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Genomic Analysis of Multidrug-Resistant *Escherichia coli* from Surface Water in Northeast Georgia, United States: Presence of an ST131 Epidemic Strain Containing $bla_{\text{CTX-M-15}}$ on a Phage-Like Plasmid

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Surface water is suspected of playing a role in the development and spread of antimicrobial-resistant (AR) bacteria, including human pathogens. In our previous study, 496 *Escherichia coli* isolates were recovered from water samples collected over a 2-year period from the Upper Oconee watershed, Athens, GA, United States, of which 34 (6.9%) were AR isolates. Of these, six isolates were selected based on their multidrug resistance (MDR) phenotypes, the presence of mobile genetic elements, and their pathogenic potential and were subjected to whole-genome sequence (WGS) analysis to enhance our understanding of environmental MDR *E. coli* isolates. This study is the first report on genomic characterization of MDR *E. coli* from environmental water in the United States through a WGS approach. The sequences of the six MDR *E. coli* isolates were analyzed and the locations of their AR genes were identified. One of the *E. coli* isolates was an ST131 epidemic strain, which also produced an extended-spectrum β-lactamase encoded by the $bla_{\text{CTX-M-15}}$ gene, carried on a plasmid that is a member of a very rarely reported family of phage-like plasmids. This is the first time an in-depth sequence analysis has been done on a $bla_{\text{CTX-M-15}}$- containing phage-like plasmid, the presence of which suggests a new emerging mechanism of AR gene transmission.

Keywords: ST131, phage-like plasmid, surface water

Introduction

Escherichia coli is a commensal organism and an occasional pathogen in the gastrointestinal tracts of humans and other warm-blooded animals and is frequently associated with intestinal and extraintestinal infections, including urinary tract infections and bacteremia.[1] *E. coli* can persist in surface water, and therefore, the occurrence of antimicrobial-resistant (AR) *E. coli* in environmental water increases concerns about the spread of AR. *E. coli* easily exchanges AR genes through horizontal gene transfer and thus serves as vectors for the dissemination of AR genes to other pathogenic bacteria in aquatic environments.[2,3] These AR bacteria may limit therapeutic options if infections occur in humans through direct ingestion or contact with contaminated water, for instance, during water-related recreational activities or indirectly through consumption of fresh produce irrigated with contaminated water.[4] In addition, food animals with access to surface water can become colonized with AR bacteria, which could be transmitted to humans through close contact with the animals or through contamination of meat during processing.[5]

E. coli producing extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC) β-lactamase, which mediate resistance to β-lactam antibiotics, including-third generation cephalosporins, are widely disseminated in health care, community settings, and food animals, and represent a public health risk.[6–10] On the contrary, information on ESBL- and pAmpC β-lactamase-producing *E. coli* from the U.S. water environments is limited with most of the water studies focusing on wastewater plant discharges. Moreover, these limited number of current studies focus on the testing of phenotypic resistance to later generation cephalosporins and detection of the genes responsible for the observed resistance

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phenotypes, while detailed analysis of the sequence data on environmental water isolates through a whole-genome approach is lacking.

Our recent study has shown that AR *E. coli* are present in the surface water of the Upper Oconee watershed, a mixed-use watershed in northeast Georgia, United States, including those producing β-lactamase (TEM-1), ESBL (CTX-M-15), and pAmpC β-lactamase (CMY-2).[11,12] For the present study, a subset of those isolates was selected based on the maximum number of different AR phenotypes, different multidrug-resistant (MDR) phenotypes (resistance to three or more classes of antimicrobial drugs; Table 1), and the presence of different AR genes and mobile genetic elements (MGEs).[12] Whole-genome sequencing (WGS) was performed on these isolates to identify the comprehensive profiles of AR genes in the isolates and their locations on MGEs. This study provides an enhanced understanding of the genetic characteristics of MDR *E. coli* isolates recovered from environmental water, such as their genetic content and phylogeny, and improves our limited knowledge on the mechanisms of AR gene transfer and the potential for AR gene transmission in the water environments.

Materials and Methods

E. coli *isolates*

Six MDR *E. coli* isolates with phenotypic resistances to most antimicrobials and presenting different combinations of AR phenotypes, AR genes, and MGEs[12] were selected for further characterization.[11]

Whole-genome sequencing

WGS of the isolates was carried out. Briefly, genomic DNA of overnight grown cultures was isolated using the GenElute™ bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO). The DNA libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, Inc., San Diego, CA), as per the manufacturer's instructions.

