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Detection of CTX-M-27 β-Lactamase Genes on Two Distinct Plasmid Types in ST38 *Escherichia coli* from Three U.S. States

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ABSTRACT Infections caused by extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* are a significant cause of morbidity and health care costs. Globally, the prevailing clonal type is ST131 in association with the $bla_{CTX\text{-}M\text{-}15}$ β-lactamase gene. However, other ESBLs, such as $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}27}$, can also be prevalent in some regions. We identified ST38 ESBL-producing *E. coli* from different regions in the United States which carry $bla_{CTX\text{-}M\text{-}27}$ embedded on two distinct plasmid types, suggesting the potential emergence of new ESBL lineages.

KEYWORDS *Escherichia coli*, β-lactamases, ST38, IncF plasmid, CTX-M-27, ESBL, extended-spectrum β-lactamases, ceftriaxone

Infections caused by multidrug-resistant organisms present a major threat to health care systems worldwide (1). Disease caused by extended-spectrum-β-lactamase (ESBL)-producing organisms (such as *Escherichia coli*) is on the rise in the United States, with estimated costs to the health care system of $1.2 billion in 2017 (2). ESBLs confer resistance to most β-lactam antibiotics, including penicillins, oxyimino-cephalosporins (e.g., ceftriaxone), and monobactams, and can cause difficult-to-treat nosocomial- and community-acquired infections (3, 4). Globally, sequence type (ST) 131 is the predominant ESBL-producing *E. coli* isolated from urinary tract infections (UTIs) and bloodstream infections (BSIs), although other STs have been associated with ESBL carriage (i.e., ST38, ST648, ST405, ST10, and ST1193) (5–7). In the United States and elsewhere, a subclone of ST131 (C2/*H*30Rx) that carries the $bla_{CTX\text{-}M\text{-}15}$ β-lactamase gene is often reported (8). Another ST131 clade (C1-M27) was first observed in 2006 in Japan and subsequently emerged as a major lineage; C1-M27 is also reported in Europe (5, 6). C1-M27 carries the $bla_{CTX\text{-}M\text{-}27}$ gene on IncF[F1:A2:B20]-type plasmids (9, 10). Little is known about the epidemiology and genetic context of the $bla_{CTX\text{-}M\text{-}27}$ gene among clonal types other than ST131.

In 2017, we performed whole-genome sequencing (WGS) of 89 ceftriaxone-resistant *E. coli* urine and sterile site isolates collected by our laboratory ("URMC ESBL"; Table 1), which serves several counties in western New York. We identified ST38 as a frequent lineage second only to ST131 (ST131, 41/89 [46.1%] isolates; ST38, 14/89 [15.7%] isolates) (11). ST38 is a phylogroup D lineage that encompasses a variety of O:H serotypes and has been described as a hybrid uropathogenic/enteroaggregative strain (12). ST38 is far less characterized than ST131, and clear CTX-M-associated ST38 lineages have not been defined, although $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ have been found in ST38 (6, 7, 13–15).

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TABLE 1 Summary of ST38 isolates included in this study

Parent collection	Description (reference no.)	Yr(s) isolated	Location	No. of ST38 isolates included in current study
URMC ESBL	89 ceftriaxone-resistant surveillance isolates (11)	2017	NY	14
CDC EIP	97 isolates from 5 EIP surveillance sites[a]	2017	NY, CO, NM	12
URMC 2018–2019	Additional ceftriaxone-resistant ST38 isolates found in clinical archive	2018–2019	NY	11
Total				35[b]

[a]Resistant to ceftazidime, cefotaxime, or ceftriaxone, and nonresistant to all carbapenems tested and confirmed to be ESBL positive.
[b]Two ST38 URMC isolates were submitted to the CDC EIP program (35 unique isolates in total).

In contrast, in our 2017 study, ST38 was strongly associated with $bla_{CTX\text{-}M\text{-}27}$, which was found in 10/14 (71.4%) of ST38 isolates compared to 11/41 (26.8%) of ST131 isolates. In ST38, $bla_{CTX\text{-}M\text{-}27}$ was typically associated with IncF[F2:A-:B10] replicon plasmids, whereas in ST131, it was found on IncF[F1:A2:B20] plasmids (11, 16).

