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Detection of the *mcr-1* Gene in a Multidrug-Resistant *Escherichia coli* Isolate from an Austrian Patient

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ABSTRACT Since colistin resistance based on the plasmid-encoded *mcr-1* gene was first described, this resistance gene in *Enterobacteriaceae* has been found worldwide. These organisms are typically of heterogeneous genetic background and show exceptional clonal diversity. We describe the first confirmation of *mcr-1* in a human *Escherichia coli* strain cultured from a surveillance stool sample of an Austrian oncology patient.

KEYWORDS Austria, colistin, *Escherichia coli*, *mcr-1*, resistance

Received 12 December 2016 **Returned for modification** 6 January 2017 **Accepted** 26 January 2017

Accepted manuscript posted online 30 January 2017

Citation Hartl R, Kerschner H, Lepuschitz S, Ruppitsch W, Allerberger F, Apfalter P. 2017. Detection of the *mcr-1* gene in a multidrug-resistant *Escherichia coli* isolate from an Austrian patient. Antimicrob Agents Chemother 61:e02623-16. https://doi.org/10.1128/AAC.02623-16.

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Since the first description of colistin resistance based on the plasmid-encoded *mcr-1* gene, the occurrence of this resistance gene in *Enterobacteriaceae* has been described in Europe and many other areas worldwide (1–3). These organisms are typically of heterogeneous genetic background and show exceptional clonal diversity (4). We describe the first confirmation of *mcr-1* in a human *Escherichia coli* strain cultured from a surveillance stool sample of an oncology patient in Austria.

The *E. coli* strain (isolate 204965) was isolated in June 2016 from a surveillance stool sample of a 60-year-old female patient with acute myeloid leukemia secondary to a myelodysplastic syndrome in Linz, Upper Austria. The patient did not present any symptoms of infection and had no recent travel history. Consecutively collected stool samples revealed the persistence of the strain for at least 3 weeks; no further screening results were available after that period. Screening cultures from other body sites, including urine and throat swab samples, revealed no further colonization. The multidrug-resistant phenotype of the isolate presented in Table 1 indicated the presence of extended-spectrum β-lactamase (ESBL) in combination with resistance to aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole (5). ESBL was confirmed phenotypically by a positive double-disk synergy test between ceftazidime and clavulanic acid.

According to our routine two-step approach for multidrug-resistant enterobacteria, extended susceptibility testing, including colistin, fosfomycin, and tigecycline, was applied to the isolate. The initial colistin MIC of 2 μg/ml (Etest, bioMérieux, France) was categorized susceptible, according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Due to the recent EUCAST warning concerning the use of colistin gradient tests, subsequent broth microdilution was done (UMIC, biocentric, France), which resulted in an MIC of 4 μg/ml, indicating resistance to colistin (6).

The presence of *mcr-1* was then confirmed by PCR, as previously described, and whole-genome sequencing (WGS) (1, 7). For WGS, high-quality genomic DNA (gDNA) was isolated from an overnight culture using the MagAttract HMW DNA kit (Qiagen,

TABLE 1 Susceptibility pattern of isolate 204965

Antibiotic	Result[a]
Ampicillin	R (>256)
Amoxicillin-clavulanic acid	R (128)
Cefuroxime	R (16)
Cefotaxime	I (2)
Ceftazidime	R (16)
Cefepime	R (16)
Ceftolozane-tazobactam	R (8)
Meropenem	S (0.032)
Gentamicin	R (32)
Tobramycin	R (8)
Amikacin	S (2)
Ciprofloxacin	R (>32)
Tigecycline	S (0.5)
Trimethoprim-sulfamethoxazole	R (>32)
Fosfomycin	S (8)

[a]S, susceptible; I, intermediate; R, resistant. Results are based on EUCAST disk diffusion method. Etest results (μg/ml) are given in parentheses.

