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

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Abstract

Salmonella 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 characterized *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. The *Salmonella enterica* 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. While 7of the *Salmonella* isolates carry the IncI1-I(Gamma) plasmid replicon. While antimicrobial resistance was not common among *Salmonella* strains, most (11/19) of the *E. coli* strains had at least one resistance gene and almost half (8/19) were multidrug resistance and carry mobile elements. Three of the 19 *E. coli* strains belong to serovars commonly associated with enteroaggregative *E. coli* (EAEC) lineage. 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 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 frequently associated with foodborne infections (1), particularly those caused by *Escherichia coli* and *Salmonella* (2-5). Salmonellosis is under-reported in Ghana, and only a few studies have investigated the plausible role of contaminated milk, meat or meat products in facilitating their transmission (6-8). There have been a few reports of meat samples contaminated with *E. coli* in Ghana (9-11). Some of these *E. coli* strains harbour virulence and antimicrobial resistance genes (ARGs), which is of public health concern however few have been characterized in detail.

There are public health and food safety implications of finding E. coli and Salmonella in food because they are invariably of faecal origin. Globally, *Salmonella* and *Escherichia coli* sourced

from milk and meat increasingly exhibit resistance to different classes of antibiotics that is commonly mediated by mobile elements (10, 12-14). The prevalence of multidrug-resistant (MDR) *S. enterica* and *E. coli* is also on the rise in clinical infections (15, 16). 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 *S. enterica* and *E. coli* isolates from milk and retail meats in Ghana (7, 9, 11).

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 determine their interrelationships and connections to pandemic clones of interest (10, 17). Next-generation sequencing (NGS) technology, the most versatile and informative approach, has gained recent prominence (18, 19). NGS is now used by PulseNet to categorize foodborne diseases, enabling nuanced epidemiological investigations (20). 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 (6, 21). In light of this gap, this study aims to characterize the resistance, virulence and plasmid profile of *Salmonella* and *E. coli* isolated from fresh different retail meats, milk, and associated samples (handler's hand swab, table, knife and faecal samples) in 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 (22, 23). 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 and *E. coli* 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. *Salmonella* isolates were additionally 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) (24), 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, extension at 72^{0}C for two minutes and 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.

90 **DNA Extraction, Library Preparation and Whole Genome Sequencing**
91 Genomic DNA of the isolates was extracted using Wizard DNA extraction kit (Promega;
92 Wisconsin, USA) in accordance with manufacturer's protocol. Using a dsDNA Broad Range
93 quantification assay, the extracted DNA was quantified on a Qubit fluorometer (Invitrogen;
94 California, USA). dsDNA libraries were prepared using NEBNext Ultra II FS DNA library kit for
95 Illumina with 96-unique indexes (New England Biolabs, Massachusetts, USA; Cat. No: E6609L).
96 DNA libraries was quantified using dsDNA High Sensitivity quantification assay on a Quibit
97 fluorometer (Invitrogen; California, USA) and fragment length analysed with the Bioanalyzer
98 (Agilent). Denatured libraries were sequenced on an Illumina MiSeq (Illumina, California, USA).
99 The raw sequence reads were *de novo* assembled using SAdes v3.15.3 (25) according to GHRU
100 protocols (https://gitlab.com/cgps/ghru/pipelines/dsl2/pipelines/assembly).
101
102 **Sequence Typing of *Salmonella* and *E. coli* Genomes**
103 Sequence reads were deposited in the *Salmonella* and *E. coli* database for *Salmonella* and *E. coli*
104 respectively on EnteroBase (26). Multi-locus sequence types (MLST) for the isolates were
105 determined and core-genome MLST calculated. The *Salmonella* genome assemblies were
106 analysed using the *Salmonella* In-Silico Typing Resource (SISTR) for the prediction of serovars and
107 serogroups (27) (https://github.com/phac-nml/sistr_cmd), while the serotyping of the *E. coli*
108 genome was done using ECtyper (28).
109
110 **Identification of AMR, Plasmids and Virulence genes**
111 PlasmidFinder (29) was utilized to identify plasmid replicons that were present in the assembled
112 genomes. AMRFinderPlus v3.10.24 (30) and its associated database (version 2022-04-04.1) were
113 used to predict the antimicrobial resistance genes carried by the isolates and the drug classes to
114 which they probably conferred resistance. Using ARIBA (31) and the virulence factor database
115 (VFDB), we were also able to identify the virulence genes that were present in the isolates.
116
117 **Single Nucleotide Polymorphism (SNP) Calling and Phylogenetic analysis**
118 For phylogenetic analysis, reference sequences for the *S. enterica* and *E. coli* genomes were
119 objectively selected from the National Center for Biotechnology Information Reference Sequence
120 (RefSeq) database (https://www.ncbi.nlm.nih.gov/refseq/) using BactinspectorMax v0.1.3
121 (https://gitlab.com/antunderwood/bactinspector). The selected references were the *S. enterica*
122 subsp. enterica serovar Fresno strain (assembly accession: GCF_003590695.1) and the *E. coli*
123 O25b:H4-ST131 strain (assembly accession: GCF_000285655.3). The sequence reads for each
124 species were then mapped to the chromosome of the reference using BWA (v0.7.17) (32) and
125 variants were called and filtered using bcftools (v1.9) (Danecek et al., 2021) as implemented in
126 the GHRU SNP phylogeny pipeline (https://gitlab.com/cgps/ghru/pipelines/snp_phylogeny).
127 Variant positions were concatenated into a pseudoalignment and used to generate a maximum
128 likelihood tree using iqtree (v1.6.8) (33). SNP distances between the genome pairs were
129 calculated using snp-dists v.0.8.2 (https://github.com/tseemann/snp-dists).
130
131 **Data availability**
132 The sequence reads were deposited in the European Nucleotide Archive with the study accession
133 PRJEB58695 (Supplementary table 1).

