mcr-1 and bla$_{KPC-3}$ in Escherichia coli Sequence Type 744 after Meropenem and Colistin Therapy, Portugal

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Escherichia coli Ec36 was recovered from a patient in Portugal after treatment with meropenem and colistin. Besides an IncF plasmid with Tn1441d-*bla*$_{KPC-3}$, already reported in clinical strains in this country, *E. coli* Ec36 co-harbored an IncX4::*mcr-1* gene. Results highlight emerging co-resistance to carbapenems and polymyxins after therapy with drugs from both classes.

The emergence of the *mcr-1* gene (*1*) and reports on its global dissemination (*2*) unveiled the danger of plasmid-associated colistin resistance. In July 2016, a 70-year-old woman was admitted to the intensive care unit of Centro Hospitalar do Baixo Vouga-EPE, Aveiro, Portugal, for abdominal pain, ostensibly from an abdominal occlusion. After emergency surgery, the patient received meropenem (20 d), fluconazole, and linezolid (both 10 d) and was transferred to the general medicine ward. After 50 days of antibacterial drug therapy, a urine specimen was positive for *Klebsiella pneumoniae* (Kp81). Further testing showed a multidrug-resistance profile, including resistance to carbapenems, but susceptibility to colistin and tigecycline (Table). The drug regimen was altered to colistin and tigecycline for 6 days, after which urine cultures were negative for *K. pneumoniae*.

Urine culture was performed as a standard procedure after 72 days. *Escherichia coli* (Ec36) was isolated, showing a resistance profile identical to *K. pneumoniae* Kp81 but expressing colistin resistance (Table). PCR screening and amplicon sequencing confirmed the presence of *mcr-1* in Ec36 and *bla*$_{KPC-3}$ in both isolates (*1,3*). All treatments were discontinued, and the patient was discharged 72 days after admission.

We sequenced the Ec36 whole genome (GenBank accession no. MUGF00000000) by using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA); we assembled it de novo by using CLC Genomics (https://www.qiagen.com/us/search/clc-genomics-workbench/) and annotated results by using RAST (http://rast.nmpdr.org/). We used tools available at the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk) to determine the sequence type, resistome, mobilome, serotype, virulence genes, and pathogenicity potential.

Strain Ec36 was assigned to sequence type 744 (ST-744) and predicted as a human pathogen with serotype O89:H10. Testing detected the virulence gene *gad*, encoding a glutamate decarboxylase involved in acid resistance. Besides *mcr-1* and *bla*$_{KPC-3}$, Ec36 harbored genes encoding resistance to aminoglycosides (*strA*, *strB*, *aacA4*, *aadA*, *aadA5*), β-lactams (*bla*$_{TEM-1B}$, *bla*$_{OXA-9}$), macrolides (*mph*[A]), chloramphenicol (*catA1*), tetracycline (*tet*[A], *tet*[B]), sulfonamides (*sul1*, *sul2*), and trimethoprim (*dfrA14*, *dfrA17*). We used Plasmidfinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/) to identify IncX4 (100%; in the *mcr-1*–encoding contig), IncFIA, IncFII, IncQ1, IncX1, and IncI1. We used pMLST 1.4 (https://cge.cbs.dtu.dk/services/pMLST/) to identify IncFIA and IncFII.

bla$_{KPC-3}$ was in a 16,455-bp contig, 100% identical to plasmid sequences from clinical *K. pneumoniae* (*4*). In Portugal, this plasmid was reported in clinical isolates of *K. pneumoniae*, *E. coli*, and *Enterobacter* (*5*). *bla*$_{KPC-3}$ was part of Tn*4401* isoform d (*4*), flanked by IS*Kpn7* and IS*Kpn6* and located in a cointegrated FIA and FII plasmid (pEc36-KPC3), co-harboring *bla*$_{TEM}$, *bla*$_{OXA-9}$, *aacA4*, and *aadA1*. We analyzed the genetic context of *bla*$_{KPC-3}$ in Kp81 and Ec36 by using a PCR-based protocol (*4*), which indicated a similar context in both strains within Tn*4401d* in a FIA-FII plasmid. As highlighted previously (*5*), results reinforce the role of Tn*4401d* on the spread of carbapenemase genes among *Enterobacteriaceae* in Portugal.

