Discovery of mcr-1-Mediated Colistin Resistance in a Highly Virulent Escherichia coli Lineage

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ABSTRACT  Resistance to last-line polymyxins mediated by the plasmid-borne mobile colistin resistance gene (mcr-1) represents a new threat to global human health. Here we present the complete genome sequence of an mcr-1-positive multidrug-resistant Escherichia coli strain (MS8345). We show that MS8345 belongs to serotype O2:K1:H4, has a large 241,164-bp IncHI2 plasmid that carries 15 other antibiotic resistance genes (including the extended-spectrum β-lactamase blalO2K-H9252 and 3 putative multidrug efflux systems, and contains 14 chromosomally encoded antibiotic resistance genes. MS8345 also carries a large ColV-like virulence plasmid that has been associated with E. coli bacteremia. Whole-genome phylogeny revealed that MS8345 clusters within a discrete clade in the sequence type 95 (ST95) lineage, and MS8345 is very closely related to the highly virulent O45:K1:H4 clone associated with neonatal meningitis. Overall, the acquisition of a plasmid carrying resistance to colistin and multiple other antibiotics in this virulent E. coli lineage is concerning and might herald an era where the empirical treatment of ST95 infections becomes increasingly more difficult.

IMPORTANT Escherichia coli ST95 is a globally disseminated clone frequently associated with bloodstream infections and neonatal meningitis. However, the ST95 lineage is defined by low levels of drug resistance amongst clinical isolates, which normally provides for uncomplicated treatment options. Here, we provide the first detailed genomic analysis of an E. coli ST95 isolate that has both high virulence potential and resistance to multiple antibiotics. Using the genome, we predicted its virulence and antibiotic resistance mechanisms, which include resistance to last-line antibiotics mediated by the plasmid-borne mcr-1 gene. Finding an ST95 isolate resistant to nearly all antibiotics that also has a high virulence potential is of major clini-
Polymyxins B and E (colistin) have been used in veterinary and human medicine for over 50 years. They have broad-spectrum activities against Gram-negative bacteria and are effective against most Enterobacteriaceae. Unfortunately, colistin is associated with both nephrotoxicity and neurotoxicity (1), and due to these adverse effects, it has seen limited use in human medicine. However, colistin has now emerged as an effective therapeutic against carbapenem-resistant Enterobacteriaceae (CRE) (2), carbapenem-resistant Acinetobacter baumannii (CRAB) (3), and Pseudomonas species (4), for which treatment options are limited (5). This overreliance on colistin for treatment of these extensively resistant infections has seen the emergence of CRE, CRAB, and Pseudomonas isolates resistant to colistin (6–10). Colistin resistance is typically mediated by chromosomal mutations resulting in modifications to lipopolysaccharide (LPS), the target site of the polymyxins, and a reduction in polymyxin affinity. These chromosomal mutations are only vertically transmissible, and until recently, the polymyxins remained one of the last classes of antibiotic where resistance was not spread horizontally from cell to cell (9–12).

In 2015, Liu et al. described for the first time a plasmid-borne transmissible colistin resistance gene, mcr-1 (13). The mcr-1 gene belongs to the phosphoethanolamine transferase family of enzymes, which function by catalyzing the 4’-phosphoethanolamine (PEA) modification of lipid A, a component of LPS (13, 14). To date, mcr-1 has been identified on both broad-host-range and narrow-host-range plasmids of different replicon types, including IncI2, IncX4, IncP, IncHI1, and IncHI2 (13, 15–17). Worryingly, carriage of mcr-1 is often associated with cocarriage of other drug resistance genes, including those for carbapenemases (18–20) and extended-spectrum β-lactamas (18, 20, 21). This coassociation of mcr-1 with other drug resistance genes is a significant step toward the emergence of pandrug resistance in the Enterobacteriaceae.

