Title
Genomic Analysis of Factors Associated with Low Prevalence of Antibiotic Resistance in Extraintestinal Pathogenic Escherichia coli Sequence Type 95 Strains.

Permalink
https://escholarship.org/uc/item/7329m056

Journal
mSphere, 2(2)

ISSN
2379-5042

Authors
Stephens, Craig M
Adams-Sapper, Sheila
Sekhon, Manraj
et al.

Publication Date
2017-03-01

DOI
10.1128/msphere.00390-16

Peer reviewed
Genomic Analysis of Factors Associated with Low Prevalence of Antibiotic Resistance in Extraintestinal Pathogenic Escherichia coli Sequence Type 95 Strains

Craig M. Stephens,a Sheila Adams-Sapper,b Manraj Sekhon,b James R. Johnson,c Lee W. Rileyb

Biology Department and Public Health Program, Santa Clara University, Santa Clara, California, USAa; Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, California, USAb; Veterans Affairs Medical Center and University of Minnesota, Minneapolis, Minnesota, USAc

ABSTRACT Extraintestinal pathogenic Escherichia coli (ExPEC) strains belonging to multilocus sequence type 95 (ST95) are globally distributed and a common cause of infections in humans and domestic fowl. ST95 isolates generally show a lower prevalence of acquired antimicrobial resistance than other pandemic ExPEC lineages. We took a genomic approach to identify factors that may underlie reduced resistance. We fully assembled genomes for four ST95 isolates representing the four major fimH-based lineages within ST95 and also analyzed draft-level genomes from another 82 ST95 isolates, largely from the western United States. The fully assembled genomes of antibiotic-resistant isolates carried resistance genes exclusively on large (>90-kb) IncFIB/IncFII plasmids. These replicons were common in the draft genomes as well, particularly in antibiotic-resistant isolates, but we also observed multiple instances of a smaller (8.3-kb) ampicillin resistance plasmid that had been previously identified in Salmonella enterica. Among ST95 isolates, pansusceptibility to antibiotics was significantly associated with the fimH6 lineage and the presence of homologs of the previously identified 114-kb IncFIB/IncFII plasmid pUTI89, both of which were also associated with reduced carriage of other plasmids. Potential mechanistic explanations for lineage- and plasmid-specific effects on the prevalence of antibiotic resistance within the ST95 group are discussed.

IMPORTANCE Antibiotic resistance in bacterial pathogens is a major public health concern. This work was motivated by the observation that only a small proportion of ST95 isolates, a major pandemic lineage of extraintestinal pathogenic E. coli, have acquired antibiotic resistance, in contrast to many other pandemic lineages. Understanding bacterial genetic factors that may prevent acquisition of resistance could contribute to the development of new biological, medical, or public health strategies to reduce antibiotic-resistant infections.

KEYWORDS Escherichia coli, ExPEC, ST95, antibiotic resistance, genomics
types 131 (ST131), ST95, ST69, ST73, and ST393. These STs are responsible for nearly half of all *E. coli* UTIs or BSIs in many regions of the world (4). Comparative genomic analysis of ExPEC strains to date has not conclusively identified essential "pandemicity" genes in these lineages (5), but there is considerable interest in understanding virulence-associated genetic features of pandemic ExPEC lineages.

In several recent surveys of ExPEC isolates from the United States, ST95 was the second most common ST, after ST131. These include reports by Adams-Sapper et al. (6), who examined bloodstream isolates from a San Francisco, CA, hospital; Bannerjee et al. (7), who examined isolates from blood, urine, and other extraintestinal sites from Olmsted County, Minnesota; and Salipante et al. (5), who examined urinary and bloodstream isolates from the University of Washington Hospital system (Seattle, WA). ST131 clinical isolates typically are multidrug resistant (MDR), expressing fluoroquinolone resistance due to chromosomal mutations and, in many instances, extended-spectrum β-lactam resistance due to acquired plasmid-borne or chromosomal genes (8, 9). In contrast, ST95 isolates are much less frequently antibiotic resistant than ST131 strains and many other clonal groups (4). Furthermore, Adams-Sapper et al. (6) noted that, at least in San Francisco, the fimH6 sublineage of the ST95 group was nearly always pansusceptible and yet very common. This implies, first, that antimicrobial resistance may not be a major factor contributing to the pandemicity of ST95 strains, and second, that there may be something distinctive about fimH6 strains with respect to acquisition or maintenance of antibiotic resistance.

We describe here the use of whole-genome sequence analysis to explore these implications. We sequenced 44 ST95 bloodstream isolates from San Francisco General Hospital (SFGH) that had been recovered between 2007 and 2011 (6, 10). These initial strains encompassed the four major sublineages of ST95, as defined by alleles of the fimH marker. We added more geographic diversity with four ST95 isolates from Minnesota (11) and five from other locations in the United States (12). Finally, we included archived sequences of 33 ST95 isolates from the Seattle area (5) in our analysis. Through this approach, we were able to identify the genetic basis for acquired antibiotic resistance genes in a large United States-based sample of ST95 isolates and to identify genomic differences between pansusceptible and resistant isolates that help explain disparities in resistance within the ST95 group.

**RESULTS**

Genome sequencing and isolate characterization. A total of 53 *E. coli* ST95 isolates were subjected to whole-genome sequencing on the Illumina MiSeq platform, followed by de novo assembly and analysis (Table 1; see Table S1 in the supplemental material). Most (83%) of the isolates were from San Francisco, CA. We also examined archived whole-genome sequences of 33 ST95 isolates from the Seattle, WA, region (5). The fimH gene, which encodes an adhesin critical for urinary tract pathogenesis, has proven to be useful as a genetic marker to increase the resolution of MLST with ExPEC strains (13, 14). Examination of fimH genotypes from genome sequences allowed assignment of the ST95 isolates to sublineages (Table 1). Alignment of scaffolded assemblies from the draft genome sequences and subsequent tree building (Fig. 1) showed the fimH sublineages to be phylogenetically coherent, with the major division being between fimH1 and fimH6 isolates, and with the fimH9 and fimH47 clusters emerging from separate branches of the fimH1 group. Among the isolates we sequenced, the fimH6 genotype was most abundant (48%), followed by the fimH1 (31%), fimH47 (17%), and fimH9 (3%) genotypes.