In the current study, we sought to determine whether the occurrence of $bla_{CTX\text{-}M\text{-}27}$ in ST38 was regional or more widespread. To address this question, 12 ST38 isolates collected in 2017 from Colorado, New Mexico, and New York by the Centers for Disease Control and Prevention (CDC) Emerging Infections Program (EIP) (17) were sequenced alongside their purified plasmids. These isolates were ceftazidime, cefotaxime, or ceftriaxone resistant, susceptible to all carbapenems tested, and confirmed to be ESBL positive ("CDC EIP" in Table 1). We compared the CDC EIP isolates to our previous ST38 *E. coli* isolated in 2017 ("URMC ESBL" in Table 1) and to 11 more recent (2018 to 2019) ST38 isolates ("URMC 2018–2019" in Table 1) from our institution in New York.

Library preparation and Nanopore- and Illumina-based sequencing for University of Rochester Medical Center (URMC) isolates (11) and EIP isolates (18) were performed as described previously. Only URMC and EIP isolates from 2017 were sequenced on the MinION platform (Oxford Nanopore Technologies, Cambridge, MA). Plasmid DNA was purified using the QIAfilter plasmid midi kit (Qiagen, Germantown, MD) from 100-ml Luria-Bertani (LB) cultures incubated for ~18 h at 37°C shaking. Read processing, assembly, single-nucleotide polymorphism (SNP) calling (GenBank accession no. NZ_CP026723.1), and phylogenetic analysis were done as described (18). The average coverage of the reference strain was 87.3% (74.7% to 95.1%). Illumina-Nanopore hybrid read assemblies were generated with Unicycler (19). Sequence contigs were screened for antibiotic resistance genes (ARGs) and putative virulence factors using ABRicate (including ResFinder and VFDB databases) (https://github.com/tseemann/abricate). Plasmids were aligned with Mauve (20), and sequence identity was depicted with Easyfig (21). This study was approved with a waiver of consent by the University of Rochester institutional review board (IRB) office.

The CDC's EIP ESBL surveillance found that 12 of 97 (12.4%) isolates typed as ST38 (6 from Colorado, 3 from New Mexico, and 3 from New York). Two of these 3 New York *E. coli* isolates were isolated from our hospital laboratory and were described previously as URMC_35 and URMC_51 (11). The third, URMC_725, was isolated from another local hospital system. An SNP-based phylogenetic tree was constructed using the 12 EIP ST38 isolates and the 23 other *E. coli* ST38 isolated and sequenced in our laboratory between 2017 and 2019 (35 isolates in total) (Table 1; Fig. 1A). Isolates appeared to group by β-lactamase type rather than state of origin. The most frequent β-lactamase gene detected was $bla_{CTX\text{-}M\text{-}27}$ (total, 17/35 [48.6%]; New York, 14/26 [53.8%]; Colorado, 1/6 [16.6%]; New Mexico, 2/3 [66.7%]), followed by $bla_{CTX\text{-}M\text{-}14}$ (total, 14/35 [40.0%]; New York, 10/26 [38.5%]; Colorado, 3/6 [50.0%]; New Mexico, 1/3 [33.3%]). Less prevalent β-lactamase genes detected included $bla_{TEM\text{-}1B}$ (6/35 [17.1%]), $bla_{CTX\text{-}M\text{-}15}$ (4/35 [11.4%]), $bla_{CMY\text{-}12}$ (1/35 [2.9%]), $bla_{DHA\text{-}1}$ (1/35 [2.9%]), and $bla_{OXA\text{-}48}$ (1/35 [2.9%]). All isolates with $bla_{TEM\text{-}1B}$ also carried one other β-lactamase gene, either $bla_{CTX\text{-}M\text{-}27}$ (1/6 [16.7%]), $bla_{CTX\text{-}M\text{-}15}$ (1/6 [16.7%]), or $bla_{CTX\text{-}M\text{-}14}$ (4/6 [66.7%]). The $bla_{OXA\text{-}48}$ carbapenemase gene