We identified the *mcr-1* gene in a 9,085-bp contig, which matched *E. coli* SHP45 100% (*1*). Genetic context analysis identified a 2,600-bp *mcr-1*–containing cassette recognized in different plasmid backbones (*6*), suggesting its mobilization between different hosts.

The IncX4 plasmid harboring *mcr-1* (pEc36_mcr-1) was divided into 2 contigs, which we subsequently cloned by using PCR and sequencing. pEc36_mcr-1 was 33,140 bp and had no other resistance genes, nor IS*Ap1l*, found originally associated with *mcr*-1 and linked to animal reservoirs (*7*). Plasmid sequence showed high similarity to pESTMCR (GenBank accession no. KU743383), pMCR1-IncX4 (accession no. KU761327), and pMCR1-NJ-IncX4 (accession no. KX447768).

We performed mating assays by using Ec36 as donor and *E. coli* J53 as recipient. Transconjugants were obtained in Plate-Count-Agar (Merck, Germany) with sodium azide

Table. MICs of antibacterial drugs for *Klebsiella pneumoniae* Kp81, *Escherichia coli* Ec36, transconjugant *E. coli* J53::*mcr-1*, and recipient strain *E. coli* J53*

Drug	MIC, mg/L (susceptibility)			
	K. pneumoniae Kp81	*E. coli* Ec 36†	*E. coli* J53::*mcr-1*	*E. coli* J53
Amikacin	≥64 (R)	16 (R)	ND	ND
Aztreonam	≥64 (R)	≥64 (R)	ND	ND
Cefepime	≥64 (R)	2 (I)	ND	ND
Ceftazidime	≥64 (R)	≥64 (R)	ND	ND
Ciprofloxacin	≥4 (R)	≥4 (R)	ND	ND
Colistin	≤0.5 (S)	8 (R)	4 (R)	0.5 (S)
Gentamicin	≥16 (R)	≥16 (R)	ND	ND
Imipenem	≥16 (R)	≥16 (R)	≤0.25 (S)	≤0.25 (S)
Meropenem	≥16 (R)	≥16 (R)	≤0.25 (S)	≤0.25 (S)
Piperacillin	≥128 (R)	≥128 (R)	ND	ND
Piperacillin/tazobactam	≥128 (R)	≥128 (R)	ND	ND
Ticarcillin	≥128 (R)	≥128 (R)	ND	ND
Ticarcillin/clavulanic acid	≥128 (R)	≥128 (R)	ND	ND
Tigecycline	1.5 (S)	1 (S)	0.25 (S)	0.25 (S)
Tobramycin	≥16 (R)	≥16 (R)	ND	ND
Trimethoprim/sulfamethoxazole	≥320 (R)	≥320 (R)	ND	ND

*MICs were determined by using VITEK2 system AST-N222 (bioMérieux, Marcy-l'Étoile, France), except colistin, for which MICRONAUT MIC-strip (BioConnections, Knypersley, UK) was used, and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org). Shaded rows indicate antibacterial drugs tested for efficacy against each of the 4 organisms. ND, not determined; R, resistant; S, susceptible.
†Strain isolated from the patient in this study.

(100 mg/L) and colistin (2 mg/L). The MIC of colistin for the transconjugant (4 mg/L) was 8 times higher than that for *E. coli* J53. We detected *mcr-1* by using PCR for the transconjugant, but not bla_{KPC-3}.

mcr-1 was previously detected in carbapenem-susceptible *E. coli* ST744 in Denmark (*8*) and in *E. coli* ST744, co-producing CTX-M–like β-lactamases, in Taiwan (*9*). Regarding clinical *mcr*-1–positive *E. coli*, >10 STs have been reported, including the high-risk ST-131 (*8,9*). Therefore, the association of a successful clone to the spread of *mcr-1* is not evident, but apparently, it is associated with successful plasmids (e.g., IncX4).