Table 1. Antimicrobial Resistance Phenotypes and Genes Identified in the *Escherichia coli* Isolates with Their Locations

Isolate	*AR phenotype*[a]	*Gene*[b]	*Location*[c]
3 mTEC	AmpCipNalStrSulTetTri	(Bla) $bla_{\text{TEM-1}}$	Plasmid
		(AGly) *strB*	Plasmid
		(Sul) *sul2*	Plasmid
		(AGly) *strA*	Plasmid
		(Tet) *tetA*	N/A
		(Tet) *tetR*	N/A
		(Tmt) *dfrA14*	Plasmid, In191
40 ECC	StrSulTet	(Sul) *sul1*	HI1, In157
		(AGly) *aadA22*	HI1, In157
		(Tet) *tetB*	HI1
238 ECC	AmpAziStrSulTetTri	(Tet) *tetA*	FII
		(Tet) *tetR*	FII
		(MLS) *mphA*	Plasmid
		(Tmt) *dfrA14*	Plasmid, In191
		(AGly) *strB*	Q1
		(Sul) *sul2*	Q1
		(AGly) *strA*	Q1
		(Bla) $bla_{\text{TEM-1}}$	Plasmid
303 ECC	AmoAmpAxoGenFoxTio	(Phe) *cmlA5*	Plasmid
		(Bla) $bla_{\text{CMY-2}}$	Plasmid
		(AGly) *aadB*	Plasmid
		(Bla) $bla_{\text{TEM-1}}$	Plasmid
367 ECC	AmpAxoNalTio	(Bla) $bla_{\text{CTX-M-15}}$	Phage-like plasmid
		(Bla) $bla_{\text{TEM-1}}$	FII
381 ECC	AziSulTetTri	(Sul) *sul2*	Chromosomal
		(Tet) *tetA*	Plasmid
		(Tet) *tetR*	Plasmid
		(MLS) *mphA*	Plasmid
		(AGly) *aadA3*	Plasmid, In1603
		(Tmt) *dfrA12*	Plasmid, In1603

[a]Antimicrobials: Amo, amoxicillin/clavulanic acid; Amp, ampicillin; Axo, ceftriaxone; Azi, azithromycin; Cip, ciprofloxacin; Fox, cefoxitin; Gen, gentamicin; Nal, nalidixic acid; Str, streptomycin; Sul, sulfisoxazole; Tet, tetracycline; Tio, ceftiofur; Tri, trimethoprim/sulfamethoxazole.

[b]Class of antimicrobials: Agly, aminoglycosides; Bla, β-lactams; MLS, macrolide–lincosamide–streptogramin; Phe, phenicols; Sul, sulfonamides; Tet, tetracyclines; Tmt, trimethoprim.

[c]''Plasmid'' indicates that the contig containing the gene was homologous to a plasmid sequence but that sequence could not be connected to a replicon type detected.

AR, antimicrobial resistant; N/A, not available.

The concentration and fragment size distribution of the DNA libraries were checked on a Qubit fluorometer, using the double-stranded DNA HS assay kit (Life Technologies, Inc., Carlsbad, CA), and Bioanalyzer 2100, using an Agilent high-sensitivity DNA kit (Agilent Technologies, Santa Clara, CA), respectively. Paired-end sequencing (2×250 bp) of the DNA libraries was performed on Illumina MiSeq using the 500-cycle MiSeq reagent kit version 2 (Illumina, Inc.). The generated reads were assembled *de novo* using the A5-miseq assembler, and the genome sequence was annotated using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline.[13,14]

WGS analysis

Plasmid replicon-associated genes were identified using Plasmid Finder, and AR genes were identified using ARG-ANNOT.[15,16] The contigs containing AR genes but not a replicon gene were compared with the NCBI nonredundant (nr) database using BLAST.[17,18] The top hits were used to determine whether they belonged to a plasmid, chromosome, or something else. Integrons were identified using INTEGRALL, and *in silico* PCR was run using previously described primers to detect integron-associated AR genes in the six sequenced isolates.[19,20] To build a phylogenetic tree, *de novo* assembled and preassembled genome sequences were annotated with Prokka v1.11., and core genome alignment was done with Roary v3.6.8.[21,22] The positions of the six isolates in different *E. coli* subgroups were inferred through the phylogenetic analysis using RAxML 7.8.6 with 100 bootstrap value.[23] The RAxML output was visualized with FigTree 1.4.2.[24] The reference sequences for each phylogroup were obtained from the NCBI GenBank.

Upon identifying a phage-like plasmid containing a $bla_{\text{CTX-M-15}}$ gene in one of the isolates, the sequence was compared with the NCBI nr database using BLAST and nine similar hits were identified. These were compared using a tree built using the Orthologous Average Nucleotide Identity Tool (OAT).[25] The contig containing the $bla_{\text{CTX-M-15}}$ gene and the two other most homologous phage-like plasmids identified from the database (GenBank accession numbers: KY515224 and KY515225) were aligned using SnapGene (GSL Biotech LLC, Chicago, IL). The identical flanking sequence of the $bla_{\text{CTX-M-15}}$ gene in all three sequences was used to define a CTX-M-15 AR cassette (ARC). This ARC was compared with the NCBI nr database using BLAST.

Because of a 249 bp direct repeat at both ends of the contig containing $bla_{\text{CTX-M-15}}$, the phage-like plasmid was believed to be circular, which was confirmed by PCR. A forward primer complementary to the 3′ end (5′-GGGGAATGATT AACCTGGTTAGC-3′) and a reverse primer complementary to the 5′ end (5′-CGGTTAACGTCATGGCAAATCCTT-3′) were designed and used in the PCR. Thermal PCR conditions consisted of an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min, elongation at 72°C for 1 min, and a final extension at 72°C for 7 min. Amplified PCR products (540 bp in size) were purified using a Qiagen QIAquick PCR purification kit (Qiagen, Germantown, MD) and sequenced using a BigDye Ready Reaction Mix (Applied Biosystems, Foster City, CA) and the ABI Prism 3130x Genetic Analyzer (Applied Biosystems) according to the manufacturer's directions.