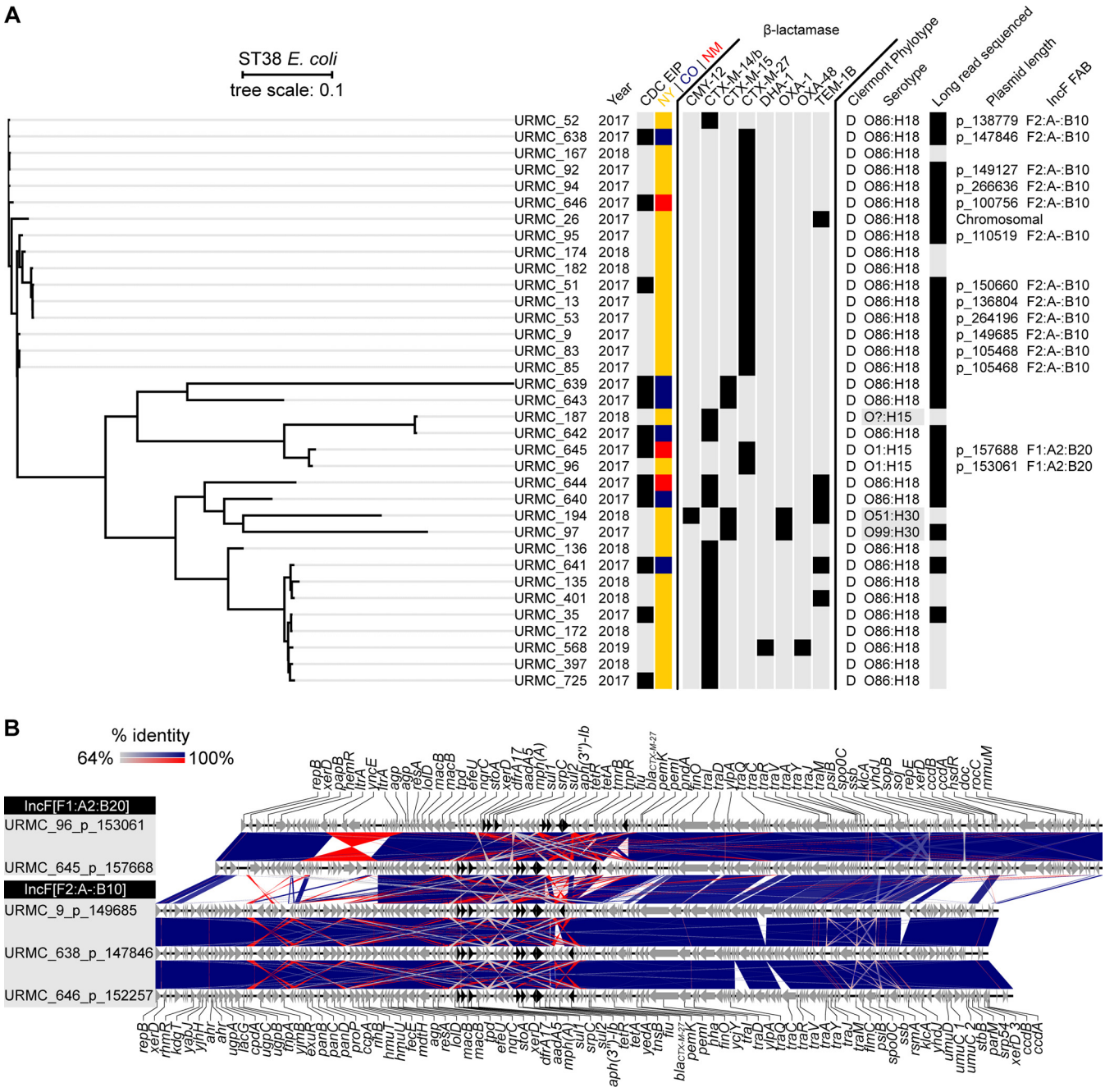

FIG 1 Whole-genome sequence SNP-based phylogenomic tree of ST38 *E. coli* isolates obtained in New York, New Mexico, and Colorado with alignments and sequence identity of related plasmids harboring the $bla_{CTX-M-27}$ gene. (A) Phylogenomic tree of 35 unique ST38 isolates showing year isolated, inclusion in the CDC EIP program, collection site, detection of β-lactamase genes (including only those with >95% sequence coverage), predicted phylotype and serotype, and plasmid name (with size in bp) and replicon type (fast atom bombardment [FAB] nomenclature). (B) Gene schematic and linear alignment of complete plasmids encoding the $bla_{CTX-M-27}$ gene from ST38 (with genes shown as arrows). Antibiotic resistance genes are show in black. All $bla_{CTX-M-27}$ plasmids identified in CDC EIP isolates are depicted (URMC_645_p_157668, URMC_638_p_147846, and URMC_646_p_152257) alongside selected previously described plasmids of the same FAB replicon type (URMC_96_p_153061 and URMC_9_p_149685) (11).

was detected in 1 ST38 *E. coli* (URMC_568) isolated from New York, which also carried bla_{DHA-1} and $bla_{CTX-M-14}$.

The median SNP distance between the 17 isolates carrying the $bla_{CTX-M-27}$ gene was 199 SNPs (or 101 SNPs, excluding URMC_96 and URMC_645) compared to a median distance of 2,727 SNPs between all isolates carrying $bla_{CTX-M-14}$. Isolates URMC_96 and URMC_645 were more distant (median of 4,153 SNPs) from the other $bla_{CTX-M-27}$-carrying *E. coli*. Long-read sequencing established that these also carried the $bla_{CTX-M-27}$

gene on plasmids (URMC_96_p_153061 and URMC_645_p_157668) of a different plasmid multilocus sequence typing (pMLST) type (IncF[F1:A2:B20]) compared to $bla_{CTX\text{-}M\text{-}27}$-carrying plasmids (IncF[F2:A-:B10]) found in the other ST38 isolates (Fig. 1B). Despite this, these different plasmid types shared some synteny (generally, ~92% identity over 42% of the conserved *tra* gene backbone versus ~100% identity over ~78% of the conserved ARG region). In addition to $bla_{CTX\text{-}M\text{-}27}$, which was