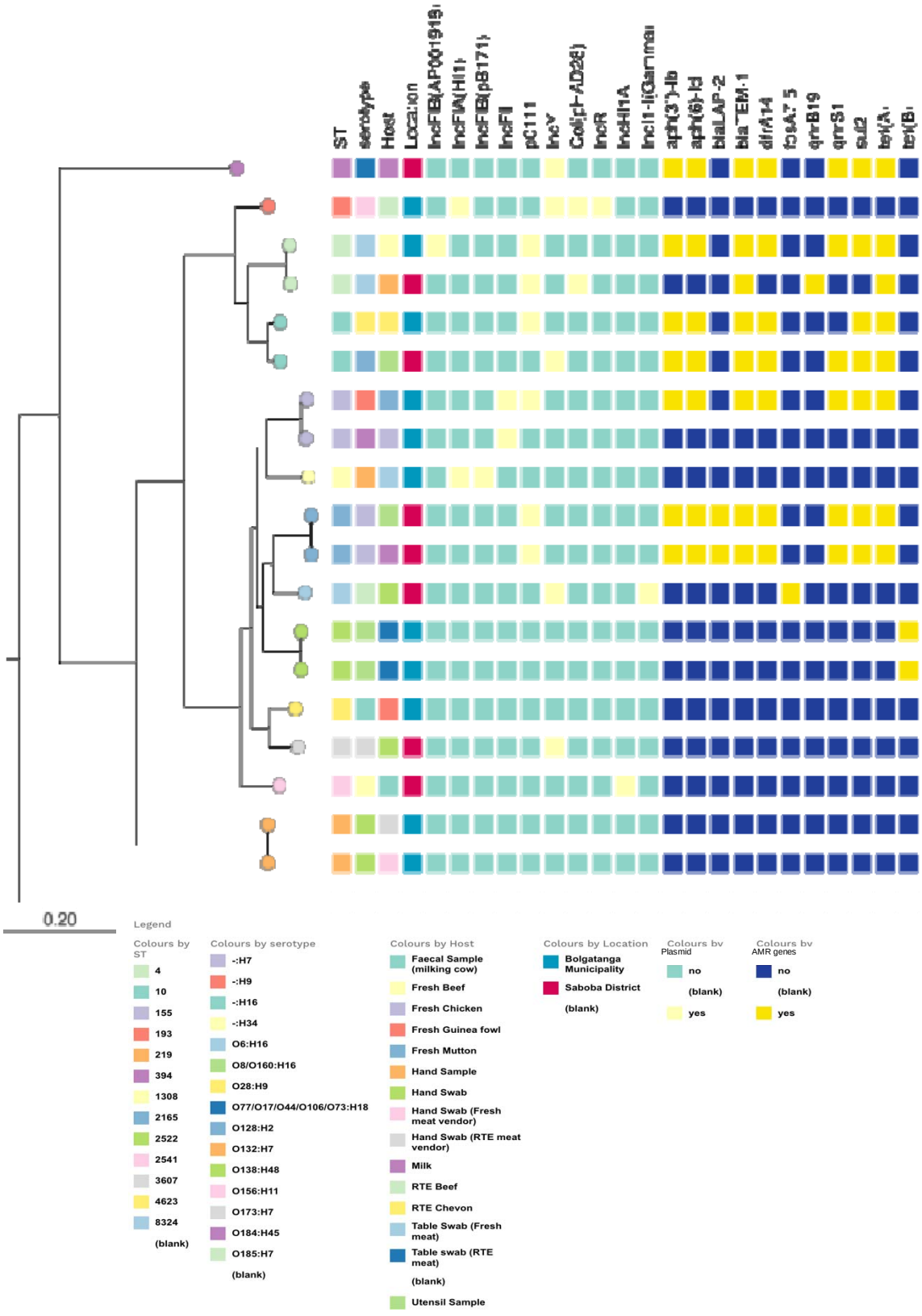
***E. coli* Serotypes, Virulence factors and Phylogeny**

A total of 19 *E. coli* isolates were identified. *E. coli* serotyping using the ECtyper revealed that the most common serotypes among the *E. coli* isolates were -:H7 (n=2), O138:H48 (n=2), O6:H16 (n=2), and O8/O160:H16 (n=2). A number of these serovars and STs are associated with pathogenicity, notably O6:H16 (41), as well as O8/O160:H16 and O77/O17/O44/O106/O73:H18 (ST394; (42-44)). The strains belonging to these lineages lacked the defining virulence genes of the respective pathotypes but did contain accessory virulence genes, as shown in supplementary table 4.