Based on their fimH genotype and antibiotic resistance phenotypes, four ST95 isolates from San Francisco were selected for long-read, single-molecule real-time (SMRT) sequencing (Pacific Biosciences) to develop high-quality, fully assembled reference genomes (15). The complete genomes were from SF-166 (fimH6 [pansusceptible, as defined in Materials and Methods]), SF-173 (fimH47 [MDR]), SF-468 (fimH1 [MDR]), and SF-088 (fimH9 [MDR]). Key characteristics of the genome sequences are presented in Table S1. The complete chromosomes of the four strains aligned readily,
### TABLE 1
Characterization of *E. coli* ST95 isolates and genomes

| FimH gene type | Isolate | Antibiotic resistance | No. of plasmid replicons present | No. of small plasmids | Full pUTI89* |
|----------------|---------|------------------------|----------------------------------|-----------------------|-------------|
|                |         |                        | IncF1 | IncFII | IncB | IncI | IncP | IncQ |           |            |
| fimH1          | SF-075  | Tet (tetA)             | 2 (B, C) | 1 | 1 | 3 |
|                | SF-094  | Amp (blaTEM-1)         | 1 (B) | 1 | 6 |
|                | SF-149  | Amp (blaTEM-1)         | 1 (B) | 1 | 3 |
|                | SF-151  | Amp (blaTEM-1), Str (strA, strB), Tet (tetA), Sul (sul2), Tmp (dfra5) | 1 (B) | 1 | 2 |
|                | SF-264  | Amp (blaTEM-1), Azm (mphA), Chl (catA1), Gen (aac3), Nor (gyrA*), Str (aadA1, aadA2, aadA5), Tet (tetA), Sul (sul1), Tmp (dfra12, dfra17) | 1 | 1 | 3 |
|                | SF-269  | Amp (blaTEM-1)         | 1 (B) | 2 | 1 | 5 |
|                | SF-305  | Amp (blaTEM-1)         | 1 (B) | 2 | 6 |
|                | SF-362  | Amp (blaTEM-1)         | 1 (B) | 1 | 4 |
|                | SF-371  | Amp (blaTEM-1)         | 1 (B) | 1 | 5 |
|                | SF-380  | None                   | 1 (B) | 1 | 2 |
|                | SF-457  | Amp (blaTEM-1)         | 1 (B) | 1 | 7 |
|                | SF-468  | Amp (blaTEM-1), Cef (blaCTX-M14), Gen (aac3), Str (strA, strB), Tet (tetA), Sul (sul2), Tmp (dfra17) | 1 | 1 | 3 |
|                | SF-495  | None                   | 1 (B) | 1 | 2 |
|                | SF-501  | Amp (blaTEM-1), Str (strA, strB), Tet (tetD), Sul (sul2), Tmp (dfra5) | 1 (B) | 1 | 2 |
|                | SF-522  | Amp (blaTEM-1), Str (strA, strB), Tet (tetB) | 1 | 6 |
|                | SF-523  | None                   | 1 (B) | 1 | 5 |
|                | SF-626  | Amp (blaTEM-1)         | 1 (B) | 2 | 6 |
|                | MVAST0234 | None               | 1 (B) | 1 | 3 |
|                | UPEC-061 | None                 | 1 (B) | 1 | ND | Yes |
|                | UPEC-094 | blaTEM-1, dfra5       | 1 | ND |
|                | UPEC-106 | None                   | 1 (B) | 1 | ND |
|                | UPEC-136 | None                   | 1 (B) | 1 | 1 | ND |
|                | UPEC-144 | blaTEM-1, strA, strB, sul2, dfra5 | 1 (B) | 1 | 1 | ND |
|                | UPEC-185 | blaTEM-1, strA, strB, sul2, dfra2 | 1 (B) | 1 | 1 | ND |
|                | UPEC-249 | None                   | 1 (B) | 1 | ND |
|                | UPEC-250 | blaTEM-1, strA, strB, sul2, dfra5 | 1 (B) | 1 | 1 | ND |
|                | UPEC-276 | None                   | 1 (B) | 1 | ND |
| fimH6          | SF-083  | None                   | 1 (B) | 2 | 0 | Yes |
|                | SF-095  | None                   | 1 (B) | 1 | 1 | Yes |
|                | SF-126  | Amp (blaTEM-1), Tet (tetA) | 3 (A, B, C) | 1 | 0 |
|                | SF-166  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-231  | Amp (blaTEM-1), Str (strA, strB, aadA5), Tet (tetB), Sul (sul1, sul2), Tmp (dfra17) | 1 (B) | 1 | 0 |
|                | SF-313  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-326  | Amp (blaTEM-1)         | 1 (B) | 1 | 2 | Yes |
|                | SF-356  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-383  | None                   | 1 (B) | 1 | 2 |
|                | SF-384  | None                   | 1 (B) | 1 | 0 |
|                | SF-403  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-421  | None                   | 1 (B) | 1 | 3 | Yes |
|                | SF-423  | None                   | 1 (B) | 1 | 0 |
|                | SF-425  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-440  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-452  | None                   | 1 (B) | 2 | 0 | Yes |
|                | SF-451  | None                   | 1 (B) | 1 | 0 |
|                | SF-518  | None                   | 1 (B) | 1 | 0 |
|                | SF-560  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-567  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-572  | None                   | 1 (B) | 1 | 0 | Yes |
|                | SF-596  | Amp (blaTEM-1), Str (strA, strB, aadA5), Tet (tetA), Sul (sul1, sul2), Tmp (dfra17) | 2 (A, B) | 2 | 2 |
|                | MVAST0098 | None              | 1 (B) | 1 | 0 |
|                | MVAST0176 | Amp (blaTEM-1)      | 1 (B) | 2 | 0 | Yes |

(Continued on following page)
with >99.9% identity across most of the genome (see Fig. S1 in the supplemental material). Most breaks in the alignment between the complete chromosomes involved prophages. Other discontinuities in the alignments involved loci for synthesis of O-antigens and P-pili. O-antigen loci are common sites of recombination and variation in \textit{E. coli}; the ST95 isolates examined in this work varied in the O-antigen loci and predicted serotypes, even within \textit{fimH} types (data not shown).

Additionally, a 40-kb segment located at approximately 0.8 Mb in SF-166 and SF-088 was moved to 4.6 Mb in SF-468 and SF-173. This segment includes the \textit{pap} genes encoding P-pili, an important ExPEC virulence factor (16).

### TABLE 1 (Continued)

| FimH gene type | Isolate | Antibiotic resistance | No. of plasmid replicons present | No. of small plasmids | Full pUTI89* |
|----------------|---------|-----------------------|----------------------------------|-----------------------|-------------|
|                |         |                       | IncFII | IncB | IncQ | IncP | IncI |                       |                       |
| USVAST184      | None    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USVAST267      | None    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| Blood-11-0031  | None    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-007      | bla\textsubscript{TEM-1}, strB | 3 (A, B, C) | 1 | ND |
| USPEC-008      | None    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-048      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-051      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-072      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-073      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-075      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-098      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-124      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-131      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-139      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-157      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-197      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |
| USPEC-255      | none    | Amp (bla\textsubscript{TEM-1}), Str (strA, strB), Sul (sul2), Tmp (dfrA5) | 1 (B) | 1 | 1 | 2 | Yes |