The mcr-1 gene has been identified in a number of different bacterial species, but to date, carriage of mcr-1 is most frequently associated with Escherichia coli (22). In a study of historical E. coli isolates in China, the emergence of the mcr-1 gene was traced back to the 1980s, which coincides with the introduction of colistin as a growth enhancer in food production (23). The study, which screened 1,611 E. coli strains of chicken origin collected from farms in China between 1970 and 2014, found that the proportion of mcr-1-positive E. coli isolates increased exponentially from 5.2% in 2009 to 30% in 2014 (23). Similar proportions of mcr-1-positive E. coli strains have been observed in surveillance studies of food and production animals globally (13, 22, 24). The carriage rates of mcr-1 are much lower in human E. coli isolates than in those from mammals and birds (13, 25). However, as extraintestinal pathogenic E. coli (ExPEC) strains that colonize humans and animals are highly similar (26–28), there is enormous zoonotic potential for mammals and birds to act as reservoirs of infection and transmit the mcr-1 gene to humans (29, 30).

E. coli sequence type 95 (ST95) is a global pandemic clone of ExPEC. In contrast to other pandemic clones, such as E. coli ST131, ST95 isolates are characterized by a low incidence of multidrug resistance (MDR) (31–33). For example, ST95 clinical isolates had the lowest level of antibiotic resistance in comparisons among the 10 most prevalent uropathogenic E. coli (UPEC) sequence types (32, 33). Similar low levels of drug resistance have been identified in ST95 clinical isolates from the United States, Canada, and France (34–37). A highly virulent O45:K1:H7 subclone of ST95 accounts for one-third of all neonatal meningitis cases in France (38). A key feature of this subclone is a ColIV-like virulence plasmid (39).

Here, we describe the first complete genome of an mcr-1-positive E. coli isolate (MS8345) of human origin. MS8345 was isolated from a patient in Qatar with a subarachnoid hemorrhage and was resistant to multiple antibiotics. We show that
TABLE 1 MICs of antibiotics for MS8345

| Antimicrobial                          | MIC (mg/liter) | Interpretation | Gene    |
|---------------------------------------|----------------|----------------|---------|
| Ampicillin                            | ≥32            | R              | TEM-1B  |
| Amoxicillin-clavulanic acid           | 8              | S              | NA      |
| Ticaricillin-clavulanic acid          | 32             | R              | NA      |
| Piperaclillin-tazobactam             | ≤4             | S              | NA      |
| Cefazolin                             | ≥64            | R              | CTX-M-1 |
| Cefoxitin                             | ≥4             | S              | NA      |
| Ceftriazone                           | 4              | R              | CTX-M-1 |
| Ceftriaxone                           | ≥64            | R              | CTX-M-1 |
| Cefepime                              | 2              | R              | CTX-M-1 |
| Colistin                              | 8b             | R              | mcr-1   |
| Meropenem                             | ≤0.25          | S              | NA      |
| Amikacin                              | ≤2             | S              | NA      |
| Gentamicin                            | ≥16            | R              | aac(3)-IIa |
| Tobramycin                            | 4              | R              | aac(3)-IIa |
| Ciprofloxacin                         | ≥4             | R              | gyrA    |
| Norfloxacin                           | 8              | R              | gyrA    |
| Nitrofurantoin                        | ≥16            | S              | dfrA    |
| Trimethoprim                          | ≥16            | R              | dfrA    |
| Trimethoprim-sulfamethoxazole         | ≥320           | R              | dfrA    |
| Polymyxin B                           | 4b             | R              | mcr-1   |

*R*, resistant; *S*, susceptible; NA, not applicable.

**RESULTS**

Identification and characterization of an *mcr-1*-positive *E. coli* strain. A single *E. coli* strain (0.37% of a total of 267 *E. coli* strains) from a diverse collection of Gram-negative bacterial pathogens in the Gulf Cooperation Council states of the Middle East was identified to possess the *mcr-1* gene. The strain, designated MS8345, was nonsusceptible to colistin, polymyxin B, multiple β-lactams (ampicillin, cefazolin, ceftriaxone, ceftazidime, cefepime), and most non-β-lactams (gentamicin, tobramycin, fluoroquinolones, trimethoprim, and trimethoprim-sulfamethoxazole). MS8345 was susceptible to meropenem, nitrofurantoin, the β-lactamase cefoxitin, and to the β-lactam/β-lactamase inhibitors amoxicillin-clavulanic acid and piperaclillin-tazobactam (Table 1).

