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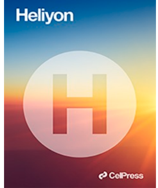

Research article

Unveiling distinct genetic features in multidrug-resistant *Escherichia coli* isolated from mammary tissue and gut of mastitis induced mice

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ABSTRACT

Escherichia coli is one of the major pathogens causing mastitis in lactating mammals. We hypothesized that *E. coli* from the gut and mammary glands may have similar genomic characteristics in the causation of mastitis. To test this hypothesis, we used whole genome sequencing to analyze two multidrug resistant *E. coli* strains isolated from mammary tissue (G2M6U) and fecal sample (G6M1F) of experimentally induced mastitis mice. Both strains showed resistance to multiple (>7) antibiotics such as oxacillin, aztreonam, nalidixic acid, streptomycin, gentamicin, cefoxitin, ampicillin, tetracycline, azithromycin and nitrofurantoin. The genome of *E. coli* G2M6U had 59 antimicrobial resistance genes (ARGs) and 159 virulence factor genes (VFGs), while the *E. coli* G6M1F genome possessed 77 ARGs and 178 VFGs. Both strains were found to be genetically related to many *E. coli* strains causing mastitis and enteric diseases originating from different hosts and regions. The G6M1F had several unique ARGs (e.g., *Qnr*S1, *sul*2, *tet*A, *tet*R, *emr*K, *bla*-TEM-1/105, and *aph*(6)-Id, *aph*(3″)-Ib) conferring resistance to certain antibiotics, whereas G2M6U had a unique heat-stable enterotoxin gene (*ast*A) and 7192 single nucleotide polymorphisms. Furthermore, there were 43 and 111 unique genes identified in G2M6U and G6M1F genomes, respectively. These results indicate distinct differences in the genomic characteristics of *E. coli* strain G2M6U and G6M1F that might have important implications in the pathophysiology of mammalian mastitis, and treatment strategies for mastitis in dairy animals.

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1. Introduction

Mastitis, inflammation of the mammary gland, is one of the most prevalent diseases that is responsible for the highest clinical and economic significance in the dairy industry worldwide [1]. The disease is primarily caused by bacterial pathogens, and the major intramammary pathogens include *Escherichia coli*, *Streptococcus* spp., *Klebsiella pneumoniae*, and *Staphylococcus aureus* [2–5]. The severity of this disease depends on host-pathogen interactions particularly when pathogenic and/or opportunistic microbes enter the germ-free environment of the mammary gland. This opportunistic encroachment is favored by the disruption of the physical barriers of the mammary quarters, immune status of the host, and migration of the gut or rumen microbes to the mammary gland through the endogenous entero-mammary axis [6,7]. A large number of microbial species have evolved novel mechanisms that facilitate their proliferation in the mammary gland and subsequent clinical manifestations [6–8].

Mastitis causing pathogens are commonly categorized as environmental or contagious [9,10], of which *E. coli* are the most important gram-negative facultative bacteria [11]. *E. coli* is a genetically and phenotypically diverse bacterial species typically colonize in the gastrointestinal tract of human and animals, where it can be a mutualist, commensal, pathogen or occasional symbiont [12]. Besides, being an important member of the normal gut flora of humans and other mammals, *E. coli* encompasses many pathotypes that cause a variety of diseases particularly during imbalances in host–bacteria relationships [13,14]. The pathotype classification of *E. coli* is based on the site of infection, symptoms of the disease, and types of virulence factors/genes [13,15]. A novel extraintestinal pathogenic *E. coli* (ExPEC) pathotype known as MPEC (mammary pathogenic *E. coli*), one of the most common etiologic agents of bovine mastitis [16]. *E. coli* commonly targets the mammary gland during the early lactating stage, which can be fatal if left untreated [17]. *E. coli* mastitis in dairy cows has severity ranging from mild (local inflammation in the mammary glands) to severe (systemic derangement) [18,19]. However, the severity of *E. coli* mastitis is primarily dependent on host factors [1,6,7]. The pathogenicity of *E. coli* mastitis is due to the presence of an arsenal of antimicrobial resistance genes (ARGs) and virulence factors [4,14,20]. The genetic structure of *E. coli* strains is usually influenced by several factors including the host and environment enabling the bacteria to acquire various antimicrobial resistance mechanisms and multidrug resistance (MDR) phenomena [20–22]. MDR *E. coli* strains have been isolated from animals in several countries, including Bangladesh [23,24]. Furthermore, detection of MDR *E. coli* strains in bovine mastitis is a critical public health concern posing a zoonotic risk for farm workers, contact people, also causing food toxin infections [20,23,25]. *E. coli* efficiently harbors a wide range of ARGs, and can transfer those genes to other pathogenic bacteria horizontally [13,26]. Virulence genes (VFGs) that code for toxins, hemolysins, adhesins, and lipopolysaccharides were identified in *E. coli* from bovine mastitis [27,28]. Although, mammary gland is not a natural or primary habitat for *E. coli*, some of the strains of this pathogen might acquire specific VFGs that help them to invade the mammary gland creating an amenable opportunistic habitat for survival, multiplication and subsequent pathogenesis [29,30]. So far, bovine mastitis associated *E. coli* are well investigated in different countries [29,30], only sporadic studies have been conducted on the molecular epidemiology, phylogenetic diversity, resistome, virulome, and metabolic potentials of *E. coli* from bovine mastitis cases in Bangladesh [4]. Moreover, no report has been published on the phylogenomic diversity, molecular typing and genomic potentials (resistome, virulome and metabolic functions) of *E. coli* isolated from mice with mastitis. Several previous studies have indicated that bovine clinical mastitis pathogens including *E. coli* can induce mastitis in germ free (GF) mice [6,7,31]. The mouse mastitis model is a good model to study bovine mastitis compared to other laboratory animals for ease of handling, keeping them in controlled environment, low maintenance cost (as compared with other mammalian experimental models), high reproductive rates, and short life cycle [6,32,33]. In addition, mouse mastitis model can allow researchers precise experimental studies in a physiologically and genetically controlled system [34]. In our previous research, we demonstrated that transplanting microbiota from clinical mastitis cow's milk and feces resulted in mastitis and distinct inflammatory changes in the gut (colon) and mammary tissues of GF mice [2]. We recently characterized the genomic features and pathophysiological potentials of a MDR non-aureus Staphylococci (NAS) strain, *Staphylococcus warneri* G1M1F, isolated from the feces of an experimentally induced mastitis mouse [35,36]. Moreover, variations in bacterial genetics, particularly strains associated with mastitis, can impact the progression of the disease in different hosts by influencing factors such as virulence, AMR, and the ability to evade host immune responses, ultimately contributing to variations in the severity and outcomes of mastitis infections. Thus, a critical question among the global researchers is that what are the specific genetic features and mechanisms underlying MDR in major pathogens (e.g., *E. coli*) of mastitis isolated from diverse samples and hosts, and how do these genetic determinants contribute to the pathophysiology of bovine mastitis? We therefore hypothesized that *E. coli* strains originating from the gut and mammary tissues exhibit distinct genomic potentials, including resistome and virulome variations, which may play a crucial role in the pathophysiology of mastitis. To address the hypothesis, we aimed to characterize *E. coli* strains isolated from mammary tissue (MT) and fecal sample (FS) of experimentally induced mastitic mice (GF), and determine their genetic relatedness based on sequence typing, phylogeny, antimicrobial resistance, virulence, and metabolic functional potentials.

2. Materials and methods

Ethical statement

The Animal Research Ethics Committee (AREC) of the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, reviewed and approved the experimental procedures of this study (Reference number: FVMAS/AREC/2023/6679, Date: January 16, 2023).

2.1. Sample collection, isolation and identification of E. coli

A cow-to-mouse mastitis model was established by the transplantation of milk and fecal microbiota from clinical mastitis cow to 42 timed pregnant GF mice (at Day 17 of their gestation) in our laboratory animal research facility (24.09°N, 90.41°E) following previously published protocols [6,7]. The challenged mice (N = 42) received 1 g/kg fecal suspension and 0.5 mL of milk suspension orally at Day 17 of their gestation while control group mice (N = 10) received a placebo with distilled water (0.5 mL/mice, orally). The mice were observed for mastitis syndrome (e.g., swollen, red and inflamed mammary glands) development, and sacrificed 10 days after microbiota transplantation (at Day 27 of their gestation) for sample collection [6]. Mammary tissue and fecal samples (n = 84; 42 from each category) from these experimentally induced mastitis mice were collected aseptically. Both MT and FS were homogenized (1:1) in PBS (Phosphate-buffered saline) and serially diluted (1:10). Dilutions were plated onto nutrient agar plates and incubated at 37 °C for 24 h. Pure colonies were isolated and subsequently streaked on Eosin Methylene Blue (EMB) agar plates (Oxoid™, Thermo Scientific, UK) and incubated at 37 °C for 24 h [3,36]. Phenotypic identification of the isolates was performed based on the colony morphology and Gram-staining (Gram -ve, formation of green metallic sheen on EMB), and biochemical tests such catalase, indole, methyl red, Voges-Proskauer (VP), oxidase, urease and triple sugar iron tests [4]. The species-level identification of 46 isolates (MT = 23, FS = 23) was performed through a VITEK-2 system (version 9.01) [37].