Irrespective of whether they belonged to a lineage commonly associated with virulence, most of the isolates contained a range of adhesins and iron utilization genes. *E. coli* extracellular protein (ECP) export pathway (*ecp/yag*) and *ompA* and type I fimbriae-encoding operon, *fim*, encoding genes seen in most *E. coli* genomes, were present in 94.7% (18/19) of *E. coli* isolates. Fimbriae encoding gene, *f17d*, often seen in enterotoxigenic *E. coli* recovered from animals, was present in the two O8/O160:H16 isolates and an O-:H7 isolate.

The phylogenetic analysis of the 19 *E. coli* isolates from this study and a reference genome (NZ_HG941718.1) based on SNP is presented in Figure 2. The range of isolates was broader than with *Salmonella* but closely related pairs of isolates belonging to the same serovar, and ST were found in three instances. Very similar (2347 SNPs) O6:H16 isolates were recovered from different food preparation table samples in Bolgatanga. One of the two isolates from milk in Saboba belonged to ST2165 and was identical (0 SNPs) to a Saboba ST2165 utensil isolate. The two ST4 isolates from fresh beef and a cow milker's hand differed by 2347 SNPs and are unlikely to be connected.

(a)


ST
serotype
Host
Location
IncFIB(AP001918)
IncFIA(HI1)
IncFIB(pB171)
IncFII
pC111
IncY
Col(pHAD28)
IncR
IncHI1A
IncI1-I(Gamma)
aph(3'')-Ib
aph(6)-Id
blaLAP-2
blaTEM-1
dfrA14
tetA(B)5
qnrB19
qnrS1
sul2
tet(A)
tet(B)
0.20
Legend
Colours by ST
4
10
155
193
219
394
1308
2165
2522
2541
3607
4623
8324
(blank)
Colours by serotype
-:H7
-:H9
-:H16
-:H34
O6:H16
O8/O160:H16
O28:H9
O77/O17/O44/O106/O73:H18
O128:H2
O132:H7
O138:H48
O156:H11
O173:H7
O184:H45
O185:H7
(blank)
Colours by Host
Faecal Sample (milking cow)
Fresh Beef
Fresh Chicken
Fresh Guinea fowl
Fresh Mutton
Hand Sample
Hand Swab
Hand Swab (Fresh meat vendor)
Hand Swab (RTE meat vendor)
Milk
RTE Beef
RTE Chevon
Table Swab (Fresh meat)
Table swab (RTE meat)
(blank)
Utensil Sample
Colours by Location
Bolgatanga Municipality
Saboba District
(blank)
Colours by Plasmid
no
(blank)
yes
Colours by AMR genes
no
(blank)
yes

(b)

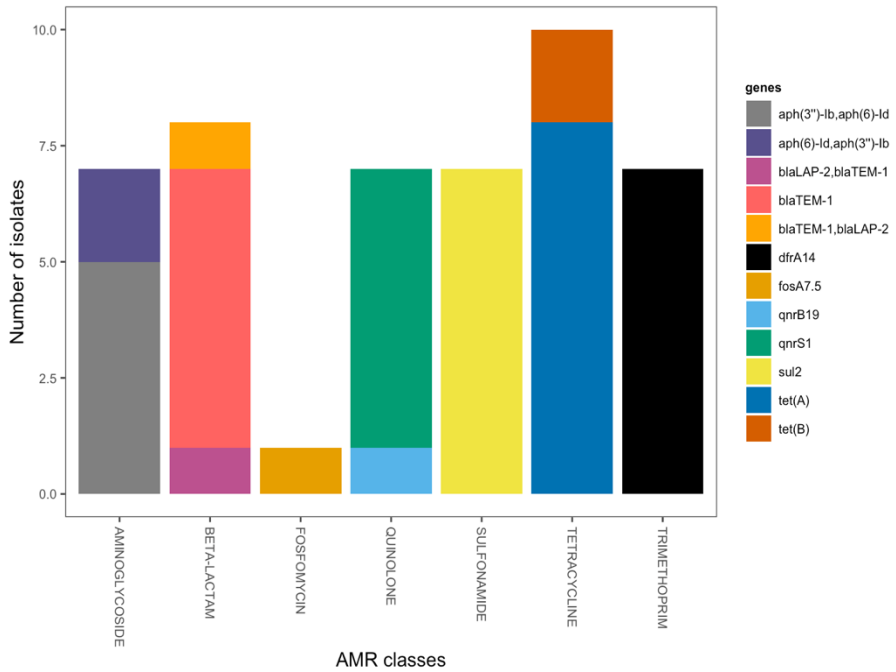


Figure 2: Phylogeny, plasmid replicons and resistance genes of *E. coli* isolates. (a). SNP-based phylogenetic tree of the *E. coli* isolates showing the range of sequence types, serovars, plasmid replicons and resistance genes detected (b) AMR determinants detected in *E. coli* and the resistance they confer