RESULTS

Salmonella Serotypes, Sequence Types (STs), Virulence Factors and Phylogeny

We used SISTR software to predict the serovars of the 14 Salmonella strains characterized in this study from whole genome sequence. The most common serotype was Fresno (n=6) followed by Give (n=3), Orleans (n=2), Plymouth (n=1), Agona (n=1) and Infantis (n=1). The *S.* Fresno and *S.* Orleans isolates were from previously unreported sequence types, now designated ST 10742 and ST 10465 respectively (Supplementary table 2). As shown in Figure 1, all the isolates from ready-to-eat pork, mutton and chicken belong to serovar Fresno. *S.* Fresno isolates were also isolated from a meat vendor's knife, as was *S.* Orleans. The three milk isolates, which were all from Saboba, belong to the serovars Plymouth (ST 565), Give (ST 516) and Agona (ST13) (Figure 1). Two more ST 516 *S.* Give isolates were recovered from the faeces of milking cow and from a milking utensil.

Phylogenetic analysis of the 14 *Salmonella enterica* isolates from this study showed that all *S.* Fresno isolates, irrespective of source, clustered together and differed by < 3 SNPs. The two *S.* Orleans isolates were identical (0 SNPs) and the three *S.* Give isolates were also identical, with the isolates originating from milk, faeces of milking cow and from a milking utensil, also in Saboba.