2.2. Antimicrobial susceptibility assay

Antimicrobial susceptibility patterns of the confirmed *E. coli* isolates (n = 46) were examined using the disk diffusion method following the guidelines of the Clinical Laboratory Standards Institute (CLSI) M100 33rd Edition (https://clsi.org/, accessed 20 May 2023). Antibiotics were selected for susceptibility testing corresponding to a panel of antimicrobial agents (CM0337, OxoidTM, Thermo Scientific, UK) commonly used by veterinary practitioners in Bangladesh. The groups of antimicrobials used were - Beta-lactams (ampicillin, 10 μg/mL; oxacillin, 1 μg/mL), Monobactams (aztreonam, 30 μg/mL), Tetracyclines (doxycycline, 30 μg/mL; tetracycline, 30 μg/ML), Nitrofurans (nitrofurantoin, 300 μg/mL), Fluoroquinolones (ciprofloxacin, 10 μg/mL; nalidixic acid, 30 μg/mL), Cephalosporins (cefoxitin, 30 μg/mL), Carbapenems (imipenem, 10 μg/mL), Aminoglycosides (gentamycin, 10 μg/mL; streptomycin, 10 μg/mL), Chloramphenicol (chloramphenicol, 30 μg/mL), Macrolides (azithromycin, 15 μg/mL), and Sulphonamides (compound sulphonamide, 300 μg/mL). Resistance was defined according to CLSI guidelines (https://clsi.org/, accessed 20 May 2023)). MDR *E. coli* isolates were defined as resistance to >3 antibiotics. We further determined the minimum inhibitory concentration (MIC) values of MDR (resistant to > 3 antibiotics) isolates (n = 20) against commonly used antibiotics according to the broth dilution method recommended by CLSI (https://clsi.org/, accessed 20 May 2023). *E. coli* ATCC 25922 was used as the control organism in the antimicrobial susceptibility tests. Based on antimicrobial susceptibility results, we selected two MDR isolates (one from MT, *E. coli* G2M6U, and one from FS, *E. coli* G6M1F) that showed resistance against the highest number (n = 10) of antibiotics. The *E. coli* G2M6U and *E. coli* G6M1F were further confirmed by 16S rRNA gene sequencing [4].

2.3. Whole genome sequencing, assembly and annotation

Genomic DNA from two MDR *E. coli* isolates (e.g., *E. coli* G2M6U and *E. coli* G6M1F) was extracted using the boiled method [36]. In brief, both isolates were incubated in nutrient broth (Biolife™, Italy) at 37 °C for 24 h, and the harvested culture was used for DNA extraction using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). NanoDrop 2000 UV–Vis Spectrophotometer (Thermo Fisher, Waltham, MA, USA) was used to check the purity and concentration of the extracted DNA. Nextera™ DNA Flex Library Prep Kit (Illumina, San Diego, USA) was used to generate libraries from 1 ng DNA, and whole genome sequencing (WGS) of the prepared libraries was performed using Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) with a 2 × 250-bp protocol. Generated raw reads (G2M6U = 74,951,320 bp and G6M1F = 84,361,784 bp) were trimmed using Trimmomatic v0.39 (with parameters leading:20, slidingwindow:4:20:20, trailing:20, and minlen = 36) [38], and quality checked using FastQC v0.11.7 [39].

De novo assembly of the clean reads was performed into the draft genome with SPAdes v3.15.5 [40]. QUAST v5.0.2 [41] and BUSCO (Benchmarking Universal Single-Copy Orthologs) v.4.1.2 with "bacteria_'odb10' data set [42] were utilized to check the quality and completeness of the assembled genomes, respectively. The NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) was used to annotate the genomes. Plasmid replicons were predicted by using PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) with the setting of the threshold for a minimum 95% identity over 60% coverage of length [43]. CRISPRimmunity (http://www.microbiome-bigdata.com/CRISPRimmunity/index/home) and PHAge Search Tool Enhanced Release; PHASTER server (http://phaster.ca/) were used to predict CRISPR (clustered regularly interspaced short palindromic repeats) arrays and phage-associated genes and genomic regions, respectively in G2M6U and G6M1F genomes.

2.4. Sequence typing, phylogenetic analysis and genomic comparison

BacWGSTdb 2.0 was used to carry out *in silico* multilocus sequence typing (MLST) analysis and bacterial source tracing using a core genome MLST (cgMLST) analysis [44]. Based on the cgMLST results, the study genomes (G2M6U and G6M1F) and 41 reference genomes (including 17 enterotoxin producing genomes) of *E. coli* (Table S1) were used in phylogenetic analysis. Genomes were aligned with MUSCLE v5.0 (https://github.com/rcedgar/muscle) [45], and a phylogenetic tree was created using PhyML v3.0 [46], and finally visualized through iTOL (v3.5.4) (http://itol.embl.de/) [47]. We further leveraged FastANI through the GTDB-tk v2 database [48] to calculate pairwise average nucleotide identity (ANI) values (as percentage) in the study genomes (G2M6U; GCA_029382245.1 and

G6M1F; GCA_029382305.1) and 18 closely related *E. coli* genomes (based on phylogenetic analysis) (Table S2). In addition, multiple genomes (n = 7) alignment and visualization were performed using BLAST Ring Image Generator (BRIG) v0.95 [49] with *E. coli* D6_113.11 (GenBank Accession No.: NZ_CCCO000000000) as reference. We compared the genomes using BRIG v0.95 with an e-value cutoff of $1e^{-5}$ [50]. Roary v3.11.2, a high speed stand-alone pipeline that rapidly builds large-scale pangenomes identifying the core and accessory genes was used to perform pangenome analysis [51]. Accordingly, four different classes of genes such as 'core' (99% ≤strains ≤100%), 'soft core' (95%≤ strains <99%), 'shell' (15%≤ strains <95%) and 'cloud' (0%≤ strains <15%) were predicted.

2.5. Genomic functional potentials analysis

To elucidate the genomic functional potentials, we analyzed the ARGs, VFGs and metabolic features in the study genomes. The ABRicate v1.0.1 (https://github.com/tseemann/abricate) bundled with multiple databases: NCBI AMRFinderPlus [52], CARD 2020 [53], ARG-ANNOT [54], ResFinder 4.0 [55], and MEGARes 2.0 [56] was used to predict ARGs in the assembled genomes. The ARGs selection criteria were set to perfect (100% identity) and strict (>95% identity) hits only to the curated reference sequences in the databases. The mobile genetic elements (MGEs) in the genomes of G2M6U and G6M1F were investigated through mobileOG-db [57]. The VFGs in both of the study genomes (with 90% nucleotide identity and query coverage) were identified using ecoli_vf (https://github.com/phac-nml/ecoli_vf) and VFDB v6.0 [58] databases bundled in the Abricate (https://github.com/tseemann/abricate). The draft genomes were also annotated using the RAST (Rapid Annotation using Subsystem Technology) server, v2.0 [59], to identify metabolic function related genes/pathways under different subsystem categories. Secondary metabolites in the study genomes were predicted using the antiSMASH v3.0 database [60].

2.6. Statistical analysis

Descriptive statistics were used to examine the distribution of antimicrobial resistance profile of the study isolates, ARGs and VFGs repertoire of the G2M6U and G6M1F genomes. The data from the antimicrobial susceptibility assay were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. Both ARGs and VFGs data were normalized by Total Sum Scaling (TSS) that uses the total read count for each gene in each sample [61]. Statistical significance was set for all tests at $p \leq 0.05$.

3. Results

3.1. Occurrence and antibiogram profile of the *E. coli* isolates

In this study, overall prevalence of *E. coli* in GF mouse mastitis was 54.76% (23/42). A total of 46 *E. coli* isolates including 23 from MT (50.0%) and 23 from FS (50%) were screened through culture, biochemical tests (catalase, indole, methyl red, Voges-Proskauer (VP), oxidase, urease and triple sugar iron tests; +ve) and VITEK-2 system (identification of *E. coli* by automating the analysis of multiple biochemical and metabolic characteristics) [37]. Of these, 82.60% (38/46) *E. coli* isolates showed multidrug resistance (resistance to > 3 antibiotics) in disk diffusion tests, mainly beta-lactams (ampicillin, oxacillin), aminoglycosides (gentamicin, streptomycin), tetracycline, macrolides (azithromycin), nitrofurans (nitrofurantoin) and fluoroquinolones (nalidixic acid) resistant profile. The isolates displayed 100% (46/46) resistance against oxacillin, aztreonam, nalidixic acid, streptomycin and cefoxitin, followed by sulphonamide (89%), ampicillin (87%), gentamicin (76.8%), tetracycline (68.26%) and azithromycin (56%) (Table 1). However, resistance rates to nalidixic acid, nitrofurantoin, gentamicin, oxacillin, azithromycin, tetracycline, ampicillin, aztreonam, cefoxitin, and sulphonamide were significantly ($p < 0.05$) higher in FS isolates (range: 50–75%) than MT isolates (range: 25 – ≤50%)

Table 1
Antibiotic susceptibility of *E. coli* isolates (n = 46) screened from mammary tissue and fecal samples of mice with mastitis.