MS8345 is closely related to a highly virulent clonal lineage associated with neonatal meningitis. The complete genome of *E. coli* MS8345 comprised a single circular chromosome 5,220,996 bp in length with an average G+C content of 50.5% and two circular plasmids: a 241,164-bp multidrug resistance (MDR) plasmid (pMS8345A) containing *mcr-1* and a 133,283-bp virulence plasmid (pMS8345B). In silico multilocus sequence typing (MLST) identified MS8345 as ST95. MS8345 was serotyped as O2:K1:H4 and possesses the fimH27 allele, placing it in the recently defined ST95 subgroup E (40). Pairwise genome comparisons revealed MS8345 to be highly similar to the human neonatal meningitis E. coli (NMEC) isolate S88 (GenBank accession number CU928161) and the avian pathogen APEC-O1 (GenBank accession number CP000468) (Fig. 1). Phylogenetic analysis demonstrated clustering of MS8345 with S88 and APEC-O1 in a clade discrete from the other completely sequenced ST95 strains (Fig. 2). The majority of ST95 complete genomes do not contain MDR plasmids, but several have been found to harbor a virulence plasmid (Fig. 2). Plasmid pMS8345B is highly similar to the ColV-like virulence plasmid pS88 (GenBank accession number CU928146) from E. coli S88 and carries an identical complement of virulence factors and iron uptake systems, specifically, etsABC, ompT, hlyF, the sitABCD operon, salmochelin (iroBCDEN), and aerobactin (iucABCD and iutA) (Fig. 3) (39).

MS8345 contains two large plasmids associated with resistance and virulence, respectively, and is phylogenetically related to strains within a discrete clade in the ST95 lineage (*E. coli* phylogroup B2) that cause meningitis and severe avian infection. The acquisition of plasmid-borne colistin resistance in this highly virulent *E. coli* lineage is of major concern to global health.
The mcr-1 colistin resistance gene is borne on a large MDR IncHI2 plasmid. The mcr-1 gene was located on pMS8345A, a 241-kb IncHI2 plasmid sharing high sequence identity (99% nucleotide identity, 89% sequence length) with the mcr-1-positive plasmid pSA26-MCR1 (GenBank accession number KU743384). pSA26-MCR1 was identified in the carbapenem-resistant (blaNDM-1-positive) E. coli ST68 strain SA26 isolated in Saudi Arabia and is highly similar (99% nucleotide identity, 84% sequence length) to the IncHI2 mcr-1-positive plasmid pHNSHP45-2 (GenBank accession number KU341381) from the Chinese pig isolate SHP45 (41) and to mcr-1-negative plasmids carried in human, poultry, and pig Salmonella isolates from China (99% nucleotide identity, 86% sequence length) (15, 41). The major difference between pMS8345A and pSA26-MCR1 is the structure and content of a large MDR region carried on both plasmids (coordinates 74,782 to 112,611 and 73,858 to 112,218, respectively). The MDR region of pMS8345A contains eight resistance genes that are not present in the MDR region of pSA26-MCR1 and includes a single copy of blaCTX-M-1 carried on an ISEcpl mobile element. In pMS8345A and pSA26-MCR1, mcr-1 is not a component of the MDR region, but instead, the mcr-1 mobile element ISApal1-mcr-1 (42) is inserted 46,815 bp and 47,761 bp downstream (Fig. 4A). The ISApal1-mcr-1 elements of pMS8345A and pSA26-MCR1 are identical (100% nucleotide sequence identity); however, in pSA26-MCR1, the gene encoding the hypothetical protein hp1, downstream of mcr-1, has been disrupted by insertion of a second ISApal1 element carrying a putative pap2-like phosphatase (15) (Fig. 4B). No additional mcr-type genes were identified.