flanked by insertion elements in an arrangement known in ST131 (i.e., IS*26*-ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}27}$-ΔIS*903D*-IS*26*) (9), other ARGs found on these plasmids putatively included those for resistance to tetracyclines [*tet(A)*], sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA17*), aminoglycosides [*aadA5*, *aph(6)-Id*, *aph(3″)-Ib*, ant(3″)-*Ia*], and macrolides [*mph(A)*]. A class 1 integron harbored the *dfrA17*, *aadA5*, *mph(A)*, and *sul1* genes (22).

This work suggests the emergence of $bla_{CTX\text{-}M\text{-}27}$ in ST38 on a newly described and conserved plasmid backbone (IncF[F2:A-:B10]) across three states from different regions of the United States. Also identified were two ST38 isolates with the gene on a plasmid type (IncF[F1:A2:B20]) already known in ST131 (10). This finding suggests two pathways for horizontal transfer of the β-lactamase among ST38. The association between $bla_{CTX\text{-}M\text{-}27}$ and ST38 may potentially result in the emergence of new ESBL-producing clones and lead to an increase in antibiotic-resistant UTIs and BSIs.

Data availability. The sequence information presented in this study has been deposited under NCBI BioProject accession nos. PRJNA692174 and PRJNA510429.

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N.D.P. conceived the study and collected clinical isolates. J.W. isolated plasmid DNA and performed sequencing. S.T. performed and managed bioinformatics analyses and pipelines. S.T., A.C., R.M., and N.D.P. analyzed sequence data. R.M., A.C., and N.D.P. analyzed overall data/results and wrote the first draft of the manuscript. N.D.P. provided funding and resources. R.A.S. did bioinformatics analysis. J.B.D. did sequencing work. D.C. performed AST and database management; J.D.L. organized CDC ESBL surveillance. All authors participated in editing and reviewing the manuscript and approved the final manuscript. All authors contributed to the article and approved the submitted version.

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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