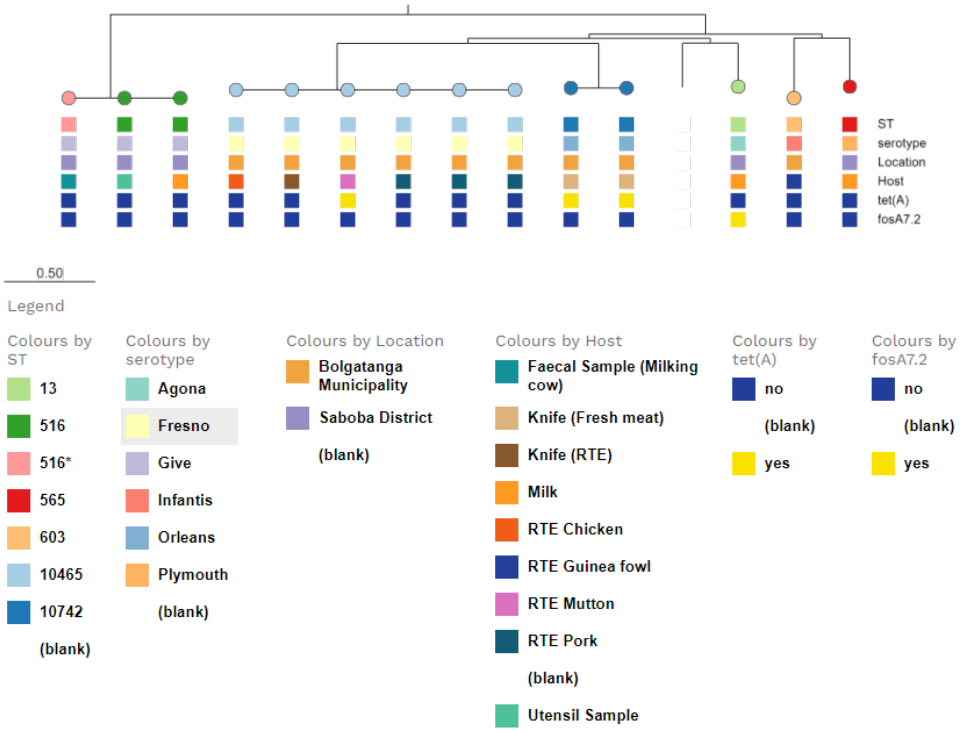


Figure 1: Core genome SNP-based Maximum likelihood tree showing phylogenetic relationships among strains sequenced in this study. The matrix below the tree shows the sequence types, serotypes, location of isolation (Bolgatanga municipality or Saboba district) as well as the source/host sample and presence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

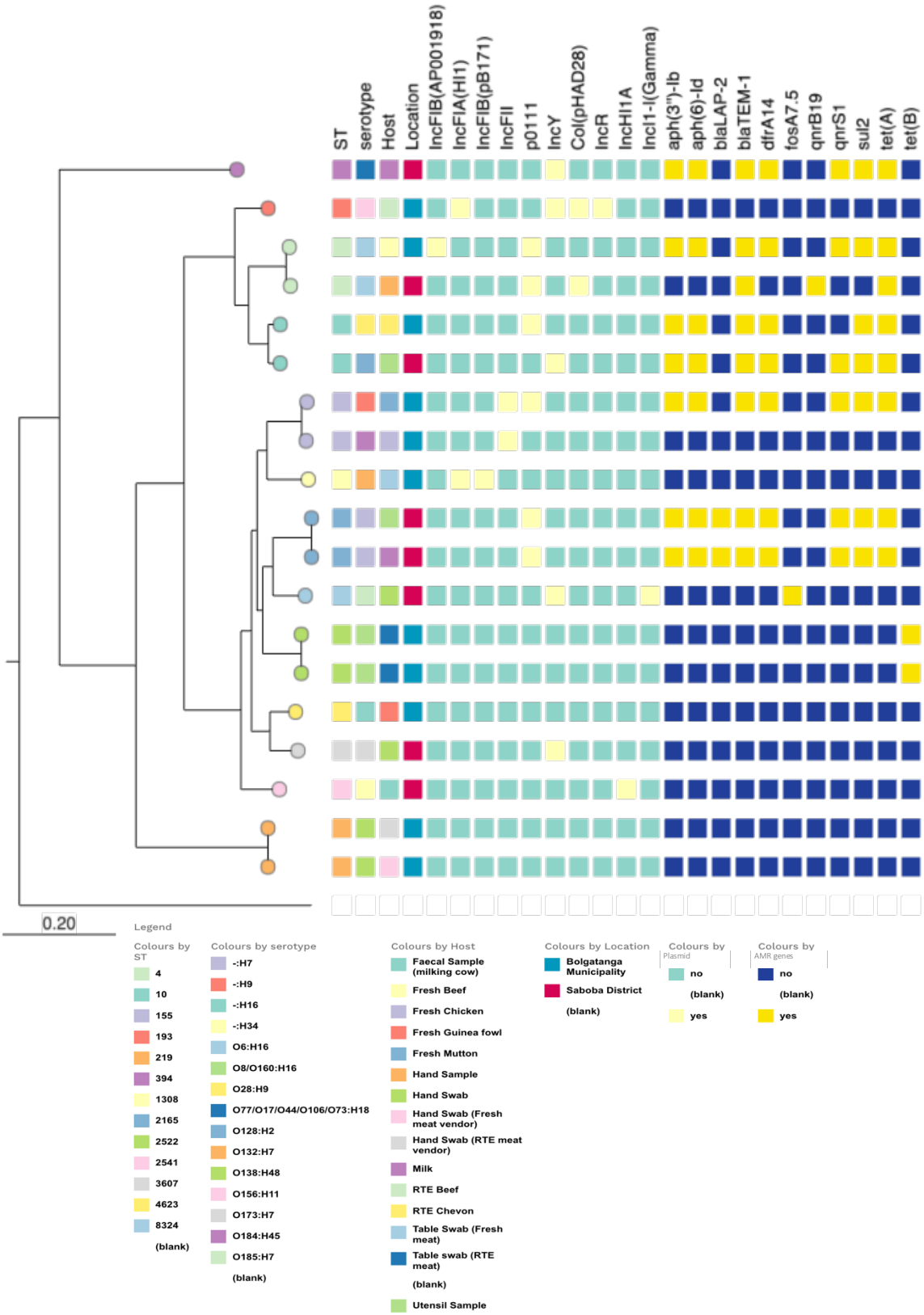
***E. coli* Serotypes, Virulence factors and Phylogeny**