Antibiotic class	Antimicrobial	MIC_{90} (μg/ml)	SIR
Aminoglycosides	Gentamicin	<12	R
	Streptomycin	<11	R
Carbapenems	Imipenem	>23	S
Monobactams	Aztreonam	>16	R
Beta-lactams	Ampicillin	<13	R
	Oxacillin	<10	R
Chloramphenicols	Chloramphenicol	<12	S
Cephalosporins	Cefoxitin	>14	R
Tetracyclines	Tetracycline	<14	R
	Doxycycline	<10	S
Fluoroquinolones	Ciprofloxacin	>21	S
	Nalidixic acid	13	R
Macrolides	Azithromycin	<13	R
Nitrofurans	Nitrofurantoin	<14	R
Sulphonamides	Compound Sulphonamide	≤12	R

MIC: Minimum Inhibitory Concentration; S: Sensitive; I: Intermediate; R: Resistant.

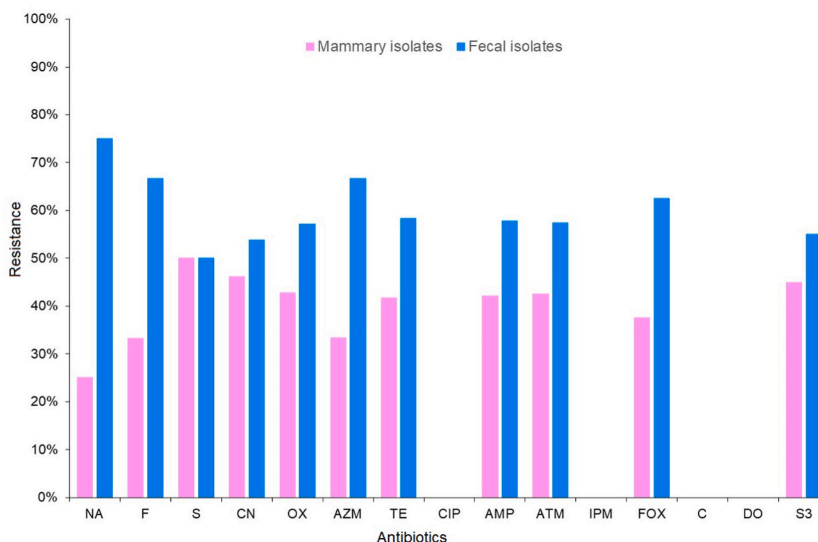

Fig. 1. Distribution of antimicrobial-resistant *E. coli* isolated from mice with mastitis. NA, Nalidixic acid; F, Nitrofurantoin; S, Streptomycin; CN, Gentamicin; OX, Oxacillin; AZM, Azithromycin; TE, Tetracycline; CIP, Ciprofloxacin; AMP, Ampicillin; ATM, Aztreonam; IPM, Imipenam; FOX, Cefoxitin; C, Chloramphenicol; DO, Doxycycline; S, Compound Sulphonamide.

(Fig. 1). In addition, *E. coli* isolates obtained from MT and FS showed similar percentage of resistance to streptomycin. Remarkably, none of the isolates showed resistance against ciprofloxacin, imipenem, chloramphenicol and doxycycline (Table 1).

3.2. Distinct genomic features of E. coli strains isolated from mammary tissue and feces of mice with mastitis

To our knowledge, this is the first WGS report of mastitis-associated *E. coli* strains isolated from MT and FS of experimentally induced mastitis mice in Bangladesh. We sequenced the genome of two representative *E. coli* isolates (G2M6U and G6M1F) with the highest MDR pattern (>7 antibiotics; beta-lactam-aminoglycoside-tetracycline-macrolide-nitrofurans-fluoroquinolone remittance). The comparative genomic features of these two strains (*E. coli* G2M6U and *E. coli* G6M1F) are summarized in Table 2. BUSCO

Table 2
General genomic features of the *E. coli* strains isolated from murine mastitis.

Features (s)	*E. coli* strains	
	G2M6U	G6M1F
Genome size (bp)	4,441,064	4,667,456
Genome coverage (x)	60	65.5
GC content (%)	50.8	50.9
Total contigs	41	57
Largest contig (bp)	409,709	640,433
Shortest contig (bp)	6753	3072
Contig N_{50} (bp)	140,681	184,919
L_{50}	10	8
Total genes	4307	4535
Coding sequences (CDSs)	4343	4451
Protein coding genes	4111	4313
RNA genes	76	84
tRNA genes	67	74
rRNAs	1	1
ncRNAs	8	9
Pseudo genes	120	138
Genes with function prediction	4209	4454
Genes assigned to SEED subsystems	1901	2020
Number of subsystems	367	378
CRISPR arrays	3	3
Number of plasmids (% identity)	0	1 (99.08)
Number of prophages	7	9
Sequence type (ST)	ST155	ST58
Number of antibiotic resistance genes (ARGs)	59	77
Number of virulence factor genes (VFGs)	159	178

assessment reveals 99.88% completeness of the both genomes. The size of the G2M6U and G6M1F draft genomes are approximately 4.44 Mbp (GC content = 50.8%, genome coverage = 60x) and 4.66 Mbp (GC content = 50.9%, genome coverage = 65.5x), respectively. The number of predicted coding sequences (CDS), tRNAs, and rRNAs were 4,343, 67 and 1, respectively in G2M6U; whereas the numbers were 4,451, 74, and 1, respectively in G6M1F genome. The final assembly of G2M6U contained 41 contigs where the largest contig assembled was 409,709 bp in length, and N_{50} was 140,681 bp for contigs larger than 1000 bp. Likewise, G6M1F contained 57 contig with the largest contig size of 640,433 bp and N_{50} of 184,919 bp. We predicted three CRISPR arrays in both of the genomes (G2M6U and G6M1F) with thirteen signature genes (e.g., *WYL*, *cas*3, *cas*8e, *cse*2gr11, *cas*7, *cas*5, *cas*6e, *cas*1, *cas*2, *csa*3, c2c9_V–U4, and *DEDDh*)). The G2M6U genome harbored seven prophage regions with 98 gene features whereas G6M1F possessed nine prophages 75 gene features. Importantly, one plasmid replicon like IncY (4012 bp) was only identified in the G6M1F genome (with 95% identity and 60% coverage) (Table 2).

3.3. Closest relatives of E. coli G2M6U and G6M1F strains

To determine the genomic epidemiological characteristics of *E. coli* strains in a global context, the phylogenetic relationships between study strains (G2M6U and G6M1F) and 515 closely related *E. coli* strains obtained from the NCBI GenBank database were analyzed using seven-gene (e.g., *adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A and *rec*A) core genome multi-locus sequence typing (cgMLST) approach of BacWGSTdb 2.0 [44]. A grapeTree was used to produce and visualize a minimum spanning tree (MST) based on findings of the cgMLST. Of the study genomes, *E. coli* G2M6U belonged to *E. coli* sequence type 155 (ST155) which differed by 834 alleles, and *E. coli* G6M1F belonged to ST58 with 952 allele difference against a pre-defined reference database (cgMLST scheme) for each species in BacWGSTdb 2.0. The cgMLST analysis revealed that the closest ancestors of G2M6U were several enterotoxins producing ST155 *E. coli* strains isolated from stool of diarrheic patients in Bangladesh (P0302293.10, P0302293.7, P0302293.2, 603936), and India (Kolkata; 503210, 503698) (Fig. 2a, Table S1). Conversely, the gut associated *E. coli* G6M1F strain showed a close evolutionary relationship with another ST58 *E. coli* strain (JL05) isolated from milk of a cow suffered from clinical mastitis in China. This strain also showed evolutionary relationship with ten other enterotoxins producing ST58 *E. coli* strains isolated from stool of diarrheic patients in Bangladesh (P0299917.2 - P0299917.9 and MP021561.3) and India (Kolkata; 503688), and another *mcr*-1 positive *E. coli* strain (LN6) isolated from healthy human's stool in China. Moreover, both of the study strains were closely related to *E. coli* strains of environmental soil and household cattle and chicken feces origin (Table S1).

