We describe a hospital-based outbreak caused by multidrug-resistant, *Klebsiella pneumoniae* carbapenemase 3–producing, *mcr-1*–positive *K. pneumoniae* sequence type 45 in Portugal. *mcr-1* was located in an IncX4 plasmid. Our data highlight the urgent need for systematic surveillance of *mcr-1* to support adequate therapeutic choices in the nosocomial setting.

Infections with carbapenemase-producing *Enterobacteriaceae* (CPE), such as *Klebsiella pneumoniae*, have been increasing since 2011 in hospitalized patients in several countries in Europe, especially those with high resistance rates ([https://ecdc.europa.eu/sites/portal/files/documents/antibiotics-EARS-Net-summary-2016_0.pdf](https://ecdc.europa.eu/sites/portal/files/documents/antibiotics-EARS-Net-summary-2016_0.pdf); [https://ecdc.europa.eu/sites/portal/files/documents/AMR-surveillance-Europe-2016.pdf](https://ecdc.europa.eu/sites/portal/files/documents/AMR-surveillance-Europe-2016.pdf)). The emergence of mobilized colistin resistance (MCR) genes is particularly concerning because colistin is being intensively used as a last resource antimicrobial drug [1,2]. In Europe, sporadic clinical CPE isolates with *mcr-1* have been reported (3,4). Because CPE has increased at an alarming pace in Portugal (5,6), we evaluated the occurrence of *mcr-1* among CPE isolated from patients admitted to Centro Hospitalar do Porto, a tertiary and university hospital in Porto, Portugal.

The Study

Using rectal swab specimens from 5,361 patients admitted to Centro Hospitalar do Porto during October 2015–July 2017, we screened for carbapenemase-positive isolates using Brilliance CRE Agar (Oxoid, Basingstoke, UK), Blue-carba test (3,4), and real-time PCR for carbapenemase genes (Xpert Carba-R; Cepheid, Sunnyvale, CA, USA) (Figure 1, panel A). We identified 283 patients with 359 CPE-positive samples available for further testing. Of the 359 isolates, 252 (75% *K. pneumoniae*-positive) were from patient fecal samples and 107 (86% *K. pneumoniae*-positive) were from other types of patient samples (e.g., blood, urine). We then screened these isolates for *mcr-1*, *blaCTX-M*-like genes, and *blaKPC* using PCR and sequencing (5,8,9). We determined the antimicrobial drug susceptibility profiles of the *mcr-1*–positive isolates by the broth microdilution method for colistin ([http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf)) and by disk diffusion for the other antimicrobial drugs using Clinical and Laboratory Standards Institute/European Committee on Antimicrobial Susceptibility Testing guidelines ([http://www.eucast.org/](http://www.eucast.org/)).

We evaluated clonal relatedness among *K. pneumoniae* isolates by multilocus sequence and *wzi* capsular typing ([http://bigbdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db = pubmlst_klebsiella_seqdef_public](http://bigbdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db = pubmlst_klebsiella_seqdef_public)) and assessed plasmid replicon content using PCR (5). We performed whole-genome sequencing with 2 isolates of the predominant *K. pneumoniae* clones by Hi Seq 2500 Sequencing System (Illumina Inc., San Diego, CA, USA) (2 × 150 bp paired-ended reads, coverage 100×). We assembled reads de novo using SPAdes version 3.9.0 ([http://cab.spbu.ru/software/spades/](http://cab.spbu.ru/software/spades/)) and annotated contigs with Prokka ([http://victbioinformatics.com/](http://victbioinformatics.com/)). We used tools from the Center for Genomic Epidemiology ([http://www.genomicepidemiology.org](http://www.genomicepidemiology.org)) to assess antimicrobial drug resistance genes and replicons and PLACNETw ([https://castillo.dicom.unican.es/upload/](https://castillo.dicom.unican.es/upload/)) for plasmid reconstruction. We located *mcr-1* in the IncX4 plasmid near the replication (*pirF*) and maintenance (*parA*) conserved regions by PCR and sequencing (Figure 2).