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 (34), as well as O8/O160:H16 and O77/O17/O44/O106/O73:H18 (ST394; (35-37)). 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
p0111
IncY
Col(pHAD28)
IncR
IncHI1A
IncI1-I(Gamma)
aph(3'')-Ib
aph(6)-Id
blaLAP-2
blaTEM-1
dfrA14
fosA7.5
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)

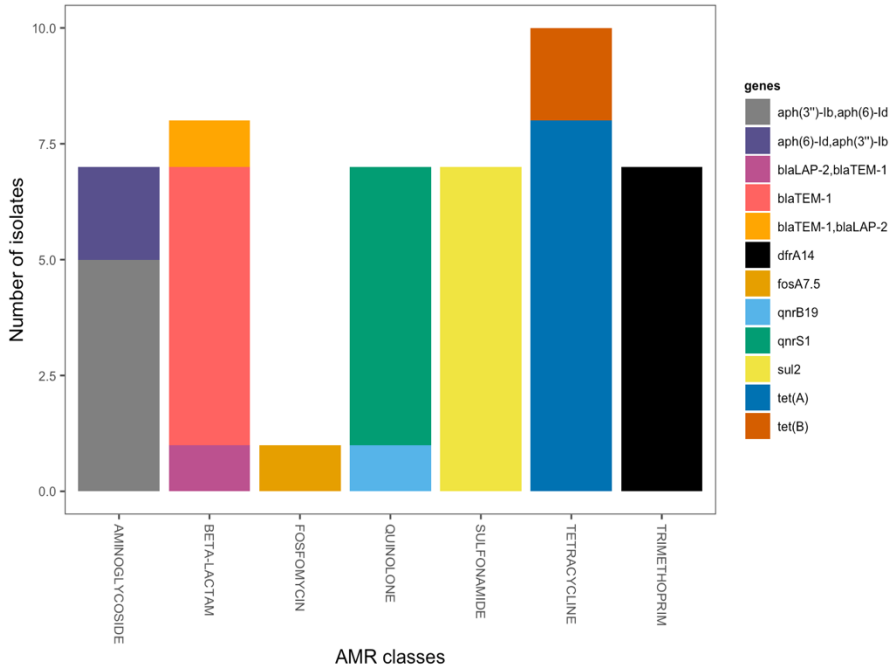


Figure 2: Phylogeny, plasmid replicons and resistance genes of *E. coli* isolates. A. SNP-based phylogenetic tree of the *E. coli* isolates showing the range of sequence types, serovars, plasmid replicons and resistance genes detected (b) AMR determinants detected in *E. coli* and the resistance they 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 resistant 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 (38). 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 strains encode pO111 or IncY replicons.

DISCUSSION

Salmonella and *E. coli* are the main causes of bacterial foodborne illnesses in Ghana (39). Retail meat, along with milk and their products are recognized as primary sources of foodborne Salmonellosis (9) and *E. coli* infection (6). Post-cooking handling practices, exposure during the