In Portugal, *mcr-1* has been reported in *Salmonella* and *E. coli* from food products and in clinical *Salmonella* isolates (*2,10*). Since bla_{KPC-3} is increasingly reported in Portugal, its co-occurrence with *mcr*-1–harboring plasmids represents a serious concern.

mcr-1 has been found in isolates that produce carbapenemases KPC, NDM, VIM, and OXA-48 (*2,7*). Carbapenemase genes usually are associated with mobile elements that encode resistance to several antibacterial drugs, and consequently produce multiresistance traits, as in *E. coli* Ec36. This scenario might predict the emergence of drug-resistant phenotypes, likely jeopardizing treatment.

In summary, we isolated KPC-3–producing and *mcr-1*–harboring *E. coli* Ec36 from a patient after treatment with meropenem, then colistin. Colistin-resistant Ec36 may have been part of the patient's gut microbiome, acquiring the bla_{KPC-3}-encoding plasmid from the KP81 strain. Although neutropenic, the patient's samples showed an asymptomatic bacteriuria. Thus, prophylactic administration of antibacterial drugs was likely avoidable.

Ms. Tacão is a research scientist at the University of Aveiro, Aveiro, Portugal. Her primary interest is microbiology, particularly bacterial genetic determinants of antibiotic resistance and their dissemination.

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Outcomes for 2 Children after Peripartum Acquisition of Zika Virus Infection, French Polynesia, 2013–2014

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Congenital Zika virus infection is associated with severe brain anomalies and impaired function. To determine outcomes, we followed 2 affected children for ≈30 months. For 1 who was symptomatic at birth, transient hepatitis developed. However, neurodevelopment for both children was age appropriate.

Zika virus, a flavivirus, is a teratogenic and neurotropic infectious pathogen (*1*). Zika virus infection during pregnancy causes congenital microcephaly and severe brain anomalies (*2*). In the newly recognized congenital Zika syndrome, infection is also associated with partially collapsed skull, retinal damage, congenital contractures, early-onset hypertonia, and signs of extrapyramidal involvement; irrespective of a clear pathomechanism, infection is also associated with intrauterine growth restriction and low birth weight (*1*). Developmental outcomes for children born with congenital Zika virus infection have been reported for infants with severe brain anomalies as consequences of early prenatal exposure (*3,4*) and include postnatal slowing of head circumference growth and impaired function.

After the first large-scale Zika outbreak in French Polynesia, October 2013–April 2014 (*5*), 2 cases of peripartum Zika virus infection in full-term neonates were reported (*6*). We report the follow-up and developmental outcomes through ≈30 months of age for these 2 children. We evaluated cognition by using the Child Development Assessment Scale (CDAS), a screening test suitable for children 0–5 years of age (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/8/17-0198-Techapp1.pdf).

Case-patient 1 was born at 38 weeks' gestation; his weight, size, and neurologic status were within reference ranges for gestational age. His mother manifested a rash, suggestive of Zika virus infection, on day 2 after delivery. Reverse transcription PCRs for Zika virus were positive in blood and saliva from the mother (day 2) and neonate (day 3) and in breast milk on day 2. The neonate was breast-fed for 2 months. He remained asymptomatic, and his neurologic development followed a typical course. At 32 months of age, CDAS scores indicated a need to monitor motor development but overall did not indicate neurocognitive problems.

Case-patient 2 was also born at 38 weeks' gestation but was small for gestational age (weight 1,925 g; height 42 cm; head circumference 32 cm). Signs of Zika virus infection (rash) appeared in the mother on day 3 and in the neonate on day 4. Reverse transcription PCRs for Zika virus of blood and urine were positive for the mother (day 1) and the neonate (days 4 and 7) and in breast milk on day 8. On day 2, laboratory testing of blood from the neonate indicated thrombocytopenia (65.0×10^9 thrombocytes/L), leukopenia (4.6×10^9 cells/L), cytolysis, and cholestasis (Table); the cholestasis resolved 4 months later. Ultrasonograms of the liver were unremarkable, and albumin levels and hemostasis remained within reference ranges. Breastfeeding was maintained for 6 months. At 30 months of age, the child's growth remained within –2 SD for weight (10,725 g) and head circumference (47 cm) and –1.5 SD for height (86 cm). CDAS scores indicated no developmental neurocognitive problems.

Follow-up of these 2 case-patients showed that peripartum Zika virus infection, the exposure situation of mother-