We identified 24 carbapenemase-producing and MCR-1–producing *K. pneumoniae* isolates from samples collected during September 2016–February 2017 from 16 hospitalized patients (Figure 1, panel B). Seventeen isolates were colonizers (i.e., bacteria of the patients’ gastrointestinal tract), and 7 were from other parts of the body (3 urine, 2 blood, 2 other biologic fluids) (Table). We recovered 1–4 isolates/patient; 10 colonizing isolates were from intensive care units. Patients (9 men, 7 women) were 50–87 years of age, and their clinical history included prolonged hospitalization (median 47 d, range 12–151 d); complicated conditions; and, for many, surgical interventions, immunosuppression, or previous antimicrobial drug...
use (usually β-lactams) favoring infection or colonization by multidrug-resistant (MDR) mcr-1–positive strains (10). Fecal samples were negative for CPE at admission (14/16 patients screened) and for a median of 15 (range 3–94) days after admission (Figure 1, panel B). Five patients had 1 or 2 extraintestinal infections with an MCR-1–producing isolate, sometimes with an isolate identical to one previously detected in their gastrointestinal tract.

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**Figure 1.** Selection for and testing of patients with *Klebsiella pneumoniae* carbapenemase 3–producing mcr-1–positive *Enterobacteriaceae*, Porto, Portugal, 2016–2017. A) Flowchart demonstrating rationale for sample selection. First, we screened for asymptomatic carriage of CPE in the gastrointestinal tract (i.e., colonization by CPE) by testing patient fecal samples with Brilliance CRE Agar (Oxoid, Basingstoke, UK); Xpert Carba-R (Cepheid, Sunnyvale, CA, USA); and VITEK 2 (bioMérieux, Marcy l’Etoile, France). Second, we tested for CPE with all patient samples available. Last, we screened the carbapenemase-producing isolates for mcr-1 to identify the final sample. *CPE isolates and complete epidemiologic and clinical data were available for ≈75% of CPE patients. †The final sample screened for mcr-1 included only nonrepetitive isolates. For fecal samples, we considered isolates repetitive when detected in the same patient in samples collected within 72 h from each other. For other types of samples, we considered isolates repetitive when detected in the same sample type collected at the same time point. ‡Four patients carried mcr-1–positive isolates either in the gastrointestinal tract or in other body sites. B) Timeline representing epidemiologic data of the 16 patients with mcr-1–positive CPE. CPE, carbapenemase-producing *Enterobacteriaceae*; ICU, intensive care unit; MED, medical unit; SURG, surgical unit; TU, transplant unit.
Colistin use and travel abroad were not recorded for any patient before mcr-1 detection; however, 5 of the 16 patients had been hospitalized in the previous 6 months. Patients were treated for CPE infection with colistin and a carbapenem, which was supplemented with fosfomycin, tigecycline, or piperacillin/tazobactam depending on clinical criteria. We missed colistin resistance initially because we used conventional antimicrobial susceptibility tests VITEK 2 (bioMérieux, Marcy l’Etoile, France) and Etest (bioMérieux), which are unreliable at detecting colistin resistance. Adequate colistin resistance monitoring (http://www.euCAST.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf) and mcr-1 screening for CPE isolates was implemented in July 2017.

Isolates carrying mcr-1.1 were resistant to colistin (MIC 4–8 mg/L), produced K. pneumoniae carbapenemase 3 (KPC-3), and most (79%) produced CTX-M-15 β-lactamase. Besides 100% resistance to third and fourth generation cephalosporins and monobactams, K. pneumoniae isolates were also frequently resistant to nalidixic acid (100%), ciprofloxacin (96%), tigecycline (96%), tetracycline (92%), tobramycin (88%), gentamicin (88%), fosfomycin (83%), trimethoprim/sulfamethoxazole (79%), and chloramphenicol (67%) (Table). All isolates were susceptible to amikacin (which was contraindicated for some patients because of renal insufficiency) and ceftazidime/avibactam (which was not available).