Conjugation assays

Conjugation assays were performed to evaluate the transferability of MGEs containing β-lactamase genes. The experiments were performed by filter mating using the two MDR *E. coli* isolates, one with $bla_{\text{CMY-2}}$ and $bla_{\text{TEM-1}}$ (303 ECC) and the other with $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ (367 ECC), as the donor strains and sodium azide-resistant *E. coli* J53 as the recipient strain. Multiple transconjugants were selected using brain/heart infusion agar plates containing cefotaxime (4 mg/L) and sodium azide (300 mg/L). Phylogenetic grouping PCR was run on the transconjugants.[26] Susceptibility testing was performed by broth microdilution to determine the minimum inhibitory concentrations (MICs) of the transconjugants for antimicrobials; AR gene PCR and plasmid replicon typing were performed on the transconjugants to verify the presence of $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-1}}$ genes and plasmid replicon types, respectively.

Electron microscopy

Negative staining and transmission electron microscopy were carried out on the phage-like plasmid-containing isolate 367 ECC, both before and after the mitomycin C treatment using a previously described method to test whether the phage-like plasmid can produce viable phage particles.[27]

Results

WGS analysis

AR genes were associated with specific replicon-type plasmids in three of the six isolates sequenced (Table 1). In the isolate 40 ECC, an IncHI1 plasmid was associated with *sul1*, *aadA22*, and *tetB*. In 238 ECC, an F plasmid contained *tetA* and *tetR*, while 367 ECC contained an F plasmid with $bla_{\text{TEM-1}}$. 238 ECC also contained another small plasmid, a Q1, which encoded only the resistance genes, *strA*, *strB*, and *sul2*, in addition to the Q1 *repA* gene. Other isolates contained resistance genes likely associated with plasmids, but they could not be linked to specific replicons by location on the same contig or homology to a plasmid of the replicon type identified in the isolate (Table 1). In the isolate 381 ECC, *sul2* was chromosomal and contained within a 25 kb insertion similar to the *E. coli* strain 6409, complete genome (GenBank accession number: CP010371.1).

In silico PCR detected a class 1 integron in four of the six genomes (3 mTEC, 40 ECC, 238 ECC, and 381 ECC) (Table 1), unlike the PCR method that detected the class 1 integron in three of the six isolates (40 ECC, 238 ECC, and 381 ECC). Three of the four class 1 integrons were identical to previously reported integrons, and one isolate had a novel integron and was assigned a new In number. An integron detected in 40 ECC, In157, was located on the HI1 plasmid and contained *sul1* and *aadA22* genes. Two isolates, 3 mTEC and 238 ECC, carried the same In191 integron that has the *dfrA14* gene as a single gene cassette, while 381 ECC displayed a novel array of gene cassettes and was assigned a new In number, In1603. The newly defined In1603 carried

aadA3 and *dfrA12* genes. Isolate 303 ECC had a sequence matching an integron, however, there was an insertion sequence in the *intI1* region, disrupting the integron containing *cmlA5*, *aadB*, and *sul1*. This *sul1* gene was a partial gene as it was disrupted by the insertion of Tn2, which is likely why the isolate did not present a sulfisoxazole-resistant phenotype.

Sequence analysis of a CTX-M-15 ESBL-producing ST131 isolate, 367 ECC, revealed that its $bla_{\text{CTX-M-15}}$ gene was located on a unique phage-like plasmid, tentatively named pSC367ECC-1 (Fig. 1). It was homologous to seven other phage-like plasmids identified in *E. coli*, the cryptic plasmid pHCM2 of MDR *Salmonella* Typhi CT-18, as well as the SSU5 phage of *Salmonella* in the NCBI nr database (Fig. 2). These phage-like plasmids consist of extrachromosomal DNA that contains both phage genes and plasmid DNA. A 2,973 bp ARC sequence containing the $bla_{\text{CTX-M-15}}$ gene, as well as a transposase and metalloprotein, was identified on pSC367ECC-1 (Fig. 1). This sequence was compared with the NCBI nr database and determined to be in 277 other NCBI records, including 182 plasmid records, of 10 different species (Table 2 and Supplementary Table S1). PCR determined that the phage-like plasmid pSC367ECC-1 was a 110,948 bp circular plasmid.

The phylogenetic positions of the six *E. coli* isolates were interpreted by comparing their genome sequences with other *E. coli* strains. The core genome obtained from Roary was used for phylogenetic tree interpretation with RAxML, and *E. fergusonii* was included as an outgroup. The *E. coli* isolates were highly diverse and clustered tightly into different phylogenetic subgroups in the phylogenetic tree (Fig. 3). The isolates 303 ECC and 367 ECC clustered together with other *E. coli* members of the phylogenetic group B2, whereas the isolates 40 ECC and 3 mTEC clustered on separate branches with members of the phylogenetic group B1. Isolates 238 ECC and 381 ECC clustered with the phylogenetic group D1 and A, respectively.