233 points of sale, and suboptimal meat storage conditions collectively contribute to an increased
234 presence of both pathogenic and spoilage bacteria in ready-to-eat (RTE) meat (40). In this study,
235 we characterized the genomes of 14 *Salmonella* and 19 *E. coli* previously isolated from Saboba
236 district and Bolgatanga Municipality in northern Ghana.
237
238 The *Salmonella* serovars (Fresno, Plymouth, Infantis, Give and Orleans) identified in this study
239 are yet to be reported in Ghana, but Guinee et al. (1961) (41) isolated *S.* Agona from cattle in
240 Ghana. Isolation of *S.* Infantis from retail poultry meat has also been reported in Ecuador (42),
241 Belgium (43) and Italy (44) and all the serovars detected in this study have been implicated in
242 human infections. The identification of Salmonella serovars not previously documented in the
243 country in meat and milk products highlights the need for heightened surveillance and preventive
244 measures to curb the spread of foodborne pathogens and reduce the risk of associated illnesses.
245
246 Unlike *Salmonella*, not all *E. coli* are potential pathogens. However, *E. coli* serve as markers for
247 faecal contamination and therefore the potential that other pathogens are present. The
248 predominant *E. coli* STs (ST4, ST10, ST219, ST2522) detected in our study have been previously
249 isolated from food animals and have been associated with pathogenicity (45-47). While none of
250 the isolates carried genes encoding ETEC heat-sensitive or heat-labile enterotoxins, f17d fimbrial
251 genes present in four of the *E. coli* genomes encode ETEC colonization factors commonly
252 associated with colonization of cattle and other ruminant isolates (48). *E. coli,* F17 fimbriae are
253 associated with pathogenic *E. coli* recovered from diarrhoea and septicaemia outbreaks in calves,
254 lambs, and humans, including from outbreaks. Additionally, two of the ST4 *E. coli* isolates from
255 this study not harbouring f17d fimbrial genes belong to the serovar O6:H16, one of the most
256 widely disseminated lineages of human enterotoxigenic *E. coli* (ETEC). O6:H16 ETEC cause
257 outbreaks, often associated with food and/or inadequate handwashing (49-51). ETEC, by
258 definition, produce plasmid-encoded heat-labile and/or heat stable toxins not present in the
259 genomes of the isolates from this study. However, the serovars O8/O160:H16 and
260 O77/O17/O44/O106/O73:H18 belong to a previously described enteroaggregative *E. coli* (EAEC)
261 lineage (35, 37). Isolates from this study belonging to the serovars O8/O160:H16 and
262 O77/O17/O44/O106/O73:H18 possess no EAEC accessory genes. These strains are from virulent
263 lineages but lack key virulence genes that are plasmid-encoded, which could mean that these
264 plasmids were lost in the food chain or during isolation but could be reacquired. Nevertheless,
265 the presence of these strains in food could increase the risk of foodborne illness.
266
267 While strains belonging to virulence-associated lineages lacked key plasmid-encoded virulence
268 plasmids, several plasmid replicons were detected in the isolate genomes. ColE, IncI1, IncF, and
269 IncX were commonly detected plasmid replicons in *Salmonella* from food animals in the US
270 according to McMillan et al. (2019) (52). In this study, the IncI1 replicon was predominant, and
271 nine of the thirteen *Salmonella* strains harboured IncI1 plasmid replicon, three harboured the
272 *tetA*. This is likely to be an instance of a successful mobile element with extraordinary local reach,
273 a few of which have been reported from West Africa, including Ghana, in the past (38, 53-55).
274 The IncI1-I(Gamma) plasmid replicon observed among *S. enterica* strains was detected in all
275 *Salmonella* isolates from Bolgatanga municipality - three different serovars - and none of the
276 Saboba district isolates. However, one *E. coli* isolate (from a recently reported ST, ST8274) from

Saboba did have this replicon. As it is an IncI1 plasmid replicon, its plasmid should be better characterized and, it should remain under surveillance because numerous articles have reported associations with multiple ARGs, such as *tetB*, *tetAR*, bla_{CMY-2}, bla_{TEM-1}, *aac3VIa*, *aphA*, *aadA* and *sul1* (52), bla_{CTXM-1} (56), *strA*, *strB*, *cmlA*, *floR*, bla_{SHV-12}, bla_{OXA-2} and *FosA3* (57) in IncI1 plasmids in *Salmonella*. As our own sequence was generated by short read only, the first step would be to generate long read sequence that could fully assemble the plasmid and make it possible to identify genetic factors supporting its success.

Among the *E. coli* isolates, the plasmid replicons pO111 was the most common replicon. A previous study by Balbuena-Alonso et al. (2022) (58), revealed that pO111 is usually associated with extended spectrum beta lactamases gene and is very common in food and clinical isolates. In this study, all isolates carrying pO111 harbour at least one beta-lactamase. Likewise, all the pO111 plasmid bearing isolates in this study carry ARGs that confer resistance to at least 4 classes of antibiotics. Altogether, these data demonstrate a concerning reservoir of resistance genes in these foodborne bacteria.

Conclusion

This study has characterized the genomes of *Salmonella* and *E. coli* in milk, meat and their associated utensils. The diverse serovars and virulence genes detected in *Salmonella* strains indicate potential pathogenicity. All *E. coli* are not always pathogenic, but they serve as indicators of faecal contamination, suggesting a potential risk for the presence of other pathogens. The presence of EAEC lineages in food is concerning as these bacteria are known to cause diarrhoea, especially in children and immunocompromised individuals. While antimicrobial resistance was not common among *Salmonella* strains, most of the *E. coli* strain had at least one resistance gene and almost half were multidrug resistance and carry mobile elements. Food contaminated with these pathogens can serve as a vehicle for 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.

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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