Additional resistance genes. The MDR island of pMS8345A carries 15 additional resistance genes and encodes three putative multidrug efflux pumps, which together provide resistance to multiple classes of antibiotics, including aminoglycosides, β-lactams, macrolides, sulfonamides, tetracycline, and trimethoprim. In addition, a further 14 resistance genes were identified on the MS8345 chromosome. Chromosomally borne resistance genes were distributed across four discrete locations and included two nearly identical copies of an ~50-kb genomic island (GI) (53,078 bp and 52,977 bp, respectively) bearing sul1, adaA1, and erm(B). Finally, mutations in gyrA (resulting in S83L and D87N) and parC (S80I) that lead to fluoroquinolone resistance were also identified. In silico resistance profiling of MS8345 correlates with the observed phenotypic resistances reported via Vitek (Table 1). A complete list of resistance genes carried by MS8345 is reported in Table 2.
The recent discovery of the transmissible, plasmid-borne colistin resistance gene \textit{mcr-1} poses a significant threat to global human health. Since the \textit{mcr-1} gene was first identified in China in 2015 (13), multidrug-resistant human and animal bacterial isolates carrying the \textit{mcr-1} gene have been reported in over 25 countries throughout Asia, Europe, the Middle East, North Africa, and North America (22). Here, we describe the first report of \textit{mcr-1}-mediated colistin resistance in an ExPEC strain of the pandemic ST95 complex from the Persian Gulf region.

Genomic analysis of MS8345 revealed that it is highly similar to the neonatal meningitis strain S88 and the avian pathogenic strain APEC-O1. Notably, these three strains are phylogenetically more related to one another than to other ST95 strains for which a complete genome is available. S88 is a representative of the highly virulent O45:K1:H7 clone, which accounts for one-third of all neonatal meningitis cases in France (38) and carries a ColV-like virulence plasmid (pS88) (39) almost identical to plasmid pMS8345B in MS8345. Although MS8345 was isolated from respiratory secretions and attempts to culture it from the patient’s bloodstream and cerebrospinal fluid were unsuccessful, the extraordinary similarity between these two strains, despite their different O and H types, suggests that they may be equally virulent.

A key feature of the ST95 lineage is the low frequency of MDR among clinical
isolates. In England, in a survey of the nine most common uropathogenic *E. coli* ST clonal lineages, ST95 clinical isolates were identified as having the lowest levels of resistance (32, 33). Similar low levels of drug resistance have been identified in ST95 clinical isolates from the United States, Canada, and France (34–37). However, despite low levels of resistance, ST95 remains a significant cause of extraintestinal *E. coli* infections worldwide. Low levels of antibiotic resistance provide for uncomplicated treatment options, but increasing levels of resistance among the ST95 strains pose a significant risk for treatment failure. Consequently, the emergence of ST95 isolates resistant to multiple antibiotics, including β-lactams and carbapenems, is worrying (37).

In this study, *in silico* antimicrobial resistance profiling of MS8345 revealed it to carry 31
resistance genes, an unusually high number for this clone compared to the number carried by other ST95 strains, which typically have fewer than five resistance genes and remain susceptible to most antibiotics (31–33). Multidrug resistance in MS8345 is attributed to a large complement of acquired resistance genes carried on the chromosome and on the MDR plasmid pMS8345A. High levels of resistance, combined with an extensive virulence profile, characterized MS8345 as a significant outbreak threat and a likely reservoir of plasmid-mediated \textit{mcr-1} trafficking in clinical environments and in the community, emphasizing the need for continuing surveillance.

Carriage of \textit{mcr-1} in human isolates has so far been rare, with less than 2% of clinical Enterobacteriaceae isolates in China and \(<0.2\%\) of clinical \textit{E. coli} isolates in Europe testing positive (13, 25). Low rates of \textit{mcr-1} carriage in human isolates might reflect the traditionally low levels of colistin usage in hospitals. In contrast, colistin is widely used to control diarrheal diseases in poultry and pigs (43). In 2018, the use of colistin in veterinary products is estimated to increase by \(-500\%\) from 1992 usage levels, with China being the largest user, consuming an estimated 12,000 tonnes in 2015 (13, 22). High rates of colistin use in animal production is almost certainly a strong driver of selective pressure for colistin resistance in animal isolates. Indeed, the rate of \textit{mcr-1} carriage in animals and in animal meat products is significantly greater than carriage rates in human isolates (22). For example, in a survey of three chicken farms in Tunisia, up to 83% of birds were estimated to be \textit{mcr-1} positive (24). Notably, poultry and retail chicken meat are recognized as reservoirs of ExPEC in humans (30, 36, 44). A study of serogroup O45 ST95 ExPEC from Spain found human and avian isolates to be highly homogeneous (30), and a study of serotype O1, O2, and O18 APEC strains in China showed that APEC O1:K1 and O2:K1 strains are a major cause of colibacillosis in