3.4. Phylogenomic relatedness between E. coli G2M6U and G6M1F strains

To elucidate the evolutionary relationship between the studied genomes (G2M6U and G6M1F) and 41 reference genomes including 17 enterotoxins producing *E. coli* genomes (based on cgMLST results), a maximum-likelihood phylogenetic tree was constructed (Fig. 3). The maximum-likelihood tree showed 12 major clades of 43 isolates from nine countries where G2M6U and G6M1F isolates clustered separately. The phylogenomic analysis showed that G2M6U (JARLTH000000000_MM_BD) and G6M1F (JARLTG010000000_MM_BD) strains clustered separately, and were more closely aligned to several *E. coli* strains previously isolated from bovine mastitis milk in France (e.g., CP027118_BM_FRA, CCCR000000000_BM_FRA), Brazil (e.g., JANKHL000000000_BM_BRA), Germany (e.g., LCVG00000000_BM_GER, LCVH00000000_BM_GER), human enterotoxigenic *E. coli* strains of Bangladesh (e.g., AQAA00000000_CHS_BD, LGND00000000_CHS_BD) India (e.g., LRLB00000000_DHS_IND), and environmental soil originated *E. coli*

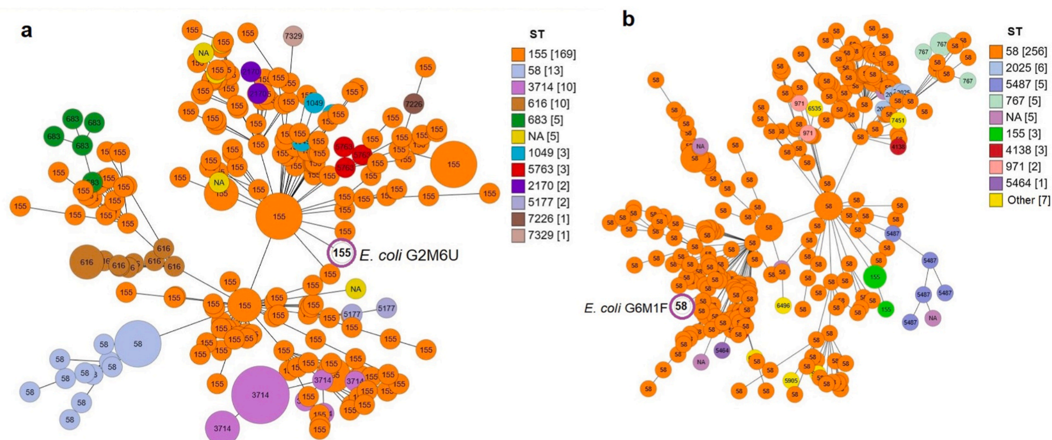

Fig. 2. Phylogeny of closest relatives of *E. coli* strains (G2M6U and G6M1F) based on core genome multi-locus sequence typing (cgMLST) analysis. The closest relative strains of G2M6U and G6M1F were identified by cgMLST allele threshold of 834 and 952, respectively. The G2M6U and G6M1F strains are the white circles highlighted in purple and the individual isolates are marked with different colors according to sequence type (ST). The numbers on the circles represent the STs, and diameter of each circle represent allelic differences. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

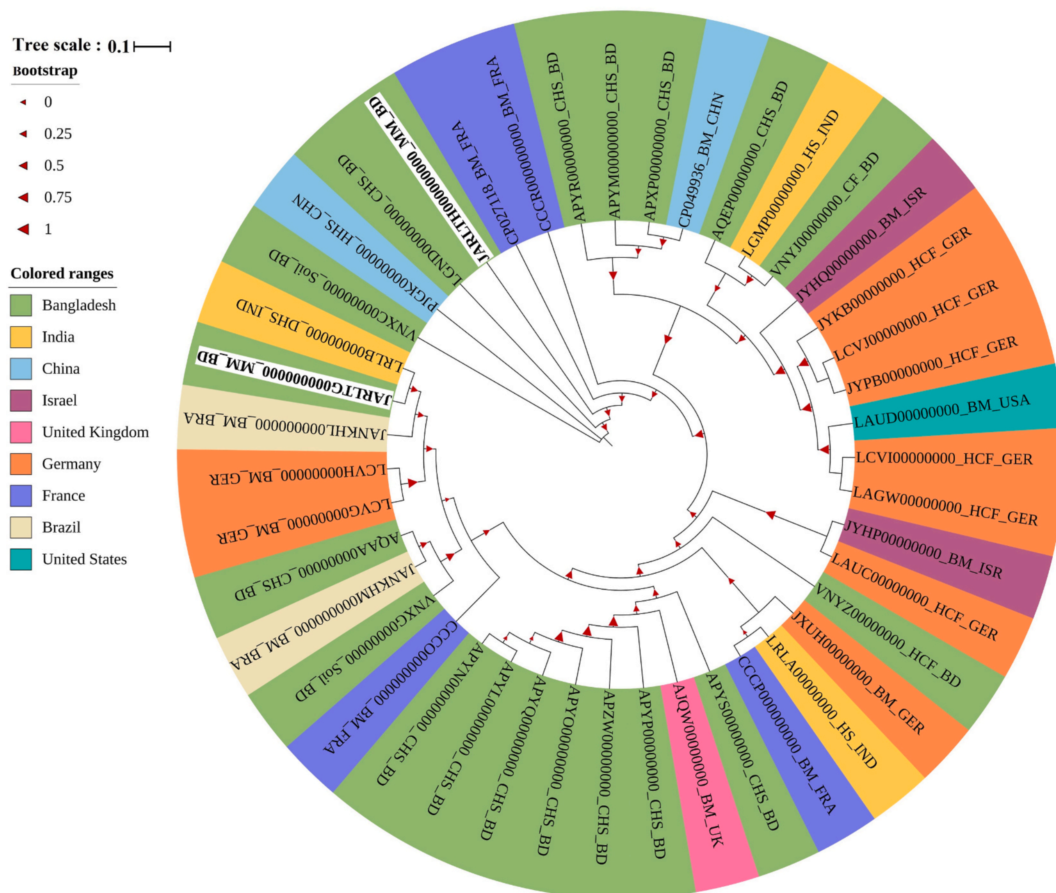

Fig. 3. The evolutionary phylogenetic relationships between *E. coli* G2M6U and G6M1F and other *E. coli* strains obtained from nine different countries of the world. Whole genome sequences of forty-one human and animal origin strains retrieved from NCBI were used for phylogenetic analysis. The mid-point rooted tree was constructed using the NCBI Tree Viewer (https://www.ncbi.nlm.nih.gov/tools/treeviewer/) and visualized with iTOL (Interactive Tree Of Life). The evolutionary relationship was inferred using the maximum-likelihood method. Different colors (e.g., green for Bangladesh, yellow for India, sky blue for China, purple for Israel, pink for United Kingdom, orange for Germany, light blue for France, colonial white for Brazil, and pastel turquoise for United States) are assigned according to the close evolutionary relatedness (clade) of the genomes. The scale bar is in the unit of the number of substitutions per site. The values on the branches are bootstrap support values based on 1000 replications. All the sequences were indicated by their accession numbers, followed by the host and country code. The country codes according to the standard abbreviation are: United States of America (USA), United Kingdom (UK), Germany (GER), Brazil (BRA), Israel (ISR), France (FR), China (CHN), India (IND) and Bangladesh (BD). The genomes of the *E. coli* strain G2M6U (JARLTH000000000_MM_BD) *E. coli* strain G6M1F (JARLTG000000000_MM_BD) are highlighted on white background. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

strains (VNXC00000000_Soil_BD, VNXG00000000_Soil_BD) (Fig. 3). These phylogenetic inferences were further supported by the findings from the average nucleotide identity (ANI) analysis which revealed that both G2M6U (GCA_029382245.1) and G6M1F (GCA_029382305.1) genomes clustered separately, and they shared 100.0% ANI with the closely related *E. coli* genomes (Fig. 4). Based on the SNP matrix-based phylogenetic inferences, G2M6U and G6M1F were found to fall in two different clades with other human enterotoxigenic and bovine mastitis associated *E. coli* strains (Fig. S1). We identified 7192 SNPs in G2M6U genome compared to a subset of 18 closely related reference genomes of *E. coli* (based on phylogenetic inference) while G6M1F was found to be more conserved with no predicted SNP (Fig. S2). In addition, a comparative genomic analysis was performed among these closely related strains where one of the bovine mastitis associated *E. coli* strains (*E. coli* 01T-32/03; NCBI GenBank accession: JANKHL000000000) was used as a reference. The genomic map obtained from the BRIG comparison did not show large scale variation between the bacterial genome sequences, and a significant number of non-homologous regions were found around the reference genome with over 95% identity (Fig. 5a). The G2M6U and G6M1F strains were found to share common regions of genetic variation with the reference strain (01T-32/03) and several enterotoxigenic *E. coli* strains of Bangladesh (603936), India (503688), China (LN6) and another environmental *E. coli* strain of Bangladesh (HH46S), at different sites on the genomes (Fig. 5a). Most of these non-homologous regions might be linked to transposable elements. We further performed pangenome analysis to better elucidate the diversity and differences in the study genomes and phylogenetically close eight reference strains. The pangenome matrix based on the presence and absence of genes

