



# Draft Genome Sequences of Multidrug-Resistant and *mcr-1.1*-Harboring *Escherichia coli* Isolated from Drinking and Well Waters Used in Syrian Refugee Camps

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**ABSTRACT** Resistance to colistin, a last-resort antibiotic, threatens the treatment of complicated infections, especially in susceptible populations such as Syrian refugees who live in makeshift camps. Two multidrug-resistant *Escherichia coli* strains with the plasmid-borne colistin resistance gene (*mcr-1.1*) were isolated from the waters used by refugees and sequenced to analyze antibiotic resistance determinants.

Many Syrian refugees live in makeshift camps with limited access to clean water, food, and proper sanitation (1–3). Previously, we showed that the refugees were exposed to multidrug-resistant *Escherichia coli* (4). These isolates were retrieved from composite water samples (1 liter) collected from the drinking reservoirs and wells that were located in the camps and used by the refugees. The water was filtered using 0.22- $\mu$ m Millipore membranes that were then placed onto the selective RAPID'E. coli 2 agar (Bio-Rad, USA) supplemented with 4  $\mu$ g/ml colistin (Sigma-Aldrich, USA) (4). The plates were then incubated at 37°C under aerobic conditions for 18 to 24 h. Isolates that had the expected *E. coli* phenotype (violet to pink colonies) were purified, and their identities were confirmed using PCR analysis that targeted an *E. coli*-specific 16S rRNA gene fragment (5). The *E. coli* exhibited resistance to important antibiotics, including colistin (polymyxin E) (Table 1), and carried the colistin resistance gene (*mcr-1.1*) on transmissible plasmids (4). To further characterize these strains, their sequence types, and other antimicrobial resistance (AMR) determinants, the genomes of two *mcr-1.1*-positive multidrug-resistant *E. coli* strains that exhibited high resistance to colistin were sequenced.

For genomic DNA isolation, the *E. coli* strains were cultured on RAPID'E. coli 2 agar as described above. Colonies were removed from the plates with inoculation loops and suspended in the buffer supplied in the QiaAmp DNA minikit (Qiagen, USA), which was used to extract the DNA as described in the manufacturer's protocol. DNA concentrations were determined using a Qubit BR double-stranded DNA (dsDNA) assay kit (Invitrogen, USA), and libraries were prepared with the Nextera XT DNA library preparation kit (6). The Qubit dsDNA high-sensitivity (HS) assay kit (Invitrogen, USA) was used to determine the concentration of the sample libraries, which were then diluted and denatured according to the Illumina "Denature and Dilute Libraries Guide" protocol A. The libraries were loaded into the MiSeq reagent cartridge (MiSeq reagent kit v2, 300 cycles) (6) and sequenced on a MiSeq sequencer (Illumina, USA) with the paired-end sequencing strategy (2  $\times$  150 bp). Trimmomatic v0.36 (7) was used to remove low-quality reads. The leading three and the trailing three nucleotides were removed from the reads, and a 4-nucleotide sliding window was used to remove nucleotides from the 3' ends when the average Phred score dropped below 20. After discarding reads shorter than 75 bp, the total number of reads was obtained for each isolate. Draft genome sequences were assembled from trimmed and filtered reads using SPAdes v3.9.0 with

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**TABLE 1** Characteristics and accession numbers of genomes of the *E. coli* strains harboring *mcr-1.1* and isolated from water sources in Syrian refugee camps<sup>a</sup>