SF isolates were from San Francisco General Hospital (6), MVAST isolates were from Minneapolis (11), USVAST isolates were from other states in the United States (10), and "Blood" and "UPEC" isolates were from Seattle (5). Antibiotic resistance phenotypes are shown for all strains other than those from Seattle, with the ResFinder-identified gene(s) (20) presumed responsible shown in parentheses. For Seattle isolates, only the genes identified are shown, as phenotypes were not independently confirmed in this study. Antibiotic abbreviations: Amp, ampicillin; Azm, azithromycin; Cef, cephalothin; Chl, chloramphenicol; Gen, gentamicin; Nor, norfloxacin; Str, streptomycin; Tet, tetracycline; Sul, sulfamethoxazole; Tmp, trimethoprim. Plasmid replicons were predicted with PlasmidFinder (25). The number of small plasmids was determined from sequence assemblies, as described in Materials and Methods. Seattle isolates are listed as "ND" (no data), as these genomes were downloaded as assembled contigs without topology or coverage data, which precluded identification of small plasmids. Prediction of the presence of pUTI89 is described in Materials and Methods. The presence of resistance genes is discordant with phenotypes reported in reference 5: UPEC-007 (reported as Tetr), UPEC-098 (reported as Amp', Sul', Tmpr), UPEC-124 (reported as Amp', Sul', Tmpr), UPEC-131 (reported as Amp', Cef', Tetr), UPEC-144 (not reported as Sul', Tmpr), UPEC-157 (reported as Amp'), UPEC-185 (not reported as Amp', Sul', Tmpr).
Because clustered regularly interspaced short palindromic repeat (CRISPR) elements are thought to impact mobile gene acquisition (including plasmids bearing antibiotic resistance) in bacterial genomes (17), we examined CRISPR loci in the fully assembled ST95 genomes. All four isolates lacked the CRISPR1 locus and had only two imperfect and identical spacers at CRISPR2 (located at ~1.12 Mb), as has been observed previously in ST95 isolates (18). CRISPR3 and CRISPR4 (located at ~3.2 Mb) were present, with a variable number of repeats (for CRISPR3, SF-088, 4, SF-166, 7, SF-173, 8, and SF-468, 5; for CRISPR4, SF-088, 2, SF-166, 6, SF-173, 6, and SF-468, 6). When draft genomes were examined, there was some variation in repeat numbers within \textit{fimH} lineages as well (data not shown).

SMRT sequencing allowed identification of DNA methylation patterns (19). All four genomes displayed adenine methylation (m6A) at GATC sites, attributable to Dam DNA methyltransferase (see Table S2 in the supplemental material). The SF-468 and SF-173 isolates shared an additional m6A motif, as did the SF-166 and SF-088 isolates. SF-088 showed m6A methylation at two additional sites. All motifs showing m6A methylation were nearly 100% methylated. The SF-166 genome was the only one to show cytosine (m4C) methylation, at roughly one out of eight RCCGGY sequences. The SF-166 genome contains three annotated DNA-cytosine DNA-methyltransferase genes: a homolog of \textit{dcm} (located at ~2.1 Mb) that was present in all of the other ST95 genomes sequenced, a component of a restriction-modification system (located at 4.12 Mb) found in all \textit{fimH6} and \textit{fimH9} isolates, and a 1.2-kb gene located at 4.16 Mb in SF-166 that was universal in \textit{fimH6} isolates but absent in the non-\textit{fimH6} ST95 genomes examined. The latter gene is hypothesized to be responsible for the unique m4C methylation observed in SF-166, but this was not confirmed experimentally.

\textbf{Antibiotic resistance.} Thirty-four (40%) of the 86 genomes we examined contained at least one resistance gene, as identified by ResFinder (20). Resistance genes were detected for seven classes of antimicrobial agents (Table 2). For the 53 isolates we

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Phylogenetic tree showing relationships between ST95 isolates based on alignment of scaffolded genome assemblies. Raw sequencing reads for each genome were trimmed and filtered for quality control and then assembled to the \textit{E. coli} SF-166 complete genome sequence (GenBank accession no. CP012633). The resulting genome sequences were aligned using progressiveMauve 2.4.0 (38), and an unrooted tree based on the alignments was generated using Archaeopteryx 0.9920. Isolate names are shown to the right, along with \textit{fimH} type and whether the isolate also contains a pUT189* plasmid.}
\end{figure}
sequenced and assessed for resistance, predictions by ResFinder correlated well with observed phenotypes. For the Seattle isolates, intact resistance genes were assumed to confer the expected phenotype. Resistance genes were not evenly distributed among fimH sublineages: only 8 (20%) of 41 fimH6 isolates contained one or more acquired resistance genes, versus 1 (33%) of 3 fimH9 isolates, 7 (47%) of 15 fimH47 isolates, and 18 (67%) of 27 fimH1 isolates (for fimH6 versus non-fimH6 isolates, \( P < 0.001 \)). With respect to geographic origin, the Seattle ST95 isolates were significantly less likely to contain genes for \( \beta \)-lactam and tetracycline resistance (\( P = 0.01 \) and 0.02, respectively) than the San Francisco isolates, a result attributable to the non-fimH6 component of the population.

The amoxicillin resistance gene \( bla_{TEM-1} \), found in 38% of isolates, was the most common acquired resistance gene. Most \( bla_{TEM-1} \) genes were located in the context of Tn3 mobile elements. The only extended-spectrum \( \beta \)-lactamase (ESBL) gene identified, \( bla_{CTX-M-14} \), was on plasmid pSF-468-2 in strain SF-468, which also contained \( bla_{TEM-1} \) on a separate plasmid (pSF-468-1). Among the 33 ampicillin-resistant isolates, 15 were resistant to at least three classes of antibiotics. The MDR isolates accounted for nearly all detected genes conferring resistance to aminoglycosides, tetracyclines, and sulfamethoxazole-trimethoprim (Table 1). Sulfonamide and trimethoprim resistance genes were typically coresident (occurring together in 12 of 15 isolates with either type of gene) and in close proximity on the same contig. One strain (SF-264) was also resistant to fluoroquinolones due to the presence of typical chromosomal mutations (in gyrA, Ser83-to-Leu and Asp87-to-Asn; in parC, Ser80-to-Ile) encountered in fluoroquinolone-resistant clinical \( E. \ coli \) isolates (21).