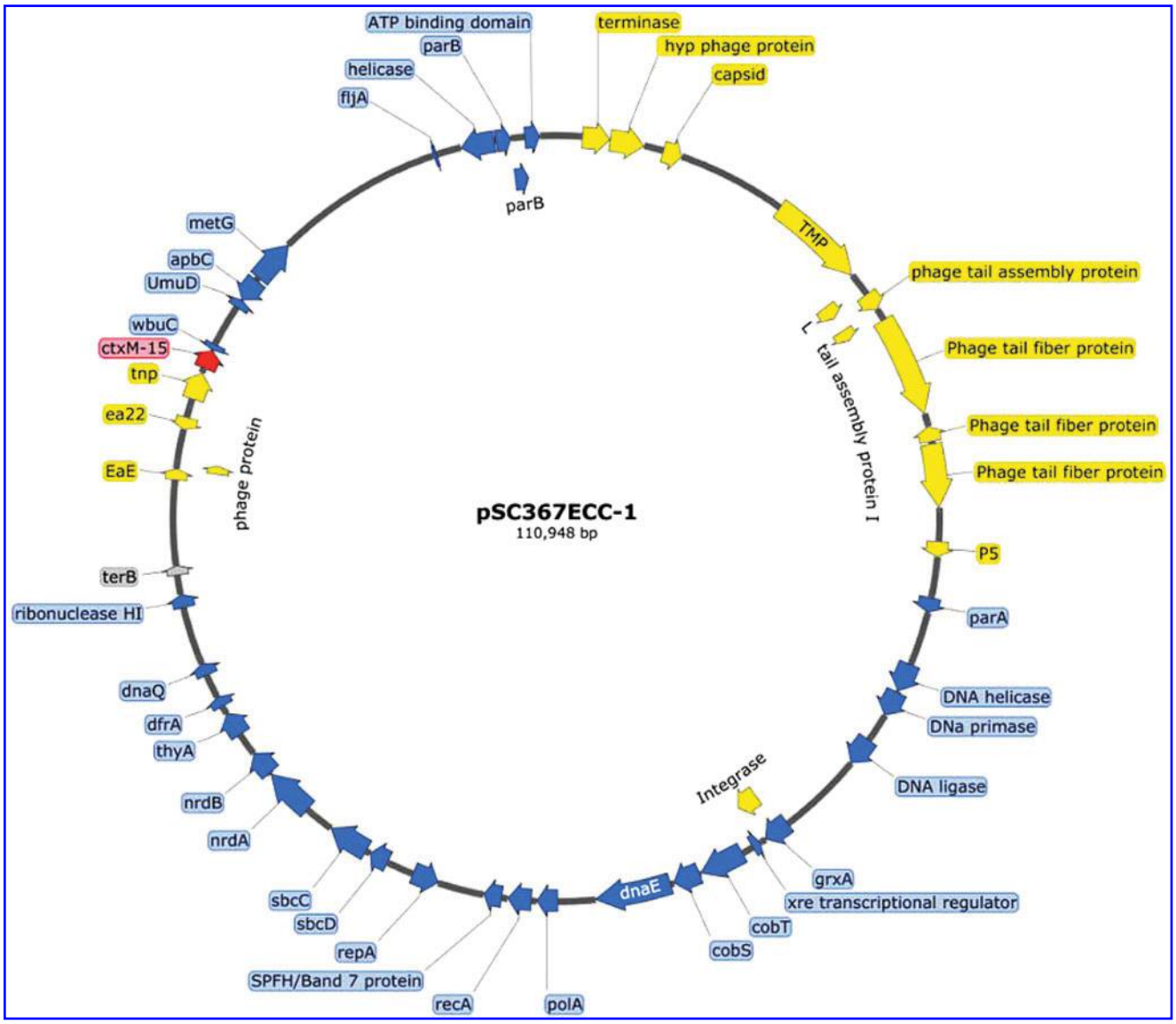

FIG. 1. Genetic map of genes and protein products for pSC367ECC-1, a phage-like plasmid containing $bla_{\text{CTX-M-15}}$ gene. Antimicrobial resistance genes are *red*, genes associated with mobile elements are *yellow*, metal resistance genes are *gray*, and other genes are *blue*. Hypothetical genes are not included in the map unless they were annotated as specific hypothetical proteins.

