A novel mcr variant, named mcr-1.2, encoding a Glnγ-to-Leu functional variant of MCR-1, was detected in a KPC-3-producing ST512 Klebsiella pneumoniae isolate collected in Italy from a surveillance rectal swab from a leukemic child. The mcr-1.2 gene was carried on a transferable IncX4 plasmid whose structure was very similar to that of mcr-1-bearing plasmids previously found in Escherichia coli and K. pneumoniae strains from geographically distant sites (Estonia, China, and South Africa).

Transferable polymyxin resistance mediated by the plasmid-borne mcr-1 gene has recently been described in Enterobacteriaceae, raising considerable concern (1). The mcr-1 gene product is a membrane-anchored enzyme able to modify the lipid A polymyxin target by addition of phosphoethanolamine, resulting in a reduction of affinity for polymyxins (1).

The mcr-1 gene has mostly been found in Escherichia coli isolates from animals, but also in those from human and food samples, and in isolates of other enterobacterial species, with a worldwide distribution (2). The gene has often been reported in strains susceptible to other antibiotics, but occasionally also in multidrug-resistant (MDR) strains (3–8), including members of high-risk epidemic lineages spreading in the clinical setting (9, 10). Thus far, however, it had never been found in Klebsiella pneumoniae strains of clonal group 258 (CG258), which is the lineage mainly responsible for the dissemination of KPC-type carbapenemases on the global scale (11, 12).

Here we describe the first detection of a novel mcr variant, named mcr-1.2, from an MDR KPC-producing K. pneumoniae strain belonging to sequence type 512 (ST512), a member of CG258.

Screening for KPC carbapenemase-producing Enterobacteriaceae in rectal swabs was carried out using the direct KPC screening test (DKST), based on direct plating of rectal swabs onto MacConkey agar in the presence of a meropenem disc and of a meropenem-plus-phenylboronic acid disc (13). Bacterial identification was carried out by matrix-assisted laser desorption ionization—time of flight (MALDI-TOF) mass spectrometry (Vitek-MS; bioMérieux, Marcy l’Etoile, France). Antimicrobial susceptibility was determined by reference broth microdilution (14) using custom plates (Trek Diagnostic Systems, Cleveland, OH, USA), and data were interpreted according to the EUCAST guidelines (EUCAST breakpoint tables v6.0). Whole-genome sequencing (WGS) and analysis were carried out as previously described (15). Plasmid finishing was achieved by a PCR-based strategy and Sanger sequencing. Sequence alignments were performed using Mauve (16), and physical maps were generated using Easyfig (17). Determination of the multilocus sequence type, plasmid replicons, and resistance gene content was performed in silico using online tools (http://www.genomicepidemiology.org/). Transfer of the colistin resistance determinant by conjugation was assayed on Mueller-Hinton (MH) agar plates (Oxoid, Basingstoke, United Kingdom) with an initial donorrecipient ratio of 0.1, using E. coli J53 (F’ met pro Azi2 [azide resistance]) as the recipient (18). After incubation at 35°C for 16 h, transconjugants were selected on MH agar supplemented with colistin (2 μg/ml) and sodium azide (150 μg/ml). The transfer frequency was expressed as the number of transconjugants per recipients (t/r). Transfer of mcr to transconjugants was confirmed by PCR targeting the mcr gene (10).

K. pneumoniae KP-6884 was isolated in 2014 from a rectal surveillance swab obtained from an Italian child admitted to the pediatric onco-hematology ward of Pisa University Hospital. The strain was preliminarily identified as a putative KPC producer by the DKST method. The child was receiving a cycle of antinecancer chemotherapy for acute lymphoblastic leukemia and had previously been admitted twice to the same ward (1 and 2 months earlier). Of note, the child had not received colistin before the first isolation of KP-6884.

KP-6884 showed an MDR phenotype, including resistance to β-lactams, fluoroquinolones, trimethoprim-sulfamethoxazole, gentamicin, and colistin, remaining susceptible only to amikacin and tigecycline (Table 1). As such, KP-6884 exhibited an unusual phenotype (resistance to gentamicin and trimethoprim-sulfamethoxazole and susceptibility to amikacin) compared to that of the most prevalent carbapenem-resistant (CRE) KPC-producing K. pneumoniae strain circulating in our setting (19, 20).

To investigate the mechanisms of resistance of KP-6884, a WGS approach was adopted. The draft genome was de novo as-
Assembled in 91 scaffolds (largest scaffold, 544,125 bp; N50, 270,452 bp; L50, 8; average GC, 57.09%), with an estimated genome size of 5,626,271 bp and average coverage of 80×. A total of 5,631 coding DNA sequences were identified using the PGAP annotation pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok).