**Plasmid content of ST95 genomes.** Each of the SMRT-sequenced and fully assembled ST95 genomes contained at least one circular plasmid larger than 90 kb. These large plasmids all contained one or more known Inc replicons (Table S1), and with one exception (pSF-166-1), all of the large plasmids contained antibiotic resistance genes. Furthermore, all of the resistance genes in the three sequenced MDR isolates were contained on large plasmids. pSF-468-1 carried \( bla_{TEM-1} \), in a Tn3-type transposon adjacent to a 17-kb region containing genes for resistance to streptomycin (strAB), sulfonamides (sul2), trimethoprim (dfrA17), and tetracycline (tetA). pSF-088-1 carried \( bla_{TEM-1}, sul2, dfrA5, strA, \) and \( strB \) in an 11-kb region, while pSF-173-1 included dfrA12, aadA2, sul1, and mphA in a 10-kb region. The large IncF plasmids (pSF-468-1, pSF-088-1, pSF-173-1, and pSF-166-1) shared roughly 40 kb of sequence encoding conserved replication and conjugation functions. pSF-468-1 also shared with pSF-088-1 another ~45 kb of sequence that included genes encoding potential virulence-associated factors, such as the Sit manganese-iron transport system and genes for aerobactin siderophore production and uptake. Virulence factors in the ST95 genomes will be reported in greater detail elsewhere.

pSF-166-1, the 114-kb plasmid from pansusceptible strain SF-166, was the only large plasmid not encoding antibiotic resistance and the only one with a full-length match (>98% identity, 100% coverage) to previously described plasmids: pUTI89 from uro-

| Antibiotic class | Resistance gene(s) identified | Proportion (%) of genes in: | All isolates | fimH6 isolates | Non-fimH6 isolates |
|-----------------|-------------------------------|-----------------------------|-------------|---------------|-----------------|
| \( \beta \)-Lactams | \( bla_{TEM-1}, bla_{CTX-M-14}, aadA1, aadA2, aadA5, aac3, strA, strB \) | San Francisco | 21/44 (48) | 4/22 (18) | 17/22 (77) |
| | | Seattle | 9/33 (27) | 3/15 (20) | 6/18 (33) |
| | | Other | 3/9 | 1/4 | 2/5 |
| Aminoglycosides | | San Francisco | 10/44 (23) | 2/22 (9) | 8/22 (36) |
| | | Seattle | 6/33 (18) | 1/15 (7) | 5/18 (28) |
| | | Other | 0 | 0 | 0 |
| Tetracyclines | tetA, tetr, tetD | San Francisco | 9/44 (20) | 3/22 (14) | 6/22 (27) |
| | | Seattle | 1/33 (3) | 0/15 | 1/18 (6) |
| | | Other | 0 | 0 | 0 |
| Sulfonamides | sul1, sul2 | San Francisco | 9/44 (20) | 2/22 (9) | 7/22 (32) |
| | | Seattle | 5/33 (15) | 0/15 | 5/18 (28) |
| | | Other | 0 | 0 | 0 |
| Trimethoprim | dfrA5, dfrA12, dfrA17 | San Francisco | 8/44 (18) | 2/22 (9) | 6/22 (27) |
| | | Seattle | 5/33 (15) | 0/15 | 5/18 (28) |
| | | Other | 0 | 0 | 0 |
| Macrolides | mphA | San Francisco | 2/44 (5) | 0/22 | 2/22 (9) |
| | | Seattle | 0/33 | 0/15 | 0/18 |
| | | Other | 0 | 0 | 0 |
| Chloramphenicol | catA1 | San Francisco | 2/44 (5) | 0/22 | 2/22 (9) |
| | | Seattle | 0/33 | 0/15 | 0/18 |
| | | Other | 0 | 0 | 0 |
pathogenic E. coli strain UTI89 (22), plasmid pRS218 from meningitis-associated E. coli strain RS218 (23, 24), and plasmid pEC14_114 from uropathogenic strain EC14 (25). This plasmid carries several candidate virulence factors and has been associated with virulence in a mouse UTI model (26) and a rat model for neonatal meningitis (24). For practical purposes, we will refer to this group of plasmids and the close relatives identified in this work as pUTI89 from now on.

Examination of our draft genomes showed that pUTI89 was common. When pUTI89 was used as the scaffold for reference-guided assemblies of reads from all 53 isolates we sequenced, a complete version of pUTI89 (defined as >98% of pUTI89 assembled) was present in 16 isolates—14 from San Francisco, one from Sacramento, and one from Minnesota (Table 1)—all of which were fimH6 strains. Among the Seattle ST95 isolates (see Materials and Methods), 11/33 likely contained pUTI89, including 1/9 fimH1 isolates, 5/15 fimH6 isolates, and 5/9 fimH47 isolates. Overall, pUTI89 was likely present in 27 (32%) of 86 ST95 genomes we examined. It was most common in fimH6 isolates (21/41 [51%]), less common in fimH47 isolates (5/15 [33%]), and rare in fimH1 isolates (1/29 [3%]) (for the three-group comparison, by \( \chi^2 \) test, \( P = 0.0014 \)).

pUTI89-containing isolates rarely contained acquired antibiotic resistance genes (2/27 [7%]) compared to the ST95 isolates lacking pUTI89 (27/59 [46%]) (\( P < 0.001 \)). Because most pUTI89-containing isolates were also in the fimH6 sublineage, and fimH6 isolates were also less likely to be resistant than non-fimH6 isolates, we examined whether these traits were independently associated with resistance frequency in this population of isolates (Table 3). Among fimH6 isolates, resistance genes were less common among isolates with pUTI89 than among those without pUTI89 (2/21 [10%] versus 6/20 [30%]), but the difference was not significant (\( P = 0.10 \)). Among non-fimH6 isolates, pUTI89 was also associated with reduced resistance frequency (0/6 resistant isolates containing pUTI89 versus 26/39 resistant isolates lacking pUTI89; \( P = 0.002 \)). Comparing all isolates lacking pUTI89, fimH6 isolates were still less likely to be resistant than non-fimH6 isolates (6/20 [70%] versus 26/39 [33%]; \( P = 0.007 \)). These data suggest that both the fimH genotype (specifically fimH6) and the presence of pUTI89 may be associated with reduced carriage of antibiotic resistance genes.

The published sequences of pUTI89 and pRS218 differ by only 16 single nucleotide polymorphisms (SNPs). Alignment of the assembled sequences from this study showed that pSF-166-1 differs from pUTI89 by 32 SNPs and from pRS218 by 40 SNPs. Many of the plasmids from San Francisco isolates were more closely related, including pSF-403-1, pSF-420-1, and pSF-440-1 (7 SNPs), which were obtained from separate patients in late 2009 and early 2010, and pSF-560-1 and pSF-567-1 (6 SNPs), obtained from separate patients in October 2010. Given the expected rate of false-positive SNPs in assemblies from MiSeq data (1 × 10^{-4}) (27) at comparable coverage, the plasmids in these isolates may in fact be identical.

Although large plasmids were assembled readily from SMRT sequencing data, they rarely assembled as circular contigs from shorter MiSeq reads, so it was not possible to reliably assess the presence of such plasmids in draft genomes. As an alternative, we used PlasmidFinder (28) to identify replicons from various incompatibility (Inc) groups in draft genome sequences. A total of 190 IncF (IA, IB, IC, or II), IncB, IncI, IncP, or IncQ plasmid replicons were detected in the 86 ST95 genomes examined (mean, 2.2 per genome)
PlasmidFinder failed to identify plasmid replicons in only five genomes (6%), four of which were fimH6 strains. Most isolates (83%) contained both IncFIB and IncFII replicons (Table 1); only 9/86 lacked an IncFII replicon, and only 12/86 lacked an IncFIB replicon. The mean number of Inc replicons per strain did not differ significantly by fimH lineage. However, replicon type exhibited a borderline significant association with fimH lineage: fimH1 isolates more frequently contained replicons other than FIB and FII (13/27 [48%]) than did fimH6 (8/41 [20%]) or fimH47 (5/15 [33%]) isolates (P = 0.051).