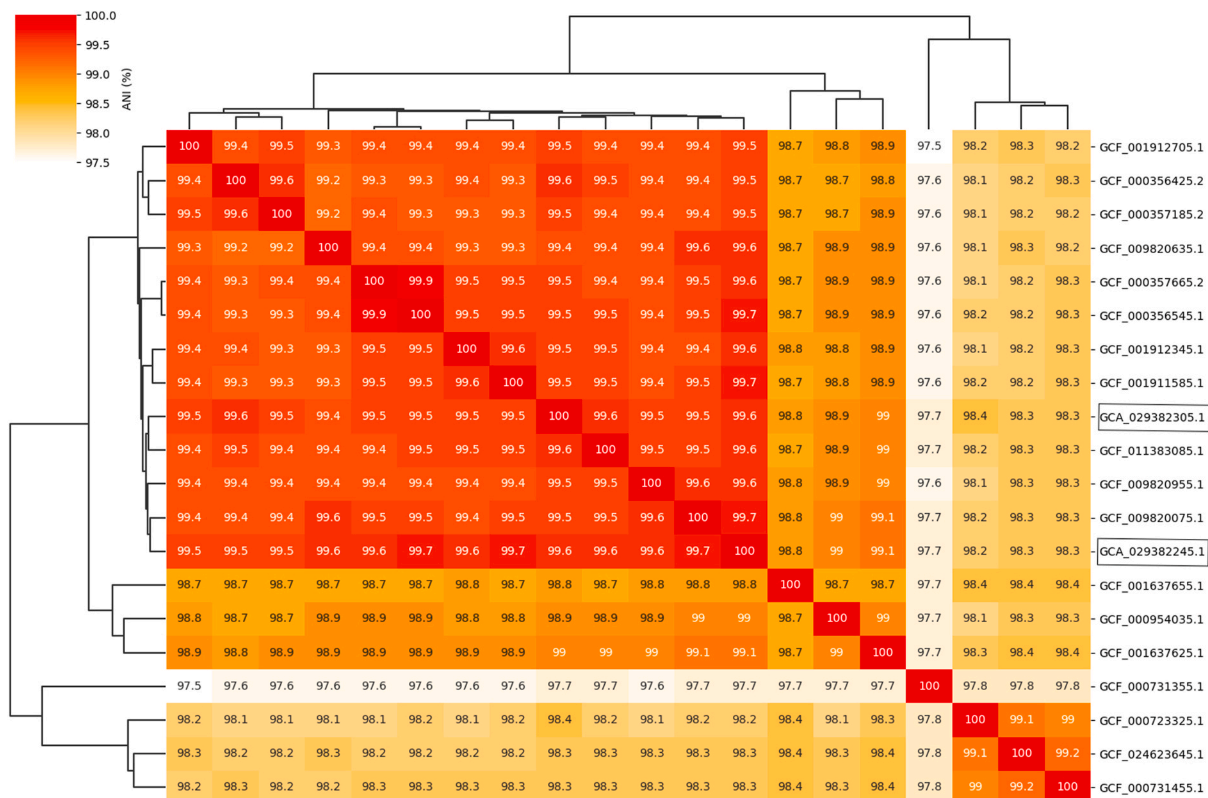

Fig. 4. Clustered heatmap of average nucleotide identity (ANI) values between study genomes (G2M6U; GCA_029382245.1 and G6M1F; GCA_029382305.1) and 18 reference genomes of the *E. coli* (previously used in phylogenetic analysis; Table S2). The numbers represent the ANI values (%) between two genome sequences. An ANI above 95% between two genomes is an indication that they belong to the same species. An ANI above 95% between two genomes is an indication that they belong to the same species. The color codes indicate ANI values, red for 100% identity and whitish yellow for 97.5% identity. Study genomes are highlighted in black box (in the Y-axis). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in ten valid strains showed clusters of genes and dendrogram of the closely related *E. coli* strains (Fig. 5b). In the pangenome dendrogram, *E. coli* strain HH34S was found as the closest relative of G2M6U whereas LN6 was the closest strain of G6M1F. A total of 7655 genes were estimated in the pangenomes. Among these, 3629 core genes were identified which were present in >99% sequences of the valid strains. We also detected 1408 shell genes and 2618 cloud genes, which were present in 15%–95% and <15% of genomes, respectively. However, no soft-core gene was found in the pangenome analysis. (Fig. 5c). Importantly, both of the studied strains (G2M6U and G6M1F), contained 43 and 111 unique genes in their genomes, and shared 68 genes with each other (Fig. 5d).

3.5. Resistome repertoire of the E. coli strains

Through a comprehensive resistome analysis, a total of 119 ARGs belonging to 18 functional classes with 7 different resistance mechanisms were predicted (Fig. 6). The composition and relative abundances of the predicted ARGs in both genomes varied significantly ($p < 0.05$), keeping higher number of ARGs in G6M1F strain. The G2M6U and G6M1F strains harbored 59 and 77 ARGs, respectively. Based on abundance information (percent identity and number of hits) of ARGs, a heatmap of abundance clustering for 30 predominantly abundant ARGs was built (Fig. 6a). Among these ARGs, *bla*TEM-1, *Qnr*S1, *sul*2, *tet*R, *aph*(6)-Id, *aph*(3″)-Ib, *bla*TEM-105, *dfr*A14, *emr*K, and *tet*A were the highly abundant genes, and these ARGs had unique association with gut associated G6M1F genome (Fig. 6a). However, the mammary tissue originated G2M6U strain of *E. coli* had relatively higher abundance of *mar*A, *acr*S, *EC*-18, *mdt*K, *bae*R, *mdt*G, *emr*R, *CRP*, *mdt*E, *PBP*2, *bae*S, *emr*B and *H-NS* genes. In resistome analysis, the highest number of ARGs (23.5%) were associated with multiple drug resistance (MDR; >3 antimicrobials), followed by fluoroquinolones resistance (12.6%), fluoroquinolone-penam-macrolide resistance (8.4%), peptide resistance (9.2%), aminoglycoside-aminocoumarin resistance (5.9%), cephalosporin, tetracycline, and macrolide resistance (3.36%, each) (Fig. 6b). By comparing the mechanism of resistance of the predicted ARGs, we found that more than 73% genes were encoding for antibiotic efflux, whereas, rest of the genes encode for antibiotic target replacement (12.6%), antibiotic inactivation (6.7%), reduced permeability of antibiotics and antibiotic target protection (~7.5%) (Fig. 6c). Furthermore, several mobile genetic elements (MGEs) were predicted in the genomes of *E. coli* G2M6U and G6M1F, notably in proximity to the ARG cassettes. This finding suggests the likelihood of horizontal transfer of ARGs (Fig. S3).