Plasmid Replicons and ARG Profiles of *E. coli*

Antimicrobial resistance determinants present in the *E. coli* isolates include those encoding resistance to aminoglycosides (*aph(3")-Ib*, *aph(6)-Id*, *aph(6)-Id*, *aph(3")-Ib*), beta-lactams (bla_{LAP-2}, bla_{TEM-1}), fosfomycin (*fosA7.5*), quinolones (*qnrB19*, *qnrS1*), sulfonamide (*sul2*), tetracycline (*tet(A)*, *tet(B)*) and trimethoprim (*dfrA14*) (Figure 2). At least 3 antimicrobial resistance genes (ARGs) which confer resistance to different classes of antibiotics were present in 8 isolates. Three isolates carried one ARG each while 8 isolates had no ARGs. Six strains carried the genes *aph(3'')-Ib, aph(6)-Id, blaTEM-1, dfrA14, sul2, qnrS1* and *tet(A).* The *dfrA14-qnrS1-tet(A) resistance* gene combination has previously been reported from Nigeria, being part of a transposon transmitted in an IncX plasmid (45). In this study however, IncX replicons were not detected. The most common plasmid replicon type detected among the *E. coli* isolates was pO111 (n=6), originally described in an *E. coli* virulence plasmid and found in the aforementioned strains belonging to virulence-associated lineages. The other plasmid replicon types detected were IncY (n=5), IncFII (n=2), IncFIA(HI1) (n=2), Col(pHAD28) (n=2), IncFIB(pB171) (n=1), IncR (n=1), IncHI1A (n=1) and IncI1-I(Gamma), which was common among the *Salmonella*, (n=1) (Figure 2). All multidrug resistant *E. coli* strains in this study encode pO111 or IncY replicons.

DISCUSSION

Salmonella and *E. coli* are the main causes of bacterial foodborne illnesses in Ghana (46). Retail meat, along with milk and their products are recognized as primary sources of foodborne

245 Salmonellosis (12) and *E. coli* infection (7). Post-cooking handling practices, exposure during the
246 points of sale, and suboptimal meat storage conditions collectively contribute to an increased
247 presence of both pathogenic and spoilage bacteria in ready-to-eat (RTE) meat (47). Within
248 Ghana, food safety has been inadequately studied in the northern region (10). In this study, we
249 characterized the genomes of 14 *Salmonella* and 19 *E. coli* previously isolated from Saboba
250 district and Bolgatanga Municipality in northern Ghana.
251
252 The *Salmonella* serovars (Fresno, Plymouth, Infantis, Give and Orleans) identified in this study
253 are yet to be reported in Ghana, but Guinee et al. (1961) (48) isolated *S.* Agona from cattle in
254 Ghana and *S.* Give (but ST524, different from ST516 in this study) has been reported from beef
255 in Nigeria (49). Isolation of *S.* Infantis from retail poultry meat has also been reported in
256 Ecuador (50), Belgium (51) and Italy (52) and all the serovars detected in this study have been
257 implicated in human infections. The identification of *Salmonella* serovars not previously
258 documented in the country in meat and milk products highlights the need for heightened
259 surveillance and preventive measures to curb the spread of foodborne pathogens and reduce
260 the risk of associated illnesses.
261
262 Unlike *Salmonella*, not all *E. coli* are potential pathogens. However, *E. coli* serve as markers for
263 faecal contamination and therefore the potential that other pathogens are present. The
264 predominant *E. coli* STs (ST4, ST10, ST219, ST2522) detected in our study have been previously
265 isolated from food animals and have been associated with pathogenicity (53-55).
266
267 While none of the *E. coli* isolates carried genes encoding ETEC heat-sensitive or heat-labile
268 enterotoxins, f17d fimbrial genes present in four of the *E. coli* genomes encode ETEC
269 colonization factors commonly associated with colonization of cattle and other ruminant
270 isolates (56). *E. coli,* F17 fimbriae are associated with pathogenic *E. coli* recovered from
271 diarrhoea and septicaemia outbreaks in calves, lambs, and humans, including from outbreaks.
272
273 Additionally, two of the ST4 *E. coli* isolates from this study not harbouring f17d fimbrial genes
274 belong to the serovar O6:H16, one of the most widely disseminated lineages of human
275 enterotoxigenic *E. coli* (ETEC). O6:H16 ETEC cause outbreaks, often associated with food and/or
276 inadequate handwashing (57-59). ETEC, by definition, produce plasmid-encoded heat-labile
277 and/or heat stable toxins not present in the genomes of the isolates from this study.
278
279 However, the serovars O8/O160:H16 and O77/O17/O44/O106/O73:H18 belong to a previously
280 described enteroaggregative *E. coli* (EAEC) lineage (42, 44). Isolates from this study belonging to
281 the serovars O8/O160:H16 and O77/O17/O44/O106/O73:H18 possessed no EAEC accessory
282 genes. These strains are from virulent lineages but lack key virulence genes that are plasmid-
283 encoded, which could mean that these plasmids were lost in the food chain or during isolation
284 but could be reacquired. Nevertheless, the presence of these strains in food could increase the
285 risk of foodborne illness.
286
287 While strains belonging to virulence-associated lineages lacked key plasmid-encoded virulence
288 plasmids, several plasmid replicons were detected in the isolate genomes. ColE, IncI1, IncF, and