| Gene   | Locus tag | Requirement(s) for resistance phenotype | Location        | Coordinates     | Resistance to antibiotic(s) |
|--------|-----------|------------------------------------------|-----------------|-----------------|-------------------------------|
| gyrA   | MS8345_02349 | S83L D87N | Chromosome | 2417780–2420407 | Fluoroquinolones               |
| sul1   | MS8345_03203 |             | Chromosome | 3283535–3284374 | Sulfonamide                    |
| aadA1  | MS8345_03205 |             | Chromosome | 3284879–3285658 | Spectinomycin, streptomycin    |
| erm(B) | MS8345_03209 |             | Chromosome | 3288275–3289012 | Erythromycin                   |
| parC   | MS8345_03375 | S80l         | Chromosome | 4136995–4137855 | Penicillin                     |
| bladEM-1B | MS8345_04043 |             | Chromosome | 4141072–4141887 | Sulfonamide                    |
| strA   | MS8345_04044 |             | Chromosome | 4141948–4142751 | Aminoglycosides                |
| strB   | MS8345_04045 |             | Chromosome | 4142751–4143587 | Aminoglycosides                |
| tetR   | MS8345_04057 |             | Chromosome | 4151677–4152433 | Tetracycline                   |
| tetA   | MS8345_04061 |             | Chromosome | 4152433–4153632 | Tetracycline                   |
| dfrA1  | MS8345_04149 |             | Chromosome | 4244602–4245075 | Trimethoprim                   |
| aadA1  | MS8345_04151 |             | Chromosome | 4245752–4246540 | Spectinomycin, streptomycin    |
| erm(B) | MS8345_04728 |             | Chromosome | 4844585–4845322 | Erythromycin                   |
| aadA1  | MS8345_04732 |             | Chromosome | 4847939–4848718 | Spectinomycin, streptomycin    |
| sul1   | MS8345_04733 |             | Chromosome | 4849219–4849887 | Sulfonamide                    |
| ampC   | MS8345_04780 |             | Chromosome | 4888466–4889999 | Cephalosporins                 |
| arr-2  | MS8345_A00270 |             | pMS8345A | 76547–76999 | Rifampin                       |
| ere(A) | MS8345_A00271 |             | pMS8345A | 77275–78501 | Erythromycin                   |
| adaA1  | MS8345_A00272 |             | pMS8345A | 78587–79378 | Spectinomycin, streptomycin    |
| blaCTX-M-1 | MS8345_A00004 |             | pMS8345A | 87010–87870 | Aminoglycosides                |
| tmrB   | MS8345_A00005 |             | pMS8345A | 87883–88425 | Tunicamycin                    |
| strA   | MS8345_A00011 |             | pMS8345A | 91706–92509 | Aminoglycosides                |
| strB   | MS8345_A00012 |             | pMS8345A | 92509–93345 | Aminoglycosides                |
| blaCTX-M-1 | MS8345_A00015 |             | pMS8345A | 94194–95054 | Penicillin                     |
| mph(A) | MS8345_A00018 |             | pMS8345A | 96389–97294 | Macrolides                     |
| sul1   | MS8345_A00025 |             | pMS8345A | 102605–103444 | Sulfonamide                    |
| dfrA1  | MS8345_A00027 |             | pMS8345A | 104015–104716 | Trimethoprim                   |
| tetX   | MS8345_A00031 |             | pMS8345A | 107415–108551 | Tetracycline                   |
| sul1   | MS8345_A00032 |             | pMS8345A | 108707–109546 | Sulfonamide                    |
| aadA1  | MS8345_A00034 |             | pMS8345A | 110050–110904 | Spectinomycin, streptomycin    |
| mcr-1  | MS8345_A00099 |             | pMS8345A | 160818–162443 | Colistin, polymyxin B          |
domestic and wild birds (35, 39, 45, 46) and can cause septicemia and meningitis in mammalian infection models (47). Here, we have shown that one of the closest relatives of MS8345 is the E. coli avian pathogen APEC-O1, highlighting the zoonotic potential of these bacteria and the potential impact of continued antibiotic misuse in animal production.