Isolate	Bacterial species	Source	SRA no. of raw sequences	GenBank accession no. of assembled genomes	Genome size (bp)	No. of contigs	GC content (%)	N <sub>50</sub> (bp)	Sequencing coverage (X)	Total no. of reads	Resistance to selected antibiotics <sup>b</sup>	Collistin MIC (μg/ml)	AMR genes detected by WGS (total no. of genes) <sup>c</sup>	ST
D1	<i>E. coli</i>	Drinking water	<a href="#">SRX7741066</a>	<a href="#">JACYT0000000000</a>	5,169,139	254	50.4	74,127	82.2	2,241,796	R: PEN, AMP, AMC, LEX, GEN, KAN, STR, TET, CIP, NOR, SXT, CHL	64	<i>aac(3)-IIa, aadA2, ant(3)-Ia, aph(3)-Ib, aph(3)-Ia, aph(6)-Ia, bla<sub>TEM-1</sub><sup>b</sup>, cmlA1, dfrA12, dfrA17, dfrA5, floR, fosA3, Inu(I), mcr-1.1, mdhA, mphA, sul1, sul3, tetA</i> (20 genes)	ST2936
D3	<i>E. coli</i>	Well water	<a href="#">SRX7741068</a>	<a href="#">JACYY0000000000</a>	4,760,848	251	50.7	59,155	79.5	1,910,610	IR: FEP, CTX S: CFM, DOR, IPM, MEM R: PEN, AMP, AMC, CTX, LEX, CFM, DOR, GEN, KAN, STR, TET, CIP, NOR, SXT, CHL	32	<i>aac(3)-IIa, aadA2, ant(3)-Ia, aph(3)-Ia, bla<sub>CFE-1</sub>, bla<sub>CMV-2</sub>, bla<sub>TEM-1</sub><sup>b</sup>, cmlA1, dfrA14, floR, mcr-1.1, mdhA, mphA, sul3, tetA</i> (15 genes)	ST1638

<sup>a</sup> The collistin MIC was quantified using the broth microdilution method. Resistance to other antibiotics was determined using the disk diffusion assay and the Clinical and Laboratory Standards Institute (CLSI) guidelines (16).  
<sup>b</sup> R, resistance; IR, intermediate resistance; S, susceptibility; PEN, penicillin; AMP, ampicillin; AMC, amoxicillin plus clavulanic acid; FEP, cefepime; CTX, cefotaxime; LEX, cephalaxin; CFM, cefixime; DOR, doripenem; IPM, imipenem; MEM, meropenem; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; TET, tetracycline; CIP, ciprofloxacin; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol. The antibiotics in the resistance profiles are arranged according to the order of antibiotics/classes listed in the CLSI guidelines (16).  
<sup>c</sup> WGS, whole-genome sequencing.

the “–careful” option (8). Contigs shorter than 200 bp were discarded. Evaluation of the draft genome quality was performed using QUAST v4.5 (9). Sequence types (STs) were determined using the assembled genomes and the PubMLST database (<https://pubmlst.org/>) with MLST software v2.16.2 (<https://github.com/tseemann/mlst>) (10). Default parameters were used for all software unless otherwise specified.

The properties of the draft genome sequences and their accession numbers are listed in Table 1. Using the ResFinder v3.0 database (11), it was shown that the *E. coli* strains carried 15 and 20 AMR genes (Table 1), including *mcr-1.1* and others that encoded resistance to important classes of antibiotics, such as aminoglycosides, diaminyrimidines, macrolides,  $\beta$ -lactams, phenicols, fosfomycin, tetracyclines, fluoroquinolones, and sulfonamides. MLST analysis revealed that the strains belonged to ST2936 and ST1638. These STs have been associated with extended-spectrum  $\beta$ -lactamase-producing *E. coli* strains and those resistant to extended-spectrum cephalosporins in poultry, as well as *mcr-1*-positive *E. coli*, perhaps suggesting the contamination of water from nearby poultry farms (12–15).

The draft genome sequences are crucial to understanding the dissemination of AMR determinants in refugee camps, highlighting the vulnerability of these populations to resistant infections and unsanitary conditions.

**Data availability.** The assembled genome sequences were deposited under accession numbers [JACYT000000000](https://ncbi.nlm.nih.gov/submit/submit-bio) and [JACYYS000000000](https://ncbi.nlm.nih.gov/submit/submit-bio).

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We declare no conflict of interest.

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