Hilden, Germany). One nanogram of gDNA was used to prepare the fragment library with the Nextera XT kit, and paired-end sequencing (2 × 300 bp) was performed on a MiSeq (both Illumina Inc., San Diego, CA, USA). There were 1,860,146 raw reads generated from 480,250,526 unassembled nucleotides. Raw reads were *de novo* assembled into a draft genome using Velvet version 1.1.07 (8). Contigs were filtered for a minimum coverage of 5 and minimum length of 200 bp, which resulted in 327 contigs with a total of 5,259,094 nucleotides at a coverage of 91-fold. There were 5,788 genes, 5,427 coding sequences, 239 pseudogenes, 122 RNA genes, and 2 CRISPR (clustered regularly interspaced short palindromic repeat) arrays identified by the NCBI prokaryotic genome automatic annotation pipeline. The PlasmidFinder, ResFinder, FimTyper, and SerotypeFinder tools from the Center for Genomic Epidemiology were used for WGS data analysis, which revealed the presence of plasmids IncHI2, p0111, IncX4, IncH12A, IncFII (pRSB107), IncQ1, and IncFIB (AP001918); *fimH* type f-54; serotype O9:H9; and the resistance genes listed in Table 2 (7, 9, 10). The $bla_{\text{TEM-154}}$-containing contig (8,459 bp) matched *E. coli* plasmid R1 transposon Tn*4* (GenBank accession number HM749966.1) to 99% (3 mismatches) (11). The presence of this complex mutant TEM-type ESBL has not yet been associated with *mcr-1* carriage (2, 12). To assess the classic multilocus sequence type (MLST), ST10 was extracted *in silico* from WGS data using the Warwick MLST scheme. Finally, the contig containing *mcr-1* (15,163 bp) was submitted to GenBank using the basic local alignment search tool (BLAST), and IncHI2 was identified as the *mcr-1*-carrying plasmid showing 99% identity (one mismatch) to plasmid pS38, an IncHI2 plasmid already described as carrying *mcr-1* (13, 14).

After finding the isolate described above and following an ECDC (European Centre for Disease Prevention and Control) rapid risk assessment, 221 suspected carbapenemase-producing *Enterobacteriaceae* (CPE) isolates from the nationwide surveillance system (CARBA-Net) for CPE, archived at the Austrian National Reference Centre for Nosocomial Infections and Antimicrobial Resistance, underwent colistin MIC deter-

TABLE 2 Resistance gene profile of isolate 204965

Antibiotic substance class	Gene (mutation) detected by WGS
Polymyxins	*mcr-1*
Oxyiminocephalosporins	$bla_{\text{TEM-154}}$
Carbapenems	None found
Aminoglycosides	*aadA1*, *aadA2*, *strA*, *strB*, *aac(3)-IIa*
Fluoroquinolones	*gyrA* (Leu83, Asn87), *parC* (Ile80)
Dihydrofolate reductase inhibitors	*sul1*, *sul2*, *sul3*, *dfrA1*
Phenicols	*cmlA1*
Tetracyclines	*tet(A)*

mination by broth microdilution (15). Seven such isolates (5 *K. pneumoniae*, 2 *Enterobacter* spp.) were resistant to colistin and were screened for the presence of *mcr-1*. None of these isolates showed a positive PCR result, which is in line with the observation that colistin resistance may be determined by multiple chromosomal and plasmid-encoded resistance mechanisms (4). To the best of our knowledge, this *mcr1*-mediated colistin resistance is the first instance described and reported in a human *E. coli* strain in Austria.

Accession number(s). This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession MSEK00000000. The version described in this paper is version MSEK01000000.

ACKNOWLEDGMENTS

We thank Sarah Widhalm for excellent technical assistance. We also thank all Austrian microbiology laboratories for referring strains to the National Reference Centre for Nosocomial Infections and Antimicrobial Resistance.

We have no conflicts of interest to declare.

CARBA-Net is funded by the Austrian Ministry of Health and Women's Affairs.

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