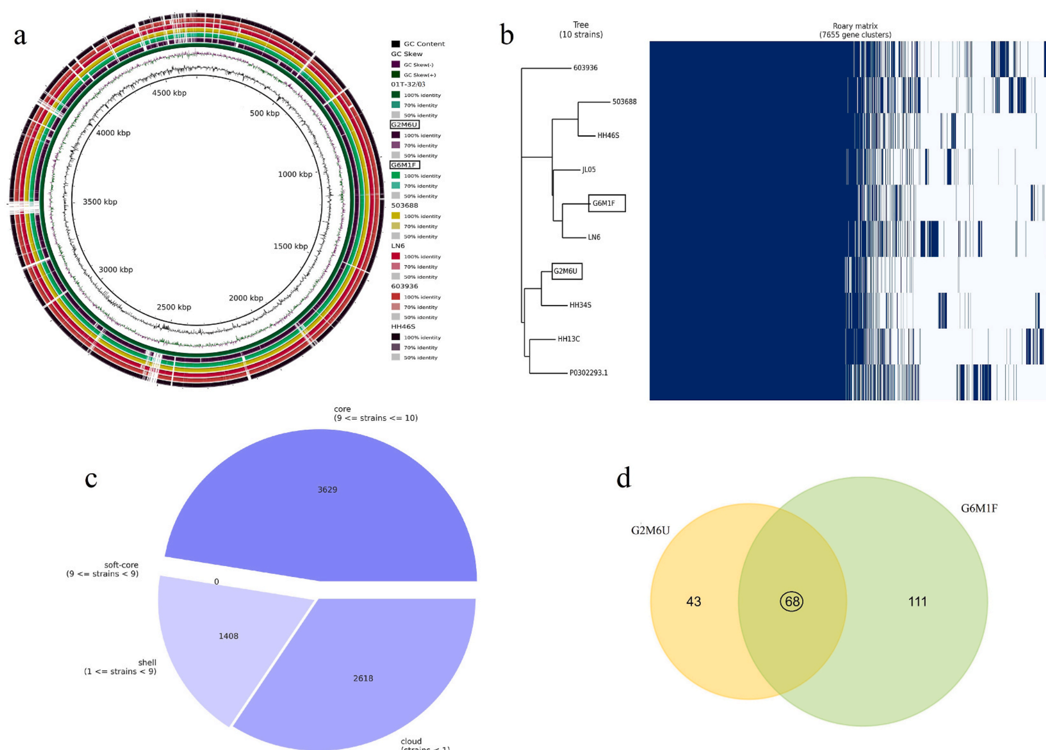

Fig. 5. Genomic characterization of *E. coli* strains (G2M6U and G6M1F). (**a**) Circular representation of the *E. coli* complete genomes. Circles (from inside to outside) 1 and 2 (GC content; black line and GC skew; purple and deep green lines), circle 3 (reference *E. coli* strain 01T-32/03; green circle); circle 4 (mapped *E. coli* G2M6U genome; deep purple circle); circle 5 (mapped *E. coli* G6M1F genome; light green circle); circle 6 (mapped *E. coli* strain 503688; olive circle); circle 7 (mapped *E. coli* strain LN6 genome; maroon circle), circle 8 (mapped *E. coli* strain 603936 genome; brick red circle), and circle 9 (mapped *E. coli* strain HH46S genome; black circle. Mapping of the genomes was done using BLASTn with an e-value cut-off 1^{e-5} (considered as a significant threshold) using BRIG 0.95. (**b**) Pangenome based (gene presence and absence) gene clustering matrix of G2M6U and G6M1F genomes (enclosed in black boxes) and closely related *E. coli* genomes from Bangladesh and beyond. (**c**) Breakdown of genes in G2M6U and G6M1F genomes. (**d**) Unique and shared genes in G2M6U and G6M1F genomes where shared genes are highlighted in a black circle. We generated the figures (**b-d**) based on the data obtained from Roary pangenome analysis using the roary_plots.py script. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. Metabolic and virulence potentials of the E. coli strains

The overall metabolic functional profile of the genomes is shown in Fig. 7a. We detected 367 and 378 metabolic features in SEED subsystems in G2M6U and G6M1F genome, respectively, with 32% coverage. Overall, there was a high degree of overlap in metabolic features predictions in both genomes. These subsystems were mostly represented by genes encoding for metabolisms of carbohydrates (17.85%), amino acid and derivatives (14.26%), protein (10.26%) and cofactors, vitamins, prosthetic groups and pigments (7.83%). In addition, 193 (4.92%) genes were annotated to be responsible for stress response, 163 (4.16%) for membrane transport, and 128 (3.26%) for regulation and cell signaling (Fig. 7a). Besides, secondary metabolite-biosynthetic gene clusters (BGCs) were predicted in both of the draft genomes. Th BGC gene clusters related to biosynthesis of *agr*D-like cyclic lactone autoinducer peptides, thiopeptide, non-ribosomal peptide synthetase cluster (NRPS)/NRP-metallophore domain, and post-translationally modified peptide product (RiPP) were predicted in G2M6U and G6M1F genomes (Fig. S4).

To better elucidate the virulence potentials, we comprehensively analyzed both of the genomes through ecoli_vf (https://github.com/phac-nml/ecoli_vf) and VFDB v6.0 databases. This virulome analysis revealed a wide array of VFGs related to intramammary infections experimentally induced mastitis in lactating mice. The study revealed significant variations ($p < 0.05$) in the composition of the detected VFGs between G2M6U and G6M1F genomes, indicating potential differences in pathogenicity, while maintaining consistent relative abundance of individual VFG ($p > 0.05$). In our study strains, 182 VFGs involved in adherence, aggregation, iron uptake, stress response, heat stability, hemolysis etc. were predicted. The G6M1F strain harbored higher number (178) of VFGs than G2M6U (159) (Fig. 7b). We also analyzed the relative abundances of these VFGs and compared the abundances in the G2M6U genome to the G6M1F genome. There were 155 VFGs, found to be shared between two genomes. However, 23 VFGs (e.g., *eha*A, *vgr*G, *hcp*, *aec*17-*aec*32, *clp*V, *vir*k, *agn*43, *int* etc.) were solely found in FS associated *E. coli* genome. Conversely, the MT related G2M6U genome had a unique association of four VFGs such as *fim*H, *fim*F, *tia* and *ast*A (Fig. 7b). Interestingly, *fde*C, *ent*A/B/C/D/E/F/S, *esp*L1, *fim*F/G/H, *omp*A, *rcs*B, and *ecp*A/B/C/D/E/R genes were found to be highly abundant in both of the study genomes. However, the only

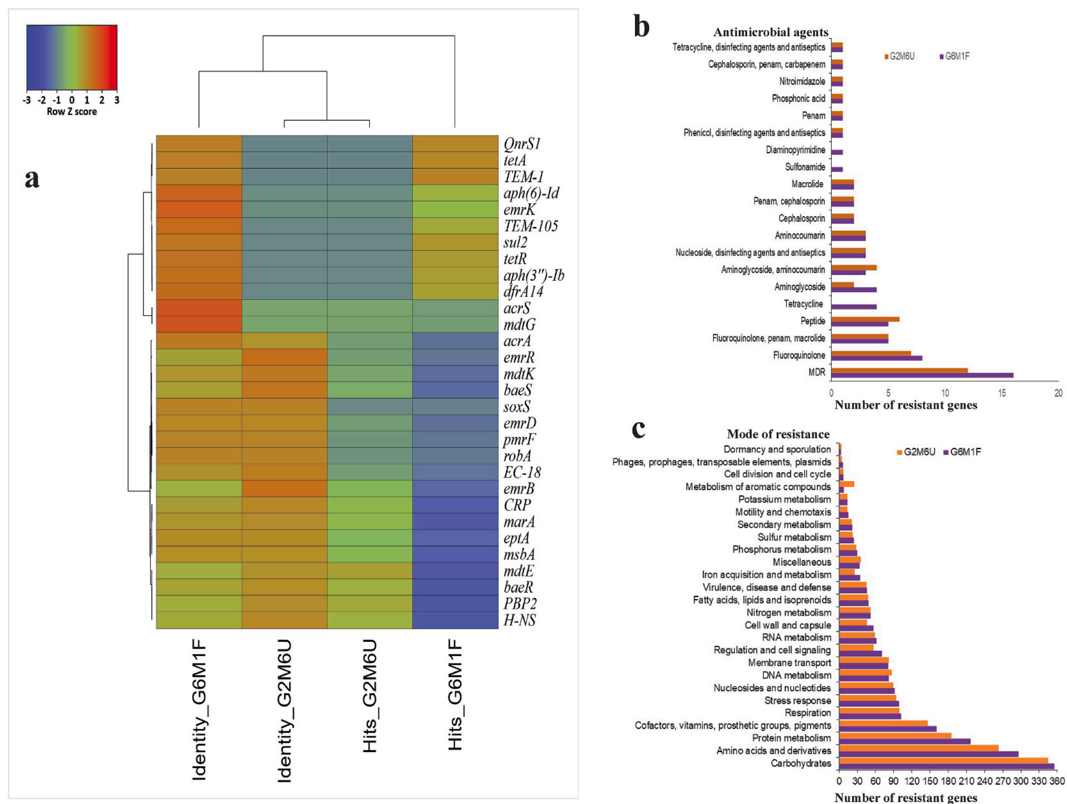

Fig. 6. An overview of the resistome in *E. coli* G2M6U and G6M1F genomes. (**a**) Clustered heatmap of top 30 abundant antimicrobial resistance genes (ARGs) based on identity and number of hits. The color bar (row Z score) at the top represents the relative abundances of the respective ARGs in G2M6U and G6M1F genomes. The color codes indicated the presence and completeness of each ARG, expressed as a value between −3 (lowest abundance) and 3 (highest abundance). The red color indicates the highest abundant patterns, while blue cells account for the least abundant ARGs in the corresponding genome. The heatmap is generated through FunRich (http://www.funrich.org/). (**b**) Bar plot of resistant antimicrobial agents (Y-axis) and associated ARGs (X-axis), and (**c**) bar plot of mode of resistance (Y-axis) and correlated ARGs (X-axis). The G2M6U and G6M1F genomes are represented in pink and blue, respectively. The color codes in the bar plots indicate strains of *E. coli* (i.e., orange for G2M6U and purple for G6M1F). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

enterotoxin producing gene (*ast*A) was not identified in the FS originated *E. coli* genome (G6M1F).