In silico analysis of the draft genome confirmed the identification of KP-6884 as *K. pneumoniae sensu stricto* (21) and revealed that it belonged to ST512.

Screening for acquired resistance determinants revealed the presence of genes encoding β-lactamases (*bla*TEM-1, *bla*SHV-11, and *bla*KPC-3), aminoglycoside-modifying enzymes (*aadA2*, *aadA5*, *aacA4*, and *aacC2d*), sulfonamide resistance (*sul1*), trimethoprim resistance (*dfrA17*), and colistin resistance (a new *mcr* gene variant). Compared to *mcr-1* (1), the sole allelic variant thus far described, the *mcr* gene from KP-6884 carried a missense mutation at position 8 (A→T) resulting in a Gln-to-Leu change in the N-terminal protein region. A BLAST search, using this novel allelic variant, here referred to as *mcr-1.2*, showed no perfect match with any of the *mcr* genes present in the nr/wgs databases (last accessed 11 May 2016). Overall, the acquired resistome of KP-6884 was

### TABLE 1 MICs for *K. pneumoniae* KP-6884, *E. coli* J53 (pMCR1.2-IT), and *E. coli* J53

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml) (category)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>&gt;8/2 (R) 8/2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;4 (R) 0.12</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;128 (R) 0.5</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;32 (R) ≤1</td>
</tr>
<tr>
<td>Eratapenem</td>
<td>&gt;1 (R) ≤1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;16 (R) ≤1</td>
</tr>
<tr>
<td>Meropenem</td>
<td>64 (R) ≤0.12</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>&gt;128/4 (R) ≤2/4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2 (R) ≤0.06</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8 (S) ≤4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;4 (R) ≤1</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&gt;4/76 (R) ≤0.5/9.5</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.5 (S) 0.5</td>
</tr>
<tr>
<td>Collistin</td>
<td>8 (R) ≤0.5</td>
</tr>
</tbody>
</table>

* R, resistant; S, susceptible.
consistent with the resistance phenotype, including the unusual gentamicin resistance justified by the presence of the aacC2d gene (Table 1). Other possible causes of colistin resistance, mediated by mutations in chromosomal genes (22, 23), were excluded by the analysis of sequence data. In detail, mrgB, pmrAB, phoPQ, and crrAB sequences did not show any genetic alteration previously or potentially associated with colistin resistance (the sequences were identical to those of colistin-susceptible strains).

Bioinformatic analysis revealed the presence of several plasmid replicons, including ColE, IncFIA-FIB, IncFII-FIB, IncX3, and IncX4. The latter was located on the same contig as mcr-1.2. Plasmid finishing resulted in a 33.3-kb-long circular molecule, representing the complete sequence of the IncX4 plasmid carrying mcr-1.2, named pMCR1.2-IT (Fig. 1). Overall, pMCR1.2-IT was very similar to other previously sequenced IncX4 plasmids carrying mcr-1, namely, pMCR1-IncX4 (GenBank accession no. KU761327) (24), pESTMCR (GenBank accession no. KU743383), and pAf48, from a clinical K. pneumoniae isolate from China, an E. coli isolate from pig sludge in Estonia, and an E. coli clinical isolate from South Africa (GenBank accession no. KX032520), respectively (25) (Fig. 1). In all these plasmids, the mcr gene was embedded in the same genetic environment, with a downstream gene encoding the PAP2 transmembrane protein and without an upstream ISApII, unlike what has been observed in other non-IncX4 plasmids (24) (Fig. 1). These findings underscored the broad intercontinental distribution of this type of resistance plasmid.

Gene transfer experiments demonstrated that the mcr-1.2 gene could be transferred by conjugation from KP-6884 to E. coli J53 with a frequency of $5 \times 10^{-5} \text{ (t/t)}$. The colistin MIC of transconjugants showed a 16-fold increase (Table 1), confirming that MCR-1.2 was functional. The MICs of transconjugants for all other agents were unchanged.

Concluding remarks. To the best of our knowledge, this is the first description of a novel MCR-1 functional variant and also the first time that an mcr-type gene has been found to be associated with an ST512 KPC-3-producing high-risk clone of K. pneumoniae. Until now, only a few cases of human infections caused by carbapenemase-producing (NDM-type, VIM-1, KPC-2, OXA-48), mcr-positive strains of E. coli and K. pneumoniae have been reported (3–6, 24, 26).

Considering that the clinical use of polymyxins is essentially restricted to the treatment of invasive infections caused by carbapenem-resistant and extensively drug-resistant Gram-negative nonfermenters, the emergence of transferable colistin resistance among CRE is a cause of serious concern, especially in settings of extended-spectrum carbapenemase producers among CRE is a cause of serious concern, especially in settings of extended-spectrum carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis 16:161–168. http://dx.doi.org/10.1016/S1473-3099(15)00424-7.