Because in the draft genomes most large plasmids did not assemble as single contigs, whether antibiotic resistance genes were plasmid associated could not always be determined definitively. However, 35 (64%) of 55 identified antibiotic resistance genes were located on the same contigs as Inc replicons or on pAnkS (see below), suggesting that most resistance genes were plasmid-borne, similar to the pattern observed in the fully assembled genomes.

Strains containing pUTI89* had the same mean number of Inc replicons (2.2/strain) as those lacking pUTI89* (Table 4). However, for pUTI89*-containing isolates, nearly all Inc replicons were IncFIB or IncFII and were presumably present on pUTI89 itself. Isolates without a complete pUTI89 were more likely to contain non-IncFIB/FII replicons or small plasmids (37/59 [63%]) than were pUTI89*-containing isolates (4/27 [15%]) (P < 0.001) (Table 4). Thus, the presence of pUTI89* was associated with a significantly lower prevalence of other plasmids.

Genomes of pansusceptible ST95 isolates were less likely to contain plasmids than those of resistant strains: the mean number of Inc replicons was lower for isolates lacking resistance genes (mean, 1.9) than for those containing one or more resistance genes (mean, 2.7) (P < 0.001 by Student's t test). In the genomes we sequenced, the number of small plasmids was also lower in isolates lacking antibiotic resistance (mean of 0.9 small plasmids/isolate) (P = 0.002). Included among the 52 pansusceptible strains were five that lacked Inc replicons and small plasmids altogether.

**Small plasmids in ST95 genomes.** Of the 53 ST95 genomes we sequenced, 26 (49%) contained small plasmids (Table 1), appearing in the draft genome assemblies as circular contigs of less than 10 kb (see Materials and Methods). PlasmidFinder identified some of these as containing replicons of colicin-producing plasmids, but most were not flagged by PlasmidFinder and lacked the Inc replicons enumerated above. Verification that these contigs represented small plasmids was demonstrated by isolation of plasmid DNA in the laboratory (see Fig. S2 in the supplemental material). fimH1 isolates were much more likely to contain at least one small plasmid (17/18 [94%]) than fimH6 isolates (6/26 [23%]) (chi-square test, P < 0.001). fimH1 isolates also contained more small plasmids per isolate (3.8/isolate; SEM, 0.4; range, 2 to 7) than fimH6 isolates (mean, 0.5; SEM, 0.2; range, 0 to 3). Isolates containing pUTI89* (many of which are fimH6 strains) also contained significantly fewer small plasmids than isolates lacking pUTI89* (Table 4).
The vast majority of small plasmids identified had no antibiotic resistance genes or known virulence factor genes. An exception was an 8.3-kb plasmid encoding ampicillin resistance that was present in six fimH1 isolates (SF-94, -149, -305, -457, -522, and -626), isolated over a 4-year period (2007 to 2011). This plasmid and the accompanying β-lactam resistance could be moved into laboratory E. coli strains by transformation, allowing it to be separated from other small plasmids present in the clinical isolates (Fig. S2). All ST95 isolates containing the 8.3-kb plasmid showed an elevated cephalothin MIC (32 μg/ml versus 12 μg/ml for other strains containing blaTEM-1), perhaps due to an increased blaTEM-1 gene dosage relative to larger, lower-copy-number plasmids in other ST95 strains.

**DISCUSSION**

The work presented here explores genomic diversity and antibiotic resistance in the E. coli ST95 lineage. Our results suggest that most antibiotic resistance in ST95 strains is associated with large conjugal plasmids. In the SMRT-sequenced SF-088, SF-173, and SF-468 strains, all resistance genes were borne on large multireplicon plasmids. This likely was true also for isolates sequenced to the draft level, since in them most resistance genes were found on contigs containing IncF replicons, as was the case for resistance plasmids in the SMRT-sequenced strains. Intriguing exceptions included six San Francisco isolates containing an 8.3-kb plasmid nearly identical to pAnkS, previously reported in Salmonella enterica isolates in Turkey (29) and Uruguay (30). None of the non-San Francisco isolates examined contained a pAnkS-like plasmid, but the temporal and geographic distribution of isolates was far from comprehensive. The pAnkS-containing strains were all isolated between 2007 and 2011; determination of whether this plasmid is still contributing to aminopenicillin resistance in San Francisco or elsewhere warrants further effort.

We also sought to shed light on why ST95 strains are less frequently antibiotic resistant than many other major ExPEC lineages. In previous work, among ST95 clinical isolates from San Francisco the fimH6 sublineage was most closely associated with pansusceptibility (6). The present analysis of an expanded set of isolates confirmed that fimH6 isolates were significantly less likely than other ST95 isolates to contain antibiotic resistance genes. Although antibiotic resistance in ST95 isolates derived largely from acquired, plasmid-borne genes, it is conceivable that both chromosomal and plasmid composition could impact the likelihood of acquiring resistance. Indeed, analysis of data in Table 3 suggests that both the fimH6 genotype and pUTI89* are associated with a reduced likelihood of resistance.

Chromosomal elements in fimH6 strains such as CRISPR or restriction-modification systems might restrict entry of new plasmids. The ST95 genomes examined here lacked CRISPR1 and contained only a rudimentary CRISPR2, as is common in the E. coli B2 group that includes ST95 (18). The CRISPR3 and CRISPR4 loci varied somewhat within and between the fimH lineages within ST95. No correlation was observed between the number of spacers or the sequences of spacers in ST95 isolates and their resistance to antibiotics or the presence of particular plasmids. Thus, there is currently no evidence to suggest that CRISPR-mediated effects on DNA acquisition could account for the patterns of antibiotic resistance observed in this work.

Multiple restriction-modification systems are found in each of the completely sequenced ST95 isolates according to REBASE (31). The genome of pansusceptible isolate SF-166 contained two fimH6-specific chromosomal regions: an ~12-kb segment at 4.11 Mb that includes a homolog of the Eco31 restriction-modification system (including separate adenine- and cytosine-specific DNA methyltransferases) and a 31-kb segment at 4.15 Mb that includes a DNA-cytosine methyltransferase. The pUTI89* plasmid in SF-166 is also annotated to encode a putative DNA methyltransferase of unknown specificity. Analysis of genome methylation patterns showed no adenine methylation unique to SF-166, but this was the only isolate exhibiting cytosine methylation, and a chromosomally encoded putative cytosine DNA-methyltransferase unique to the fimH6 lineage was identified. Whether cytosine methylation in fimH6
strains has any role in limiting plasmid entry, and thereby affecting acquisition of antibiotic resistance, is an open question warranting further study.