4. Discussion

Escherichia coli has emerged as a pathogen that not only causes mastitis in cattle [16,23] but also causing mastitis in women [62], mice [63] and other mammals [64,65] worldwide. In a previous study, we demonstrated that bovine mastitis pathogens (isolated from milk and feces) have the potential to induce mastitis in pregnant mice through a comprehensive cow-to-mouse mastitis model [6]. Additionally, Ma et al. reported that fecal transplantations from mastitic cows to mice also induced mastitis [7]. However, there have been limited studies on the role of individual pathogens present in microbiomes in the pathogenesis of murine mastitis. In the current study, we demonstrated distinct genetic features in *E. coli* isolated from mammary gland (MT) and gut (FS) of experimentally induced mastitis mice. This study has predicted the sequence type, evolutionary phylogeny, ARGs, VFGs, and genomic metabolic potentials of the isolates of *E. coli* from MT and FS. The findings of our research shed light on genetic relatedness of *E. coli* which is known as a predominant pathogen in mammalian mastitis [16,23,62,66,67].

We examined 46 isolates *E. coli* screened from MT and FS lactating mice suffered from induced mastitis through culture, biochemical tests and ribosomal (16S rRNA)-gene sequencing. The *in-vitro* antimicrobial assays showed that more than 80% *E. coli* isolates (including G2M6U and G6M1F) were MDR, showing resistance to > 3 antibiotics belonging to different classes. The in vitro antibiogram profiling revealed that *E. coli* isolates (n = 46) showed the highest resistance to oxacillin, aztreonam, nalidixic acid, streptomycin, cefoxitin, sulphonamide, ampicillin and gentamicin (75.0 = 100.0%) and moderate resistance to tetracycline (68.26%) and azithromycin (50.0–70.0%). These results underscore the concerning prevalence of antibiotic resistance among *E. coli* isolates associated with mastitis, and are similar to one of our previous studies where we reported that *E. coli* isolated from clinical mastitis milk exhibited the highest resistance against tetracycline, doxycycline, nalidixic acid, and ampicillin (77.0–93.0%) and moderate resistance to chloramphenicol, nitrofurantoin, gentamicin, and ciprofloxacin (40.0–63.0%) [4]. In this study, *E. coli* isolates from FS

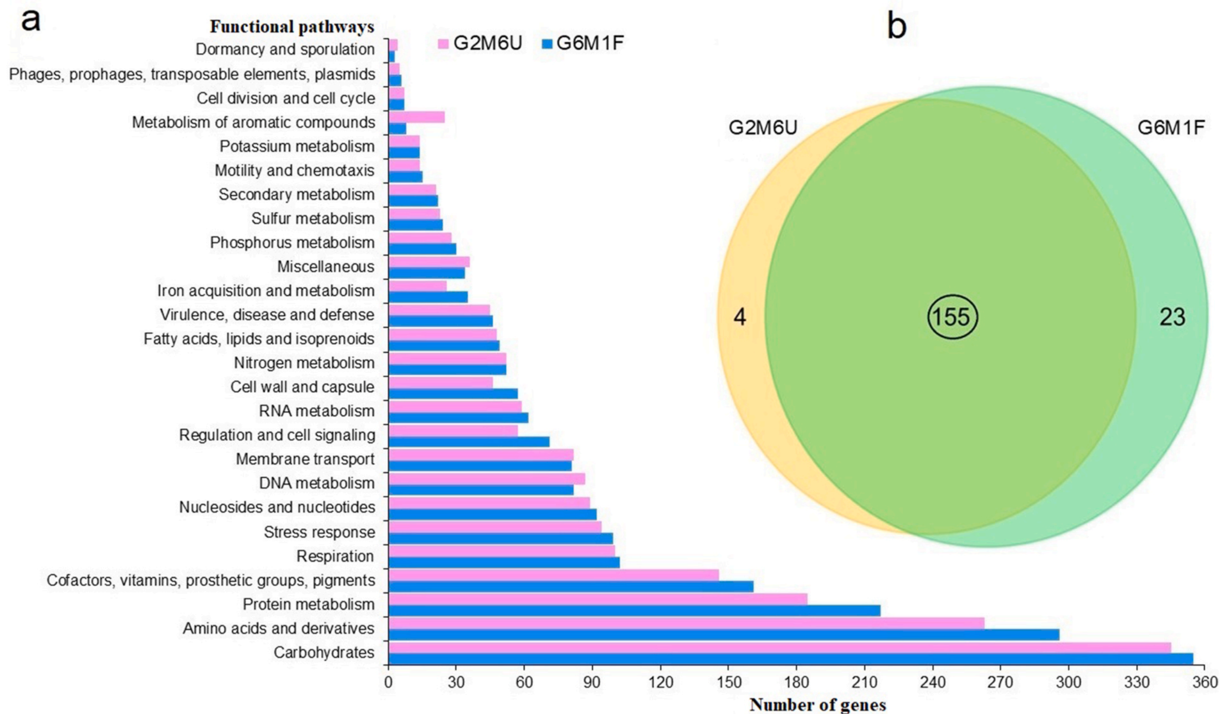

Fig. 7. An overview of the metabolic functions and virulence factor genes (VFGs) predicted in *E. coli* G2M6U and G6M1F genomes. (**a**) Bar plot showing metabolic functional pathways (Y-axis) and the assigned number of genes (X-axis), where G2M6U and G6M1F genomes are represented in pink and blue, respectively. (**b**) Venn diagram showing unique and shared virulence factor genes (VFGs) in G2M6U and G6M1F genomes. Shared VFGs in both genomes are highlighted in a black circle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

comparatively showed higher resistance but all *E. coli* isolates either from MT or FS showed susceptibility to ciprofloxacin, imipenem, chloramphenicol and doxycycline. These findings of high MDR patterns in murine mastitis associated *E. coli* strains are in line with many of previous studies on bovine and bubaline mastitis [4,20,68]. In a recent study Satpathy et al. from India reported that *E. coli* isolated from milk of indigenous Beetal goats showed the highest resistance to the beta-lactam group of antibiotics [69]. Mastitis associated *E. coli* also reported to show higher resistance against beta-lactam antibiotics in Canadian dairy herds [70], and amoxicillin, tetracycline and third-generation cephalosporin in the dairy herds of France [71], corroborating the findings of this study. A series of previous studies reported that antimicrobial resistance against mastitis causing bacteria could vary according to the type and origin of bacteria and hosts [20,23,64,68]. Nowadays, AMR is an increasingly serious threat to the dairy sector of Bangladesh compromising sustainable dairy development because of irrational overuse and widespread misuse of antibiotics [72]. The consistency between the current findings and the previous study suggests a persistent pattern of antibiotic resistance, emphasizing the need for careful antibiotic stewardship and surveillance.

The advent of whole genome sequencing (WGS) for bacterial pathogens have provided a new platform to study their molecular epidemiology and potential for virulence [36,73]. The draft genomes analyzed in this study exhibited high quality genome features for analysis, with 41–57 contigs and N_{50} values ranging from 140 to 184 kb for contigs larger than 1000 bp. While both genomes contained multiple prophage regions with more than 75 gene features, only the G6M1F genome harbored a plasmid replicon, specifically the IncY plasmid, which is commonly associated with beta-lactam resistance in *E. coli* [74]. Our investigation of the genomes also revealed the presence of three CRISPR arrays in each genome harboring 12 signature genes. CRISPR arrays, which have been identified in many bacterial pathogens (including *E. coli*) causing mastitis, play a significant role in host adaptive immune response and virulence [66]. The genomic data from this study showed that the *E. coli* isolate from MT (G2M6U strain) was genetically distinct from the one from FS (G6M1F strain) in murine mastitis. Based on core genome sequence typing, the G2M6U and G6M1F genomes were categorized as ST155 and ST58, respectively. Both strains exhibited a close evolutionary relationship with *E. coli* strains associated with enterotoxin production in humans and mastitis in cows. Our findings on core-genome typing (ST155 and ST58) are consistent with several previous studies that have reported the association of ST155 and ST58 *E. coli* strains with bovine mastitis cases [75–78].

An important finding of this study is the distinct genomic disparity observed between the G2M6U and G6M1F isolates. Further investigations of the closely related isolates by pairwise comparison of the ANI and number of core genome SNPs interestingly revealed genomic disparity between G2M6U and G6M1F. The G2M6U genome was found to underwent more than 7200 SNPs in its genomes. These results were consistent with the cgMLST, phylogenetic and ANI analyses, which provided evidence for a potential association between murine mastitis-associated *E. coli* strains and human enterotoxins producing *E. coli* lineages [79,80]. The phylogenetic

placement of the murine mastitis-causing *E. coli* isolates aligns with the core genome phylogeny. The analysis showed that G2M6U and G6M1F clustered separately, and they were more closely related to human enterotoxigenic *E. coli* strains from Bangladesh and India, bovine mastitis-causing strains from China (Jia 2020), France [81], and Brazil [82], as well as environmental soil-derived *E. coli* isolates from Bangladesh (https://rb.gy/pa88n). It is noteworthy that *E. coli* isolates assigned to MPEC phylogroups are commonly found as commensal microorganisms in the gut or in the environment [83]. This suggests that the association of enterotoxigenic and environmental *E. coli* strains in bovine and murine mastitis could be due to their ability to independently cause mastitis in different scenarios through evolutionary selective pressure. Additionally, the opportunistic recruitment of these strains from the normal gut commensal microbiota via the potential entero-mammary axis may contribute to the development of mastitis [6,7,84]. Therefore, our study provides important insights into the genomic differences between G2M6U and G6M1F isolates, supporting the association between murine mastitis-associated *E. coli* strains and human enterotoxigenic *E. coli* lineages. The findings also highlight the potential role of environmental and commensal *E. coli* strains in the development of mastitis in bovine and murine populations.