Recent reports describing mcr-1-positive plasmids from Enterobacteriaceae have shown that the ISApal1-mcr-1 gene element contains a putative pap2 gene located immediately downstream of mcr-1 (hypothetical protein MS3845_A200 in pMS3845A) (48, 49). The PAP2-like family of phosphatases is capable of modifying lipid A by replacing the negatively charged phosphate groups with a positively charged amine group (50). Changing the charge on LPS is a recognized strategy employed by some bacteria to increase resistance to cationic antimicrobial peptides, such as the polymyxins (51). Although MS3845_A200 does display some homology to putative PAP2 family proteins from other species (BLASTp, 86% amino acid identity, 55% query coverage), it does not possess any functional domains associated with this superfamily and at best represents a nonfunctional fragment of pap-2. However, over the course of our study, we identified an intact pap2-like gene associated with an ISApal1 element (ISApal1–pap2-like), carried on the mcr-1-positive plasmid pA26-MCR1, which has inserted into a hypothetical protein highly similar to MS8345_A200 (15). Whether this pap2-like gene (unannotated, coordinates 158363 to 158905) is functional is currently unknown; however, its potential impact on colistin sensitivity provides an intriguing avenue for further research.

In summary, we provide the first report of an mcr-1-positive isolate of the E. coli ST95 lineage. Using long-read sequencing data enabled us to resolve the complete genome sequence, including the precise context of mcr-1 on an IncHI2 plasmid and the resistance gene profile across three MDR genomic regions. The emergence of colistin resistance in this highly virulent ExPEC lineage is of serious concern to global human health.

MATERIALS AND METHODS

MCR-1 real-time PCR screening. A total of 694 isolates, comprising Acinetobacter baumannii (n = 130), Klebsiella pneumoniae (n = 162), E. coli (n = 267), Pseudomonas aeruginosa (n = 128), Citrobacter freundii (n = 3), and Enterobacter cloacae (n = 4) isolates mainly from the Gulf Cooperation Council states of the Middle East, were tested by PCR. Isolates were prepared using a simple heat denaturation step to release nucleic acids, as previously described (52). Heat-denatured suspensions were pooled (10 isolates per pool) for PCR testing. Isolate pools were then simultaneously tested by two different real-time PCR assays: one using the QuantTect SYBR Green PCR kit (Qiagen, Australia) as the basis for the reaction mix with previously described primers (CLRS-F and CLRS-R) (13) and the other using the Quantitative Tact Probe PCR kit with primers (ACAACTCCTGGCTTTGCTGA and CGATACGATGATAACAGCGTG) and a TaqMan probe (FAM-TGCTCTTTGGCGCGATGCTACT-DQ, where FAM is 6-carboxyfluorescein and DQ is dark quencher) designed as part of this study. PCR assays were run simultaneously and returned identical results. All isolates from any pool providing a positive result were then tested individually. From this screening exercise, we identified a single E. coli isolate (MS8345) that contained the mcr-1 gene.