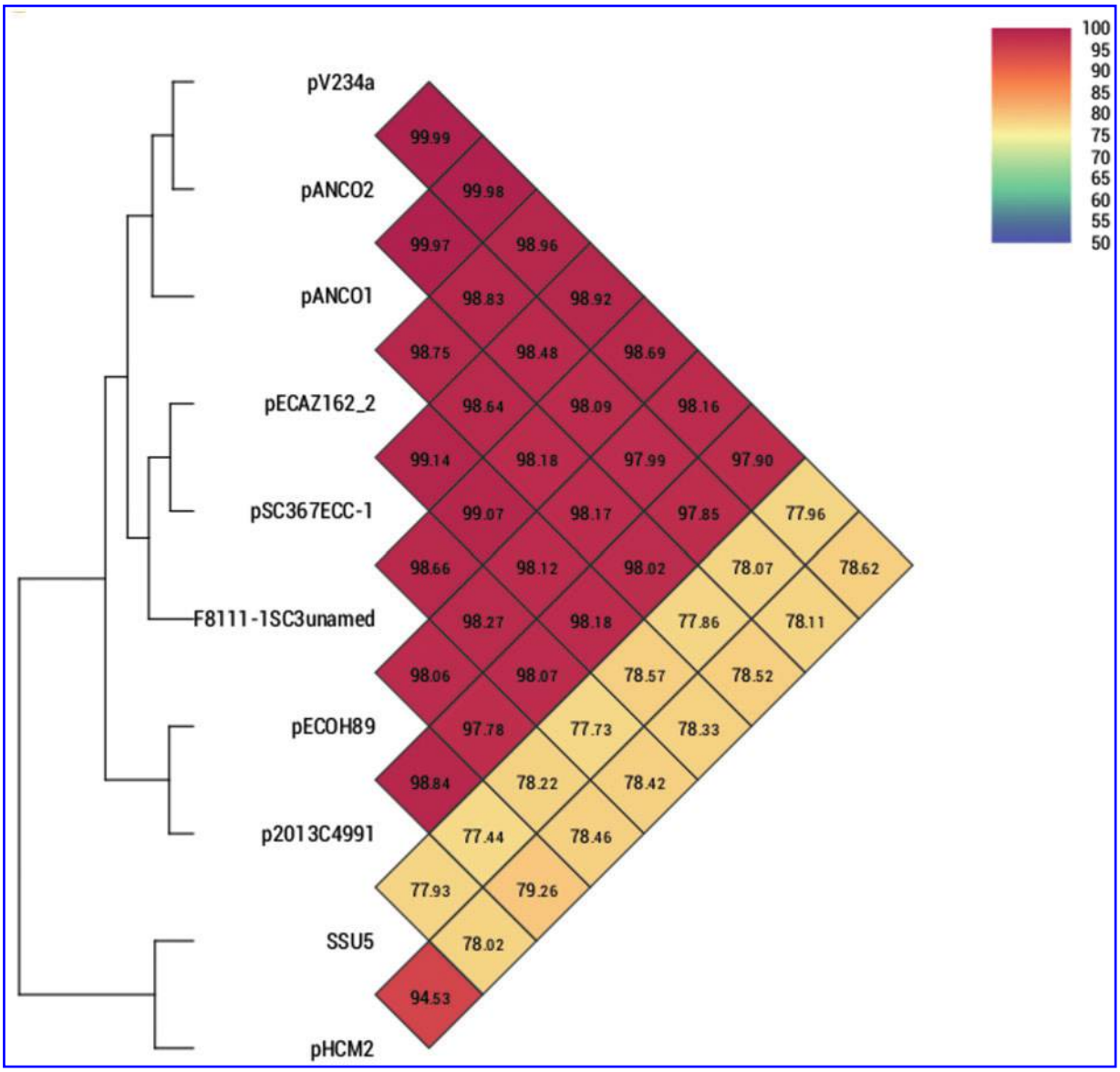

FIG. 2. Average nucleotide identity and phylogenetic tree of phage-like plasmid, pSC367ECC-1 based on OrthoANI with closely related *Escherichia coli* plasmids (F8111-1SC3:CP024271, p2013C4991:CP027358, pANCO1:KY515224, pANCO2:KY515225, pECAZ162_2:CP019013, pECOH8:HG530657, pV234a:LC056430), *Salmonella* plasmid (pHCM2:NC_003385), and *Salmonella* phage (SSU5:JQ965645).

Electron microscopy

Phage particles were not observed using negative staining or transmission electron microscopy, indicating that the phage portion of the phage-like plasmid cannot be activated even after mitomycin C treatment was used to induce the phage lytic cycle (data not shown).

Conjugation assays

A conjugation assay was conducted to determine whether $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-1}}$ were transferable to *E. coli* J53, and therefore, if pSC367ECC-1 is conjugative. Conjugation experiments demonstrated that one of the two *E. coli* isolates tested, 303 ECC, was able to transfer its β-lactam resistance. PCR identified the presence of plasmid replicon I1α and FIB, as well as $bla_{\text{CMY-2}}$ and $bla_{\text{TEM-1}}$, in the donor as well as its transconjugants. MICs of antimicrobials for the transconjugants were increased compared with the recipient, *E. coli* J53, and with the donor for some antimicrobials, including cefotaxime and ceftazidime (Table 3). Mating of 367 ECC with J53 did not yield any transconjugants, indicating pSC367ECC-1 is not self-transferable under the *in vitro* conjugation conditions used in this assay.