Many ST95 isolates, particularly within the fimH6 sublineage, contained a large multireplicon plasmid nearly identical to pUTI89, designated here generically as pUTI89*. Our data showed that the presence of pUTI89* was correlated with a lower likelihood of antibiotic resistance (Table 3) and a reduced content of other plasmids (Table 4). The preferential association of pUTI89* with the fimH6 sublineage seems likely to at least partially explain why pansusceptibility is so common in this group (6).

We hypothesize that the pUTI89* plasmid (IncF) inhibits the acquisition or maintenance of other plasmids in the same cell. Incompatibility due to replicon competition could reduce the pool of resistance-bearing plasmids able to establish themselves in cells already containing the multireplicon pUTI89*. For example, in the ST95 strains we examined, blaTEM-1 was usually located on contigs containing IncF replicons, and such plasmids would be incompatible with a resident pUTI89*. Resistance genes also could be introduced on plasmids from incompatibility groups other than IncF: e.g., the blaTEM-1, carrying IncB plasmid in pUTI89*-containing isolate SF-335. However, strains containing pUTI89* also were also less likely to harbor plasmids from non-IncF incompatibility groups (Table 4). Thus, factors other than replicon competition may be operative and should be tested experimentally.

Several pUTI89-like plasmids were known previously from the literature. Plasmid pRS218 is derived from *E. coli* strain RS218, which was originally isolated from a neonatal meningitis case in San Francisco in 1974 (32), roughly three decades earlier and thousands of miles separated from the UPEC strains bearing the closely related pUTI89 and pEC14_114 plasmids, which were isolated in St. Louis, MO, and St. Paul, MN, respectively. Both DebRoy et al. (25) and Cusumano et al. (26) have presented PCR-based evidence that pUTI89-like plasmids are common in UTI isolates. pUTI89 and the closely related pRS218 have also been shown to have roles in pathogenesis. A UTI89 derivative cured of pUTI89 was impaired in a mouse UTI virulence model (26), and an RS218 derivative cured of pRS218 was impaired in a rat pup model of neonatal meningitis (23). Genes on pUTI89* that could be involved in pathogenesis include those encoding a potential cytotoxin (senB) and systems possibly involved in iron uptake (cjr) (26). More detailed dissection of this plasmid has not yet been done due to difficulties working with it. pUTI89 was found to be highly stable during laboratory culture, and presumably is “in the wild” as well, due at least in part to a stabilization system related to the ParM ATPase (33) that prevents plasmid loss (26).

In summary, genomic analysis of ST95 ExPEC strains revealed an extensive plasmidome accounting for acquired antibiotic resistance and an intriguing phenomenon in which a large plasmid (pUTI89), perhaps in collaboration with chromosomal genes, may inhibit a subset of ST95 strains from acquiring other plasmids and associated antibiotic resistance. A deeper understanding of these pansusceptible strains could potentially be exploited to devise biological strategies to combat drug-resistant Gram-negative bacterial infections.

**MATERIALS AND METHODS**

**Strains and media.** The 44 *E. coli* ST95 isolates with “SF” designations (Table 1) were drawn from the collection of Gram-negative bacterial strains isolated from bloodstream infections at San Francisco General Hospital (SFGH) between 2007 and 2011, as described by Adams-Sapper et al. (10). Isolates MVAST0098 (urine), MVAST0176 (blood), MVAST0234 (urine), and MVAST0326 (urine) were obtained at the Minneapolis Veterans Affairs Hospital in Minneapolis, MN, in 2010 and 2011 (11). Isolates designated “USVAST” were obtained in 2011 in VA hospitals from the following locations as described by Colpan et al. (12): USVAST184, Ann Arbor, MI; USVAST245, Seattle, WA; USVAST267, Sacramento, CA; USVAST356, Dallas, TX; and USVAST406, Jackson, MS. All of the USVAST isolates were obtained from urine specimens. Strains were routinely cultivated on Luria-Bertani agar. Antimicrobial susceptibility testing of the SFGH isolates (10) was performed by a Microscan WalkAway Gram-negative panel (Dade Behring, Inc., Siemens USA, Deerfield, IL). Extended-spectrum β-lactamase (ESBL) production was confirmed by a double-disc diffusion assay according to 2011 Clinical and Laboratory Standards Institute guidelines, based on cefotaxime and ceftazidime in Luria-Bertani agar plates with and without clavulanic acid. “Pansusceptible” was defined herein as susceptibility to β-lactams (ampicillin and cephalexin), aminoglycosides (gentamicin, kanamycin, and streptomycin), chloramphenicol, quinolones (nalidixic acid and norfloxacin), FS/
macrolides (azithromycin), tetracyclines, sulfonamides (sulfamethoxazole), and trimethoprim. "Multidrug resistant" (MDR) was defined herein as resistance to at least three of these classes.

**Genomic DNA sequencing.** Genomic DNA was prepared from all strains using the Qiagen blood and tissue DNeasy kit. Library preparation and sequencing using single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences) have been described elsewhere (15). Analysis of DNA methylation patterns in the SMRT data (19) was done using analysis tools provided by the manufacturer. Library preparation for the MiSeq platform followed a standard protocol for Illumina-compatible libraries (Wafergen Biosystems). Samples were fragmented by a Covaris S220 ultrasonicator to generate an average insert size of 800 bp. After appropriate fragmentation was verified by an Agilent Bioanalyzer, samples were loaded on the Wafergen Apollo 324 NGS Library Prep system. Wafergen PrepX library kits were used for end repair, A-tail addition, adapter ligation, and size selection using AMPure XP beads. Sample concentration was quantified with a Qubit fluorometer. Libraries were PCR amplified to incorporate index tags and flow cell-binding regions. Final libraries were quantified by Qubit, Bioanalyzer, and quantitative PCR (qPCR) and then sequenced via a 300-bp paired-end run on a MiSeq instrument using V3 chemistry and standard Illumina analysis software.

**Bioinformatic analysis.** For draft genomes, MiSeq reads were screened and trimmed based on length and quality using BBduk within the Geneious software package, version R9 (Biomatters, Ltd.). The trimming process also removed any residual adapter sequences. Trimmed paired reads ($4 \times 10^8$ to $1.2 \times 10^9$) were assembled de novo into contigs with the Geneious assembler. The number of reads used in each assembly was sufficient to give a minimum of 25-fold coverage, averaged across all contigs. (A maximum of 45-fold coverage was used.) Contigs that were <1 kb in length, with <2-fold read coverage, or with <80% high-quality base calls were eliminated from subsequent analysis. Annotation of contigs for the analysis presented here was done by the RAST server (34), but sequences submitted to GenBank were annotated by the NCBI prokaryotic genome annotation pipeline. Previously assembled genomes of ST95 ExPEC isolates from the Seattle area (5) were downloaded from NCBI for analysis. Prediction of acquired antibiotic resistance genes employed ResFinder v2.1 (20). Identification of potential plasmid replicons was done with PlasmidFinder v1.3 (28). Identification of insertion elements was done with ISFinder (35), and identification of lysogenic prophage used PHAST (36).