Case record. MS8345 (also designated HZ-QTR-HMC-19) is an extended-spectrum β-lactamase (ESBL)-producing E. coli strain isolated in Qatar with subarachnoid hemorrhage. His admission was complicated by hydrocephalus, requiring insertion of an extraventricular drain, and severe sepsis with multi-organ dysfunction following gastrointestinal perforation. However, he had no clear signs of ventilator-associated pneumonia at the time that MS8345 was isolated. During his admission, he had exposure to vancomycin, piperacillin-tazobactam, meropenem, and caspofungin but did not receive any treatment associated with pneumonia at the time that MS8345 was isolated. During his admission, he had exposure to vancomycin, piperacillin-tazobactam, meropenem, and caspofungin but did not receive any treatment associated with pneumonia at the time that MS8345 was isolated. During his admission, he had exposure to vancomycin, piperacillin-tazobactam, meropenem, and caspofungin but did not receive any treatment associated with pneumonia at the time that MS8345 was isolated. During his admission, he had exposure to vancomycin, piperacillin-tazobactam, meropenem, and caspofungin but did not receive any treatment associated with pneumonia at the time that MS8345 was isolated. During his admission, he had exposure to vancomycin, piperacillin-tazobactam, meropenem, and caspofungin but did not receive any treatment associated with pneumonia at the time that MS8345 was isolated.
Genome sequencing and assembly. Genomic DNA (gDNA) from MS8345 was sequenced on a PacBio RSII instrument (The Doherty Institute for Infection & Immunity, The University of Melbourne) using a single SMRT cell, a 15-kb insert library, and the P6 polymerase and C4 sequencing chemistry. De novo assembly of the raw PacBio sequencing data were done using the hierarchical genome assembly process (HGAP version 2) and quiver (53) from the SMRT Analysis software suite (version 2.3.0 [http://www.pacb.com/devnet/]) with default parameters. Following de novo assembly, the completeness of the chromosome and plasmids was visually verified using Contiguity (https://github.com/mjsull/Contiguity) (54). The complete chromosome and plasmids were then subjected to a polishing phase, during which the raw PacBio sequencing reads were mapped back onto the assembled circular contigs (BLASR (55) and quiver) to validate the assembly and resolve any remaining errors. gDNA from MS8345 was also prepared as Nextera XT libraries and sequenced on an Illumina NextSeq sequencer at the Australian Centre for Ecogenomics. The raw Illumina sequencing reads were used to resolve 1,046 single-nucleotide insertion and deletion errors associated with homopolymer tracts. Illumina reads were aligned to the complete genome of MS8345 using bwa version 0.7.12 (56), and a corrected consensus was called using Pilon version 1.18 (57).

Multilocus sequencing typing. In silico sequencing typing was performed using MLST version 2.8 (https://github.com/tseemann/mlst) and the E. coli typing scheme available from PubMLST (https://pubmlst.org/) (58).

In silico serotyping. Determination of O and H antigens was performed using SerotypeFinder version 1.1 (59). K antigen was determined using Kaptive (60) and an in-house database of E. coli capsule genes.

Genome annotation and comparative genomics. In silico functional annotation of MS8345 was performed using prokka (Prokka, Prokaryotic Genome Annotation System, http://vicbioinformatics.com/). Identification of antimicrobial resistance genes was performed using ResFinder version 2.0 (61). Additional screening for antimicrobial resistance genes was performed by screening the raw Illumina reads against the ARG-ANNOT database (62) using srst2 (63), and chromosomal genes associated with antibiotic resistance were manually inspect for point mutations known to contribute to a resistance phenotype (e.g., gyrA). Chromosome and plasmid comparisons were performed using BLASTn (64), the Artemis comparison tool (65), and Easyfig (66).

Phylogeny. MS8345 belonged to ST95. To determine the phylogenetic relationship of MS8345 to other ST95 isolates, we carried out phylogenetic analysis. Briefly, the complete genomes of MS8345, UTI89 (GenBank accession number CP000243), S88 (GenBank accession number CP004657), IHE3034 (GenBank accession number CP001969), PMV1 (GenBank accession number HG428755), UM146 (GenBank accession number CP002167), and other E. coli isolates were aligned using Parsnp (67). Recombinant regions were filtered from the alignment using Gubbins v2.1.0 (68), and core single-nucleotide polymorphisms (SNPs) were determined. A maximum-likelihood tree was estimated using RAxML (69) under the GTR+I+G model.

Ethics approval. The permission for publication was granted by the Medical Research Centre at HMC (reference number MRC/0765/2017).

Accession number(s). The complete genome of MS8345 (chromosome and plasmids) has been deposited in GenBank under the accession numbers CP025401, CP025402, and CP025403. PacBio and Illumina sequence read data have been deposited in the Sequence Read Archive (SRA) under the accession numbers SR6364639 and SR6364638, respectively.

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B.M.F., H.M.Z., P.N.A.H., D.L.P., and S.A.B. conceived and planned the study. B.M.F. performed the analysis with input from D.M.W., L.R., E.J., N.S., A.D., M.A.M., K.C., E.T., L.S., H.H.Y., and J.L. B.M.F. produced an initial draft of the manuscript. B.M.F., H.M.Z., P.N.A.H., M.A.S., and S.A.B. wrote the final draft of the manuscript. All authors contributed to, reviewed, and approved the final draft of the manuscript.

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