Discussion

WGS analysis revealed that the ST131 367 ECC isolate contained $bla_{\text{CTX-M-15}}$ on a rarely reported phage-like plasmid, with this study being only its second presentation in the literature.[28] This particular phage-like plasmid could be part of an emerging lineage of phage-like plasmids. The phage-

Table 2. National Center for Biotechnology Information Database Results of Organisms with the Cassette Sequences Containing *bla*$_{CTX-M-15}$

Bacterial species	*Genetic location*	*No. of isolates*
Citrobacter freundii	Plasmid	2
Enterobacter cloacae	Plasmid	2
	Unknown	2
Escherichia coli	Plasmid	67
	Unknown	38
Enterobacter sp.	Plasmid	1
Enterobacteriaceae sp.	Plasmid	1
Klebsiella Aerogenes	Plasmid	1
K. pneumoniae	Plasmid	86
	Unknown	54
Pseudomonas aeruginosa	Plasmid	2
Salmonella Enterica	Plasmid	12
	Unknown	2
Shigella Sonnei	Plasmid	6
Vibrio Alginolyticus	Plasmid	1

like plasmid from isolate 367 ECC, named pSC367ECC-1, shared >70% identity with both the SSU5 phage of *Salmonella* and the cryptic plasmid pHCM2 of MDR *Salmonella* Typhi CT-18. In all, seven whole sequences of the phage-like plasmid were found in the NCBI database, all in *E. coli* isolates, which shared >97% homology with pSC367ECC-1. Only four of these seven phage-like plasmids contained *bla*$_{CTX-M-15}$; two were isolated from wildlife feces in Colorado, United States, one was isolated from a sewage in India, and the remaining one was isolated from a human in Germany.[28,29] The 2,973 bp CTX-M-15 ARC identified in isolate 367 ECC and the 4 additional phage-like plasmids were not unique to these plasmids or *E. coli*. The cassette was also identified on an F plasmid in at least 22 *E. coli* isolates, one of which was annotated as ST131[12], indicating that the F plasmid and phage-like plasmid could have acquired the gene from a similar source.[30] In addition, isolate 367 ECC has an F plasmid that contains *bla*$_{TEM-1}$ but does not contain *bla*$_{CTX-M-15}$, which suggests that these plasmids could have exchanged *bla*$_{CTX-M-15}$ in the past.

Phylogenetic analysis based on the sequences of the six selected *E. coli* isolates showed that the isolates did not cluster with one specific phylogenetic group, but belonged to different phylogenetic groups. This analysis confirmed that the MDR *E. coli* isolates from the streams near Athens, GA, United States, in the Upper Oconee watershed, are highly diverse and they fall into four main phylogenetic groups (A, B1, B2, and D).[31] The isolates analyzed by WGS in this study were only a fraction of the total isolates, which limits any meaningful conclusions. However, the fact that all of the six isolates were MDR and two of them carry resistances to important antimicrobials, including an ESBL gene and a pAmpC β-lactamase gene, it is concerning that these isolates fall into phylogenetic