289 IncX were commonly detected plasmid replicons in *Salmonella* from food animals in the US
290 according to McMillan et al. (2019) (60). In this study, the IncI1 replicon was predominant, and
291 nine of the thirteen *Salmonella* strains harboured IncI1 plasmid replicon, three harboured the
292 *tetA*. This is likely to be an instance of a successful mobile element with extraordinary local
293 reach, a few of which have been reported from West Africa, including Ghana, in the past (45,
294 61-63). The IncI1-I(Gamma) plasmid replicon observed among *Salmonella* isolates was detected
295 in all *Salmonella* isolates from Bolgatanga municipality - three different serovars - and none of
296 the Saboba district isolates. However, one *E. coli* isolate (from a recently reported ST, ST8274)
297 from Saboba did have this replicon. As it is an IncI1 plasmid replicon, its plasmid should be
298 better characterized and, it should remain under surveillance because numerous articles have
299 reported associations with multiple ARGs, such as *tetB*, *tetAR*, bla_{CMY-2}, bla_{TEM-1}, *aac3VIa*, *aphA*,
300 *aadA* and *sul1* (60), bla_{CTXM-1} (64), *strA*, *strB*, *cmlA*, *floR*, bla_{SHV-12}, bla_{OXA-2} and *FosA3* (65) in IncI1
301 plasmids in *Salmonella*. As our own sequence was generated by short read only, the first step
302 would be to generate long read sequence that could fully assemble the plasmid and make it
303 possible to identify genetic factors supporting its success.
304
305 Among the *E. coli* isolates, the plasmid replicons pO111 was the most common replicon. A
306 previous study by Balbuena-Alonso et al. (2022) (66), revealed that pO111 is usually associated
307 with extended spectrum beta lactamases gene and is very common in food and clinical isolates.
308 In this study, all isolates carrying pO111 harbour at least one beta-lactamase gene. Likewise, all
309 the pO111 plasmid bearing isolates in this study carry ARGs that confer resistance to at least 4
310 classes of antibiotics. Altogether, these data demonstrate a concerning reservoir of resistance
311 genes in these foodborne bacteria.
312
313 **Conclusion**
314 This study has characterized the genomes of *Salmonella* and *E. coli* in milk, meat and their
315 associated utensils. The diverse serovars and virulence genes detected in *Salmonella* strains
316 indicate potential pathogenicity. Although not all *E. coli* strains are pathogenic, their presence
317 serves as an indicator of faecal contamination, suggesting the potential presence of other
318 harmful pathogens. The presence of EAEC strains in food is concerning as EAEC is a well-known
319 cause of diarrhoeal diseases, particularly in children and immunocompromised individuals,
320 making its presence in food a serious concern. While antimicrobial resistance was not common
321 among *Salmonella* strains, most of the *E. coli* strain had at least one resistance gene and almost
322 half were multidrug resistance and carry mobile elements. Moreover, there have been recent
323 reports of resistant *Salmonella* and E. *coli* from meat and milk elsewhere in West Africa (49).
324
325 A recent scoping review reported weak enforcement of food safety regulations as well as a lack
326 of infrastructure, knowledge and skills to implement them (10, 11, 46). Food contaminated with
327 and *Salmonella* and *E. coli* can serve as a vehicle for their transmission, posing a significant
328 public health risk. We recommend that food safety regulation be strengthened in northern
329 Ghana and elsewhere in West Africa. It is also important to increase awareness among
330 consumers so that food is handled in such a way to prevent pathogen transmission. There is an
331 additional need for continuous surveillance and preventive measures to stop the spread of
332 foodborne pathogens and reduce the risk of associated illnesses in Ghana.

Supplementary Materials

Supplementary Table 1: Accession numbers for genomes generated in this study

Supplementary Table 2: Novel *Salmonella* allelic profile and assigned ST.

Supplementary Table 3: *Salmonella* strain metadata including serotype, ST, plasmid replicon, AMR and virulence profile.

Supplementary Table 4: *E. coli* strain metadata including serotype, ST, plasmid replicon, AMR and virulence profile.

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