To determine whether a pUTI89-like plasmid was present in ST95 isolates from Seattle (5), pUTI89 was used to query the archived draft genomes using BLAST. Contigs or fragments greater than 500 bp (allowing for the possibility of misassembly) that were >98% identical to the pUTI89 sequence were aggregated. If >98% of the total 114-kb pUTI89 sequence could be generated by combining nonoverlapping contigs or fragments from an isolate, we considered that isolate to be positive for the pUTI89 plasmid.

Plasmids less than 10 kb were not well represented in the long-read data, as the template DNA had been size selected to be greater than 5 kb. These plasmids emerged from de novo assembly of 300-base MiSeq reads. Contigs were designated "small plasmids," analogous to the "small cryptic plasmids" identified in ESBL-producing *E. coli* strains by Brolund et al. (37), if (i) they assembled de novo as circular sequences, (ii) they showed read coverage at least 50% higher than the mean coverage of the top five chromosomal contigs in the same assembly, (iii) they were <10 kb in size, and (iv) BLAST searches of GenBank identified primarily known plasmids, and the contig used as the query covered 80% or more of the plasmid sequence in GenBank. (The Geneious de novo assembler can produce a circular contig if the reads orphan and those reads do not intersect with each other anywhere else in the contig.) The archived genomes of the Seattle isolates could not be used for identification of small plasmids through this approach, as topology and read coverage data were not available for these assemblies.

**Statistical analysis.** Comparison of population frequencies (e.g., frequency of pansusceptibility) and calculated $P$ values used the chi-square test, with a significance cutoff of $P = 0.05$. Comparison of population means (e.g., mean number of small plasmids per isolate) used Students t test, with a significance cutoff of $P = 0.05$.

**Plasmid DNA isolation and transformation.** Plasmid DNA was isolated from *E. coli* cultures using the ZR Plasmid Miniprep Classic kit (Zymo Research). Plasmid DNA was analyzed on 1% agarose gels. DNA was transformed in chemically competent OneShot *E. coli* TOP10 cells (Invitrogen) by the manufacturer’s protocol, and colonies were selected on LB agar plus ampicillin (50 $\mu$g/ml).

**Accession number(s).** Complete genome sequences have been deposited in DDBJ/EMBL/GenBank under the following accession numbers: SF-468 chromosome and plasmids, CP012625 to CP012630; SF-173 chromosome and plasmid, CP012631 and CP012632; SF-166 chromosome and plasmid, CP012633 and CP012634; and SF-088 chromosome and plasmids, CP012635 to CP012638. The whole-genome shotgun sequencing projects described here have been deposited in DDBJ/ENA/GenBank under the accession numbers shown in Table S3.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00390-16.

**FIG S1**, DOCX file, 0.3 MB.

**FIG S2**, DOCX file, 0.2 MB.

**TABLE S1**, DOCX file, 0.1 MB.

**TABLE S2**, DOCX file, 0.1 MB.

**TABLE S3**, DOCX file, 0.1 MB.
ACKNOWLEDGMENTS

We thank Brian Johnston in the Johnson lab for sharing ST95 isolates. We thank Jeffrey Skerker for assistance with analysis of methylation sites from SMRT sequencing data and Nicole Tarlton for thoughtful comments on the manuscript. Finally, we thank Taylor Kelly, Charles Lee, Christopher Alvarado, Jack McBride, and Eric Jedel for contributions to bioinformatics analysis of the ST95 isolates.

This work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 Instrumentation grants S10RR029668 and S10RR027303. The work was supported by the R. B. Roberts Fund provided to the Riley lab. This material is also based in part on work supported by Office of Research and Development, Medical Research Service, Department of Veterans Affairs, grant 1 T01 CX000920-01 (J.R.J.).