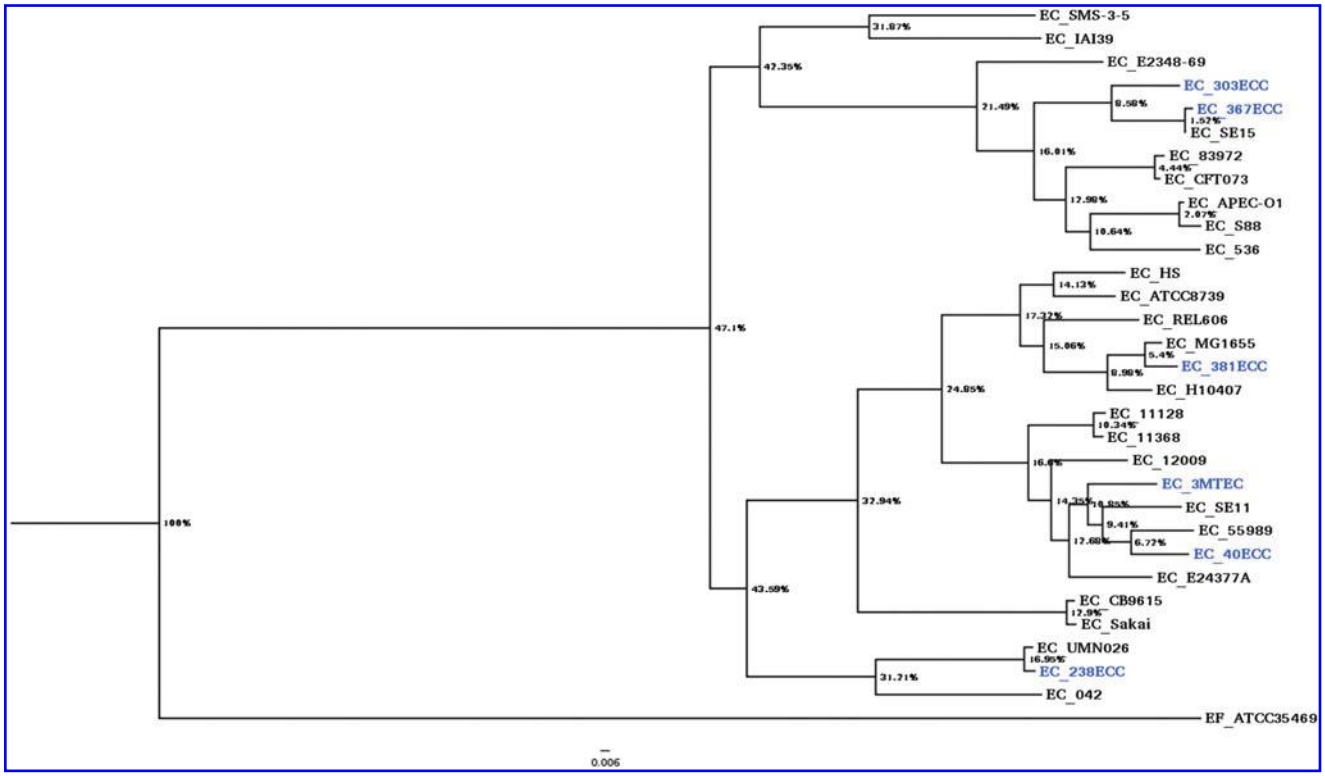

FIG. 3. Core genome-based phylogenetic tree inferring position of *E. coli* strain (*blue*) 303 ECC, 367 ECC, 381 ECC, 3 MTEC, 40 ECC, and 238 ECC in different *E. coli* phylogenetic groups as indicated by the *brackets* and *labels* on the *right side* of the figure. The genomes of *E. fergusonii* are included as an outgroup. The tree was obtained by maximum likelihood analysis of 45,897 alignment patterns. Evolutionary time is scaled by 100; lower values imply relatively recent branching. The scale indicates the number of substitutions per site.

TABLE 3. PHENOTYPIC AND GENOTYPIC ANTIMICROBIAL RESISTANCE PROFILES OF A CMY-2-PRODUCING *ESCHERICHIA COLI* ISOLATE AND ITS TRANSCONJUGANTS IN THE CONJUGATION EXPERIMENT

	Phylogenetic group	*MIC (μg/mL)*												*β-Lactamase gene*		*Plasmid replicon type*	
		AMO	*AMP*	*FAZ*	*FOT*	*F/C*	*FOX*	*POD*	*TAZ*	*T/C*	*TIO*	*AXO*	*CEP*	*CHL*	*GEN*	*P/T4*	bla_{CMY-2}	bla_{TEM-1}	*I1α*	*FIB*
Recipient J53	C	8	≤8	≤8	≤0.25	≤0.12	≤4	0.5	0.5	0.25	0.5	≤1	≤8	8	≤4	≤4	-	-	-	-
Donor 303 ECC	B2	32	>32	>16	8	8	>32	>32	16	8	>8	16	>16	16	>16	<4	+	+	+	+
Transconjugants																				
303 ECC-T1	C	>32	>32	>16	32	32	>64	>32	128	64	>8	>64	>16	32	>16	>64	+	+	+	+
303 ECC-T2	C	>32	>32	>16	32	32	>64	>32	128	64	>8	64	>16	32	>16	16	+	+	+	+
303 ECC-T3	C	>32	>32	>16	64	32	>64	>32	>128	128	>8	>64	>16	32	>16	>64	+	+	+	+
303 ECC-T4	C	>32	>32	>16	16	32	>64	>32	128	128	>8	64	>16	32	>16	64	+	+	+	+
303 ECC-T5	C	>32	>32	>16	8	4	>32	>32	32	16	>8	16	>16	16	>16	<4	+	+	+	+
303 ECC-T6	C	>32	>32	>16	8	8	>32	>32	32	8	>8	16	>16	16	>16	<4	+	+	+	+

MIC, minimum inhibitory concentration; AMO, amoxicillin/clavulanic acid; AMP, ampicillin; FAZ, cefazolin; FOT, cefotaxime; F/C, cefotaxime/clavulanic acid; FOX, cefoxitin; POD, cefpodoxime; TAZ, ceftazidime; T/C, ceftazidime/clavulanic acid; TIO, ceftiofur; AXO, ceftriaxone; CEP, cephalothin; CHL, chloramphenicol; GEN, gentamicin; P/T4, piperacillin/tazobactam constant 4.

groups that are predominantly associated with human commensals (A, B1) and virulent extraintestinal strains (B2, D).[32,33] Not surprisingly, 303 ECC with bla_{CMY-2} pAmpC β-lactamase gene and 367 ECC with $bla_{CTX-M-15}$ ESBL gene belonged to a pathogenic phylogenetic group B2. Two isolates, 238 ECC and 381 ECC, turned out to fall under different phylogenetic groups in this tree from what they were originally assigned using a PCR method.[11] They were originally assigned to groups E and C, but using the more sensitive sequencing method, they were reassigned to D1 and A, respectively. Previous studies frequently reported MDR ST69 *E. coli* strains that belong to the ''virulent'' phylogenetic group D, and the isolate 238 ECC, which is an MDR isolate that belongs to ST69, could be the same clone and fall under the same phylogenetic group D.[34,35]