All but 1 K. pneumoniae isolate belonged to sequence type (ST) 45 and carried wci101/K24, a clone that has been infrequently detected among clinical MDR K. pneumoniae isolates in Portugal (5,6) but has circulated among KPC-3 producers (without mcr-1) during the same period (L. Peixe, unpub. data). We detected 1 mcr-1–positive K. pneumoniae (capsular type KL122) ST1112 isolate from the pus of an abdominal wall abscess in a patient having mcr-1–positive ST45 in previously collected fecal and urine samples (Table). The 2 whole-genome–sequenced K. pneumoniae ST45 isolates had genes encoding resistance to aminoglycosides [aac(6’)-Ib-cr,aac(3)-IIda]; β-lactams (blaKPC-3, blaSHV-1, blaOXA-1), fluoroquinolones [qnrB66, aac(6’)-Ib-cr, qoxAB], and other antimicrobial drugs [catB4,tet(A)]; 1 of the 2 isolates possessed additional genes aph(4)-Ib, strAB, blaTEM-1β, blaCTX-M-15, catA1, sul2, and dfrA14.

In all mcr-1–positive isolates, the gene was located in an IncX4-type plasmid (Figure 2). Comparative genomics revealed that this plasmid (pAN_M1A) is circulating among diverse hosts (humans, pig, poultry) and the environment in many different countries, including Portugal (11). We identified blaKPC-3 in a Tn4401d isoform in an ≈58-kb

![Figure 2. Alignment of representative mcr-1–harboring IncX4 plasmids from different isolation sources and geographic regions. The mcr-1–harboring plasmid pAN_M1A was used as a reference plasmid. The outermost circle is an annotation of the reference plasmid and shows the direction of transcriptional open-reading frames. The pil loci and other genes (gray), replication-associated genes (dark blue), antimicrobial drug resistance gene (red), and insertion sequence (green) are indicated. The strategy for PCR mapping of mcr-1–carrying plasmids is indicated by red arrows. Primer P1 targets pirF, P2 mcr-1 (3.3 kb), P3 mcr-1, and P4 parA (2.1 kb).]
IncN-ST15 plasmid, a minority platform in our previous survey (5); \textit{bla}_{CTX-M,15} was associated with multireplicon plasmid IncFI\textsubscript{K}-FIA-FIB. We deposited this whole-genome shotgun project at DDBJ/European Nucleotide Archive/GenBank under accession no. PEHI00000000.

We found that 5.7% (16/283) of hospitalized patients had gastrointestinal tracts colonized with \textit{mcr-1}-positive CPE, and in 1.8% (5/283) of these patients, an infection developed; these rates are comparable with those reported in China (up to 6.2% for fecal colonization, 1% for infections) (10,12). In China, only 1 outbreak involving \textit{mcr-1}-carrying clinical isolates has been reported (13), and in Europe, a low occurrence (<1%) and sporadic clinical cases have been reported (3,4). Colistin is a critical last resource antimicrobial drug; prolonged carriage of \textit{mcr-1}-positive MDR strains (especially by patients at discharge) represents a risk for subsequent infections and dissemination to other \textit{Enterobacteriaceae} species. Of note, identifying CPE asymptomatic carriers at discharge is a practice recommended in Portugal, though not mandatory.
Considering the absence of CPE at admission, nosocomial acquisition and in-hospital dissemination of KPC-3-producing strains carrying mcr-1 is plausible; however, we cannot rule out that other *K. pneumoniae* lineages or *Escherichia coli* might have been the source of mcr-1. Although the prevalence of colonization of humans by mcr-1–positive strains is unknown in Portugal, previous detection of mcr-1 in livestock, such as *K. pneumoniae* ST45 in pigs, suggests transmission through the food chain and wider dispersion of MCR-1–producing *Enterobacteriaceae* (8,11,14,15).

**Conclusions**

We report the emergence of mcr-1 in MDR KPC-3-producing *K. pneumoniae* associated with an unnoticed outbreak. High rates of CPE and colistin use (2,5,6) together with an ongoing community-based dissemination of mcr forebodes of future similar events. Our data stress the need for a concerted action involving different professionals and healthcare institutions to monitor and contain the spread of mcr across human and veterinary niches, the food chain, and the environment.

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**References**


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