One of the key findings of this study is the ability to predict both the collection of antibiotic resistance genes (resistome) and virulence factor genes (virulome) in the genomes analyzed. The number and composition of antibiotic resistance genes (ARGs) and virulence factor genes (VFGs) consistently remained higher in the *E. coli* strain originating from FS. The in vitro resistance pattern of the isolates being studied aligns with the resistome profile obtained from whole genome sequencing data. The resistome profile, which includes the number of ARGs, resistant antibiotics, and mechanisms of resistance, observed in these isolates is consistent with that of MDR bacteria previously reported in mastitic cows [8,20], buffalo cows [68] and humans [85]. We observed that the majority of the ARGs were associated with MDR, indicating resistance to three or more antimicrobials. These ARGs primarily confer resistance through antibiotic efflux pumps, followed by target replacement/protection, inactivation, and reduced permeability mechanisms. Therefore, resistance to multiple drugs mediated by efflux pumps appears to be a widespread resistance mechanism in *E. coli,* likely due to the unethical overuse of antibiotics in dairy animals and the extensive application of toxic chemicals and metals in agricultural settings. Both *E. coli* strains exhibited variations in the composition and relative abundances of ARGs. For example, genes conferring resistance to quinolones (*Qnr*S1), sulphonamides (*sul*2), tetracyclines (*tet*A, *tet*R, *emr*K), beta-lactams (*bla*TEM-1/105), and aminoglycosides (*aph*(6)-Id, *aph*(3″)-Ib) were solely associated with G6M1F. In mastitis-causing bacteria, the composition and diversity of ARGs can vary greatly, likely due to differences in genetic diversity and selective pressures influencing ARG maintenance [4]. These ARGs can easily spread through their host bacteria to different hosts inhabitant of other ecosystems [86,87]. The ARGs identified in this study are of particular concern because the use of this classes of antibiotics in veterinary medicine, especially for food animals, may contribute to the development of antibiotic resistance in humans.

We aimed to identify metabolic functional genes and/or pathways in the two strains of *E. coli.* Our analysis predicted several important genes and proteins in the genomes of G2M6U and G6M1F that are associated with different subsystem categories and metabolic functions, supporting their implications with mastitis pathogenesis [88,89]. We found that similar metabolic features identified in the same SEED subsystem varied between the MT and FS strains, suggesting their possible involvement in early colonization and disease progression [18]. The studied strains possessed a higher number of genes involved in metabolism of carbohydrates, proteins, cofactors and vitamins, stress response, membrane transport, regulation and cell signaling, as well as virulence, disease, and defense. Previous research has indicated that bacterial metabolites play a role in modulating host immune functions and disease pathophysiology [6,18,90]. Additionally, we identified several gene clusters involved in secondary metabolite biosynthesis in the genome sequences of G2M6U and G6M1F. These secondary metabolites could potentially serve as a valuable source of novel bioactive compounds. Previous studies have shown that secondary metabolites produced by *E. coli* contribute to its pathogenicity [91], although the specific role may vary depending on the type and quantity of these bioactive compounds. Therefore, further investigation should be conducted to explore the secondary metabolites produced by *E. coli* and their pathogenic properties in mastitis.

The WGS analysis of G2M6U and G6M1F revealed a wide variety of VFGs present in the genomes. These VFGs play a crucial role in mastitis-causing *E. coli* by helping them evade host defenses and successfully colonize the udder [92,93]. Additionally, they contribute to the subsequent development of mammary gland pathogenesis by sensing specific metabolites produced by the pathogens [94]. It is worth noting that both genomes studied contain several VFGs associated with adhesion and invasion (*fde*C, *omp*A), stress response (*rcs*B), binding activity (*fim*F/G/H), enterobactin production (*ent*A/B/C/D/E/F/S), host cells response (*esp*L1) and *E. coli* common pilus (ECP) operon (*ecp*A/B/C/D/E/R). These genes are associated with several enteric diseases [28,95], suggesting the pathogenic potentials of the both genomes. An important finding from this study is the identification of a heat-stable enterotoxin producing gene (*ast*A) in *E. coli* strain G2M6U, which is typically associated with the production of factors that facilitate the colonization this pathogen outside of the intestines [95]. However, the specific mechanisms through which these VFGs contribute to microbial colonization in the udder are not well understood. Nevertheless, our data strongly suggest that the adhesion and binding abilities, enterobactin and heat-stable enterotoxin production, as well as multiple antibiotic resistance through efflux pumps, are essential genomic attributes that enable *E. coli* strains to induce inflammation in the mammary gland. We investigated a number of genomic features in *E. coli* strains G2M6U and G6M1F, particularly focusing on their roles in AMR development, colonization, and virulence. Through a comparative analysis with unique genes in each strain, we identified conserved elements among annotated genes in *E. coli* genomes. This analysis reveals shared and unique genetic traits, providing key insights into molecular resistance mechanisms. It guides targeted therapeutic development and has significant implications for microbiology and infectious diseases. Additionally, the research provides insights into epidemiological patterns, genetic diversity predictions, and potential diagnostic markers for pathogenic strains. This comprehensive perspective on genetic factors is valuable for informing public health interventions. Specifically, the study enhances our understanding of AMR in *E. coli*-associated mastitis in dairy cattle, offering insights into prevalence, resistance patterns, genetic mechanisms, treatment efficacy, and broader implications for One Health. These findings are crucial for guiding appropriate treatment strategies and improving our understanding of the AMR situation in dairy animals.

Our study is constrained by a relatively fewer number of *E. coli* genomes (n = 2) sequenced from mammary tissue and gut (feces) of experimentally induced mastitis mice, making it challenging to generalize findings to a firm conclusion. Addressing this limitation requires future research with a larger sample size, employing robust experimental design and statistical analysis for a more comprehensive understanding of the pathophysiology of *E. coli* associated mastitis in different hosts. Exploring differences in the genomic characteristics of a wider number of *E. coli* genomes sequenced from diverse sample categories may reveal the emergence of new variants and their association with mammalian mastitis.

5. Conclusion

Herein this study, we demonstrated a distinct genetic characteristic in two MDR *E. coli* strains, G2M6U and G6M1F, isolated from mammary tissue and fecal samples of experimentally induced mastitis mice. These strains exhibited a diverse resistome, containing ARGs that confer resistance through efflux pumps and beta lactamase enzymes, contributing to their MDR genotype. Additionally, both strains carried a virulome, containing important VFGs involved in adhesion, invasion, stress response, enterobactin production, host cell response, and heat-stable enterotoxin production. Phylogenetic and comparative genome analysis revealed genetic similarities between these two strains are related to human enterotoxigenic, bovine mastitis-causing, and environmental soil origin *E. coli* strains of diverse geographical regions. This suggests the emergence of novel variants with potential associations to mammalian mastitis. However, further investigations are needed to understand the different virulence strategies employed by gut and mammary gland-associated *E. coli* strains in mastitis pathogenesis across different hosts and demographics. These findings have implications for the development of new strategies for mastitis prevention, treatment, and control.

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Data availability statement

This whole genome shotgun projects of G2M6U and G6M1F has been deposited at the NCBI GenBank under the accession number JARLTH000000000 and JARLTG000000000, respectively. Sequencing data were deposited in GenBank and the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA946194.

CRediT authorship contribution statement

M. Nazmul Hoque: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Golam Mahbub Faisal:** Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation. **Shobnom Jerin:** Methodology, Investigation, Data curation. **Zannatara Moyna:** Methodology, Investigation, Formal analysis, Data curation. **Md Aminul Islam:** Writing – review & editing, Methodology. **Anup Kumar Talukder:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Mohammad Shah Alam:** Writing – review & editing, Supervision. **Ziban Chandra Das:** Writing – original draft, Validation, Resources, Project administration, Investigation, Funding acquisition. **Tofazzal Isalm:** Writing – review & editing, Validation, Supervision, Software, Resources. **M. Anwar Hossain:** Writing – review & editing, Validation, Supervision, Conceptualization. **Abu Nasar Md Aminoor Rahman:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26723.

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