Conjugation assays were performed to determine if genetic elements containing bla_{CMY-2}, $bla_{CTX-M-15}$, and bla_{TEM-1} were transferable to *E. coli* J53, which is resistant to sodium azide but susceptible to third-generation cephalosporins. The assay result showed that the 303 ECC isolate was able to transfer its I1α and FIB plasmids as well as β-lactamase genes bla_{CMY-2} and bla_{TEM-1} to the recipient *E. coli*. Its transconjugants acquired the donor's AR profile, exhibiting resistance to amoxicillin/clavulanic acid, ampicillin, cefazolin, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, ceftiofur, ceftriaxone, cephalothin, and gentamicin. Some of the transconjugants exhibited decreased susceptibility or resistance to chloramphenicol and piperacillin/tazobactam. This result shows that bla_{CMY-2} and bla_{TEM-1} are on MGEs that can be transferred to a recipient cell. The sequence result showed that bla_{CMY-2} is on a plasmid carrying a disrupted class 1 integron with AR genes *cmlA5*, *aadB*, and *sul1* on it, and the transferability of bla_{CMY-2} supports the plasmid localization of the gene. On the contrary, isolate 367 ECC was not able to transfer its $bla_{CTX-M-15}$ and bla_{TEM-1} to the recipient *E. coli*, and this shows that pSC367ECC-1 is not self-transmissible under the conditions of this *in vitro* assay.

The sequence analysis showed that pSC367ECC-1 carried genes encoding phage-related proteins, including ones that encode phage tail fiber proteins; hence, mitomycin C treatment and electron microscopy of 367 ECC were carried out in an attempt to visualize phage particles and determine if these genes encode an active, inducible lytic phage. The result was unsuccessful, which indicates that pSC367ECC-1 is not an intact phage and is indeed a plasmid. This result was somewhat expected since the phage-like plasmid was missing genes critical for host lysis. However, the fact that the *E. coli* isolate 367 ECC failed to conjugate or produce an activate phage in the laboratory does not guarantee that it would not do so in its natural environment. *E. coli* with a very similar phage-like plasmid has been identified in Colorado, United States, and this might suggest that the phage-like plasmids could be mobile and may already be circulating, mediating the spread of AR genes, including ESBL genes.[28] In addition, it is possible that the phage-like plasmid lost genes involved in host cell lysis but could acquire these genes again through coinfection and recombination with an active phage (phage rescue) to become an active lytic phage. This represents a new vehicle of AR gene transmission that needs further monitoring and investigation.

WGS has enabled us to assess the true nature of the MDR *E. coli* isolates. For the ESBL isolate, with the previous

detection of $bla_{CTX\text{-}M\text{-}15}$ gene and FIB plasmid replicon type using PCR methods[12], it was expected that the isolate contained the ESBL gene on its F plasmid as the current literatures suggest.[30,36] However, the analysis of WGS data allowed us to detect a phage-like plasmid and led us to further analyze its sequence. In addition, of the six isolates sequenced in this study, only three of them were previously found to carry class 1 integrons using a PCR method.[12] However, WGS analysis has shown that a higher proportion of the *E. coli* isolates carried integrons, with four isolates carrying class 1 integrons and an isolate carrying a disrupted integron sequence. The sequence analysis not only confirmed the presence of *intI1* by *in silico* PCR and the detection of AR genes in the downstream of *intI1* genes but also showed that the detection of integrons using the PCR method could underestimate the actual presence of integrons. WGS seems to be more efficient in detecting integrons, in particular those whose sequences are disrupted. WGS has proven to be an invaluable tool to reveal all genetic determinants of AR and phylogenetic diversity, as well as to uncover the new possible mechanism of AR transmission that other currently available methods, such as PCR, could not provide.

Environmental water receives inputs from several different point and nonpoint sources, such as wastewater treatment facilities and agricultural lands, and serves as a spot where diverse bacteria circulate and a reservoir where AR genes could develop and be transferred. The current study investigated MDR *E. coli* in stream waters in the Upper Oconee watershed, GA, using a WGS approach. The whole genomes of six MDR isolates, including an ESBL- and a pAmpC β-lactamase-producing *E. coli*, were sequenced and analyzed. Our findings revealed that environmental water carries MDR isolates of both commensal and pathogenic *E. coli* with a high degree of genomic flexibility, harboring AR genes on their MGEs. These environmental isolates can potentially spread to humans, rendering treatment of infections difficult. In addition, the presence of MGEs, such as plasmids and integrons, in the MDR *E. coli* would increase the chance of AR gene dissemination in the environment. In addition, the presence of the phage-like plasmid might suggest a new emerging mechanism of AR gene transmission and warrants attention. This study showed the contamination of environmental water with MDR *E. coli* of both nonpathogenic and pathogenic strains of human lineages, highlighting the role of surface water as a possible vehicle for MDR and pathogenic *E. coli* and a threat to the public health. These results confirm that more attention needs to be focused on the environmental water in our current effort to understand and fight the spread of AR.

GenBank Accession Numbers

These whole-genome shotgun projects have been deposited at DDBJ/ENA/GenBank under BioProject numbers PRJNA489694 and PRJNA489704, and the accession numbers are as follows: 3 mTEC (PRJNA489704)—QXQG00000000; 40 ECC (PRJNA489704)—QXQF00000000; 238 ECC (PRJNA489704)—QXQE00000000; 303 ECC (PRJNA489704)—QXQD00000000; 367 ECC (PRJNA489694)—QXNO00000000; and 381 ECC (PRJNA489704)—QXQC00000000. The versions described in this article are the first versions.

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Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Table S1

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