REFERENCES

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13:269–284. https://doi.org/10.1038/nrmicro3432.
2. Laupland KB. 2013. Incidence of bloodstream infection: a review of population-based studies. Clin Microbiol Infect 19:492–500. https://doi.org/10.1111/1469-0691.12144.
3. Kaper JB, Nataro JP, Mobley HL. 2004. Pathogenic Escherichia coli. Nat Rev Microbiol 2:123–140. https://doi.org/10.1038/nrmicro818.
4. Riley LW. 2014. Pandemic lineages of extraintestinal pathogen Escherichia coli. Clin Microbiol Infect 20:380–390. https://doi.org/10.1111/1469-0691.12646.
5. Salipante SJ, Roach DJ, Ritzman JO, Snyder MW, Stackhouse B, Butler-Wu SM, Lee C, Cookson BT, Shendure J. 2015. Large-scale genome sequencing of extraintestinal pathogenic Escherichia coli strains. Genome Res 25:119–128. https://doi.org/10.1101/gri.180190.114.
6. Adams-Sapper S, Diep BA, Perdreau-Remington F, Riley LW. 2013. Clonal composition and community clustering of drug-susceptible and -resistant Escherichia coli isolates from bloodstream infections. Antimicrob Agents Chemother 57:490–497. https://doi.org/10.1128/AAC.01025-12.
7. Banerjee R, Johnston B, Lohse C, Chattopadhyay S, Tchesnokova V, Sokurenko EV, Johnston JR. 2013. The clonal distribution and diversity of extraintestinal Escherichia coli isolates vary according to patient characteristics. Antimicrob Agents Chemother 57:5912–5917. https://doi.org/10.1128/AAC.01065-13.
8. Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan MD, Gomes Moriel D, Davies M, Rogers BA, Dougan G, Rodrigo-Baño J, Pascual A, Pitout JD, Upton M, Paterson DL, Walsh TR, Schenber MA, Beatson SA. 2014. Global dissemination of a multidrug resistant Escherichia coli clone. Proc Natl Acad Sci U S A 111:5694–5699. https://doi.org/10.1073/pnas.1322768111.
9. Totsika M, Beatson SA, Sarkar S, Khan MD, Bachmann N, Szubert M, Sidjabat HE, Paterson DL, Upton M, Schenber MA. 2011. Insights into a multidrug resistant Escherichia coli pathogen of the globally disseminated ST131 lineage: genome analysis and virulence mechanisms. PLoS One 6:e26578. https://doi.org/10.1371/journal.pone.0026578.
10. Adams-Sapper S, Servegeanu-Selezneva J, Tarot S, Raphael E, Diep BA, Perdreau-Remington F, Riley LW. 2012. Globally dispersed mobile drug-resistance genes in Gram-negative bacterial isolates from patients with bloodstream infection in a US urban general hospital. J Med Microbiol 61:968–974. https://doi.org/10.1099/jmm.0.041970-0.
11. Drekonja DM, Kuskowski MA, Anway R, Johnston BD, Johnston JR. 2016. The niche for Escherichia coli sequence type 131 among veterans: urinary tract abnormalities and long-term care facilities. Open Forum Infect Dis 3:138. https://doi.org/10.1093/ofid/ofw138.
12. Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, Kuskowski MA, Tchesnokova V, Sokurenko EV, Johnston JR, VICTORY (Veterans Influence of Clonal Types on Resistance: Year 2011) Investigators. 2013. Escherichia coli sequence type 131 (ST131) subclone H30 as an emerging multidrug resistant pathogen among veterans. Clin Infect Dis 57:1255–1265. https://doi.org/10.1093/cid/cdt503.
13. Dias RC, Moreira BM, Riley LW. 2010. Use of fimH single-nucleotide polymorphisms for strain typing of clinical isolates of Escherichia coli for epidemiologic investigation. J Clin Microbiol 48:483–488. https://doi.org/10.1128/JCM.01858-09.
14. Weisiman SJ, Johnson JR, Tchesnokova V, Billig M, Dykhuizen D, Riddell K, Rogers P, Qin X, Butler-Wu S, Cookson BT, Fang FC, Scholes D, Chattopadhyay S, Sokurenko E. 2012. High-resolution two-locus clonal typing of extraintestinal pathogenic Escherichia coli. Appl Environ Microbiol 78:1353–1360. https://doi.org/10.1128/AEM.00663-11.
15. Stephens CM, Skerker JM, Sekhon MS, Arpin AK, Riley LW. 2015. Complete genome sequences of four Escherichia coli ST95 isolates from bloodstream infections. Genome Announc 3:e01241-15. https://doi.org/10.1128/genomeA.01241-15.
16. Wulft B. 2003. The role of P fimbriae for Escherichia coli establishment and mucosal inflammation in the human urinary tract. Int J Antimicrob Agents 21:605–621. https://doi.org/10.1016/S0924-8579(02)00328-X.
17. Gammeau JE, Dupuis M, Villon M, Romero D, Barrangou R, Boyaval P, Painim' Alav, Horvat P, Mapadany AH, Melin C. 2010. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468:67–71. https://doi.org/10.1038/nature09523.
18. Touchon M, Rocha EP. 2010. The small, slow and specialized CRISPR and anti-CRISPR of Escherichia and Salmonella. PLoS One 5:e11126. https://doi.org/10.1371/journal.pone.0011126.
19. Flusberg BA, Webster DR, Lee JH, Travers KJ, Olivares EC, Clark TA, Korlach J, Turner SW. 2010. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nat Methods 7:461–465. https://doi.org/10.1038/nmeth.1459.
20. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MW. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
21. Shigemura K, Tanaka K, Yamamichi F, Shirakawa T, Miyake H, Fujisawa M. 2012. Does mutation in gyrA and/or parC or efflux pump expression play the main role in fluoroquinolone resistance in Escherichia coli urinary tract infections? A statistical analysis study. Int J Antimicrob Agents 40:516–520. https://doi.org/10.1016/j.ijantimicag.2012.07.019.
22. Chen SL, Hung CS, Xu J, Reigstad CS, Mgrini V, Sabo A, Blisari D, Bieri T, Meyer RR, Ozersky P, Armstrong JR, Fulton RS, Lateille JP, Spieth J, Hooton TM, Mardis ER, Hultgren SJ, Gordon JI. 2006. Identification of genes subject to positive selection in uropathogenic strains of Escherichia coli: a comparative genomics approach. Proc Natl Acad Sci U S A 103:5977–5982. https://doi.org/10.1073/pnas.0600938103.
23. Wijetunge DS, Katani R, Kapur V, Kariyawasam S. 2015. Complete genome sequence of Escherichia coli strain RS218 (O18:H7:K1), associated with neonatal meningitis. Genome Announc 3:e00804-15. https://doi.org/10.1128/genomeA.00804-15.
24. Wijetunge DSS, Karunathilake KEM, Chaudhari A, Katani R, Dudley EG, Kapur V, DeRoy C, Kariyawasam S. 2014. Complete nucleotide sequence of pRS218, a large virulence plasmid that augments pathogenic potential of meningitis-associated Escherichia coli strain RS218. BMC Microbiol 14:203. https://doi.org/10.1186/1471-2180-14-203.
25. Cusumano CK, Hung CS, Chen SL, Hultgren SJ. 2010. Virulence plasmid harbored by uropathogenic Escherichia coli functions in acute stages of
pathogenesis. Infect Immun 78:1457–1467. https://doi.org/10.1128/IAI.01260-09.
27. Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerdlow HP, Gu Y. 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genomics 13:341. https://doi.org/10.1186/1471-2164-13-341.
28. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
29. Sahin F, Karasartova D, Gerceker D, Aysev AD, Erdem B. 2008. A novel Salmonella typhimurium plasmid, pAnkS: an example for plasmid evolution in antibiotic resistance. Mikrobiyol Bul 42:383–388.
30. Vignoli R, Cordeiro NF, García V, Mota MI, Betancor L, Power P, Chabalgoity JA, Schelotto F, Gutkind G, Ayala JA. 2006. New TEM-derived extended-spectrum beta-lactamase and its genomic context in plasmids from Salmonella enterica serovar Derby isolates from Uruguay. Antimicrob Agents Chemother 50:781–784. https://doi.org/10.1128/AAC.50.2.781-784.2006.
31. Roberts RJ, Vincze T, Posfai J, Macelis D. 2015. REBASE—a database for DNA restriction and modification. Nucleic Acids Res 43:D298–D299. https://doi.org/10.1093/nar/gku1046.
32. Achtman M, Mercer A, Kusecek B, Pohl A, Heuzenroeder M, Aaronson W, Sutton A, Silver RP. 1983. Six widespread bacterial clones among Escherichia coli K1 isolates. Infect Immun 39:315–335.
33. Salej J, Gayathri P, Löwe J. 2010. The ParMRC system: molecular mechanisms of plasmid segregation by actin-like filaments. Nat Rev Microbiol 8:683–692. https://doi.org/10.1038/nrmicro2425.
34. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.
35. Sigui èr P, Pérochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32–D36. https://doi.org/10.1093/nar/gkj014.
36. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. https://doi.org/10.1093/nar/gkr485.
37. Brolund A, Franzén O, Melefors O, Tegmark-Wisell K, Sandegren L. 2013. Plasmidome-analysis of ESBL-producing Escherichia coli using conventional typing and high-throughput sequencing. PLoS One 8:e65793. https://doi.org/10.1371/journal.pone.0065793.
38. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss, and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
39. Alikhan NF, Petty NK, Ben Zakour NL, Beaton SA. 2011. BLAST Ring Image generator (BriG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.