mcr-1 in Carbapenemase-Producing Klebsiella pneumoniae in Hospitalized Patients, Portugal, 2016–2017

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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.


The Study

Using rectal swab specimens from 5,361 patients admitted to Centro Hospitalar do Porto, a tertiary and university hospital in Porto, Portugal.

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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.

**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 \textit{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 \textit{mcr-1} screening for CPE isolates was implemented in July 2017.

Isolates carrying \textit{mcr-1}.1 were resistant to colistin (MIC 4–8 mg/L), produced \textit{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, \textit{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 \textit{K. pneumoniae} isolate belonged to sequence type (ST) 45 and carried \textit{wzi}101/K24, a clone that has been infrequently detected among clinical MDR \textit{K. pneumoniae} isolates in Portugal (5,6) but has circulated among KPC-3 producers (without \textit{mcr-1}) during the same period (L. Peixe, unpub. data). We detected 1 \textit{mcr-1}–positive \textit{K. pneumoniae} (capsular type KL122) ST1112 isolate from the pus of an abdominal wall abscess in a patient having \textit{mcr-1}–positive ST45 in previously collected fecal and urine samples (Table). The 2 whole-genome–sequenced \textit{K. pneumoniae} ST45 isolates had genes encoding resistance to aminoglycosides [\textit{aac(6)\text{\textsuperscript{r}}}, \textit{bla} \textit{carbapenemase 3}], β-lactams (\textit{bla} \textit{KPC-3}, \textit{bla} \textit{SHV-1}, \textit{bla} \textit{TEM-1B}), fluoroquinolones [\textit{qnrB66}, \textit{aac(6)\text{\textsuperscript{r}}}, \textit{qoxAB}], and other antimicrobial drugs [\textit{catB4},\textit{tet(A)}]; 1 of the 2 isolates possessed additional genes \textit{aph(4)\text{\textsuperscript{r}}}, \textit{strAB}, \textit{bla} \textit{TEM-1B}, \textit{bla} \textit{CTX-M-15}, \textit{catA1}, \textit{su1}, and \textit{dfrA14}.

In all \textit{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 \textit{bla} \textit{KPC-3} in a \textit{Tn4401d} isofrom in an ≈58-kb
IncN-ST15 plasmid, a minority platform in our previous survey (5); \textit{bla}_{KPC-3} \textit{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.
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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