

Three cases of *mcr-1*-positive colistin-resistant *Escherichia coli* bloodstream infections in Italy, August 2016 to January 2017

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We describe three cases of bloodstream infection caused by colistin-resistant *Escherichia coli* in patients in a tertiary hospital in Italy, between August 2016 and January 2017. Whole genome sequencing detected the *mcr-1* gene in three isolated strains belonging to different sequence types (STs). This occurrence of three cases with *mcr-1*-positive *E. coli* belonging to different STs in six months suggests a widespread problem in settings where high multidrug resistance is endemic such as in Italy.

A new plasmid-mediated transferable resistance determinant, the *mcr-1* gene, encoding a phosphoethanolamine transferase, has been described in November 2015 by Liu et al. for the first time [1,2]. The plasmid carrying *mcr-1* is mobilised to an *Escherichia coli* recipient by conjugation [1,2]. Since that description, the *mcr-1* gene has been detected in isolates recovered from animals, in the food chain and in humans in many countries in Europe and in many other areas worldwide [3-8].

Here we describe three cases of human bloodstream infection in Italy caused by *E. coli* harbouring the *mcr-1* gene. All three patients were hospitalised in a 1,000-bed hospital, in Pavia, in the period between August 2016 and January 2017.

Case description

Case 1

In July 2016, a woman in her 70s with a pancreatic ductal carcinoma diagnosed 5 years earlier, was admitted in a respiratory disease unit for pleural effusion. It was known that she had bone and liver metastases, was splenectomised, and had received 18

chemotherapy cycles in the previous 5 years. Four days after admission she experienced fever (38.8° C), vomiting and abdominal pain associated with increased inflammatory markers: procalcitonin 60 ng/mL (norm: 0.00–0.50 ng/mL), C-reactive protein (CRP) 23.29 mg/dL (norm: 0.00–0.50 mg/dL) and highly elevated white blood cells (WBC) 38.92 x 10³ uL (norm: 4–10 x 10³ uL). *E. coli* was isolated from urine and blood cultures. The two isolates showed the same antimicrobial susceptibility profiles. Both showed resistance to colistin. The susceptibility profile of the isolate obtained from blood is shown in the Table.

The patient was empirically treated with meropenem intravenously and her clinical condition rapidly improved. She was discharged after 20 days of hospitalisation. She had never received previous treatment with colistin and did not report any previous close contacts with farm animals. She had not travelled abroad since 2008.

Case 2

In August 2016, a woman in her mid-60 with a diagnosis of non-Hodgkin's Lymphoma was admitted at the haematological unit of our hospital for distention of the ureter, renal pelvis and calices due to blockage of urine flow by bulky lymph nodes. She had been treated with two cycles of chemotherapy in the previous 6 weeks with poor response. She underwent nephrostomy after admission and one day later she developed fever (38.0° C) and chills. She had a severe pancytopenia with less than 0.800 x 10³/uL (norm: 4–10 x 10³ uL) WBC and increase of CRP 3.50 mg/dL (0.00–0.50 mg/dL).

The patient received intravenous empirical treatment with piperacillin/tazobactam and vancomycin. *E. coli*

TABLE

Antibiotic susceptibility according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints [9] of clinical *Escherichia coli* isolates obtained from human cases with bloodstream infections, Pavia, Italy, August 2016–January 2017 (n = 3 cases)

| Case | Isolation date | Sequence type | MIC mg/L (S/I/R) | | | | | | | | | | | | |
|--------|----------------|---------------|------------------|--------|--------|----------|--------|--------|-----------|---------|------------|--------|----------|-----------|----------|
| | | | CST | AMK | AMP | CAZ | CTX | FEP | CIP | FOS | MEM | GEN | TZP | SXT | TGC |
| Case 1 | 7 Aug 2016 | 131 | 4 (R) | <4 (S) | ≤2 (S) | ≤0.5 (S) | ≤1 (S) | ≤1 (S) | ≤0.25 (S) | ≤16 (S) | ≤0.125 (S) | ≤1 (S) | ≤4/4 (S) | ≤1/19 (S) | ≤0.5 (S) |
| Case 2 | 12 Aug 2016 | 3941 | 4 (R) | <4 (S) | >8 (R) | ≤0.5 (S) | ≤1 (S) | ≤1 (S) | >1 (R) | ≤16 (S) | ≤0.125 (S) | >4 (R) | ≤4/4 (S) | >4/76 (R) | ≤0.5 (S) |
| Case 3 | 22 Jan 2017 | 1851 | 4 (R) | <4 (S) | >8 (R) | ≤0.5 (S) | ≤1 (S) | ≤1 (S) | ≤0.25 (S) | ≤16 (S) | ≤0.125 (S) | ≤1 (S) | ≤4/4 (S) | >4/76 (R) | ≤0.5 (S) |

AMK: amikacin; AMP: ampicillin; CAZ: ceftazidime; CIP: ciprofloxacin; CST: colistin; CTX: cefotaxime; FEP: cefepime; FOS: fosfomycin; GEN: gentamicin; I: intermediate; MEM: meropenem; MIC: minimum inhibitory concentration; R: resistant; S: susceptible; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; TZP: piperacillin–tazobactam.

was isolated from urine and blood cultures. As with Case 1, the two isolates obtained showed the same antimicrobial susceptibility profiles, both indicating resistance to colistin. The susceptibility profile of the isolate obtained from blood is shown in the Table.

The CRP decreased to normal range within 3 days and the fever disappeared within 24 hours. The patient died 5 days later due to a massive cerebral haemorrhage. She had never received previous treatment with colistin and did not report any previous close contact with farm animals. In the previous 12 years she had not travelled abroad.

Case 3

In January 2017, a woman in her early 80s with fever (>38.5°C), diarrhoea and abdominal pain was admitted at the infectious diseases unit of the same tertiary hospital. In 2012, she underwent mastectomy and chemotherapy for breast cancer. Blood cultures were drawn upon admission and she received intravenous empirical treatment with piperacillin/tazobactam. *E. coli* was isolated from blood cultures. The Table shows the antimicrobial profile; the strain was colistin-resistant but susceptible to other commonly used antimicrobials (Table). She had 10.03 × 10³/uL WBC (norm: 4–10 × 10³ uL) and high inflammatory markers: procalcitonin 50.50 ng/mL (norm: 0.00–0.5 ng/mL), CRP 28.41 mg/dL (norm: 0.00–0.5 mg/dL).

Her clinical condition rapidly improved and she was discharged from hospital after 8 days. She had never been treated with colistin, did not report any previous close contact with farm animals and she had never travelled abroad.

Microbiological findings

Blood samples for cultures were collected in BD BACTEC culture aerobic/anaerobic vials and were incubated into BACTEC FX automated blood culture system (Becton Dickinson and Company, Franklin Lakes, New

Jersey, United States), according to the manufacturer's instructions.

Positive blood cultures were subjected to Gram-staining and subcultured into aerobic sheep blood agar plates, chocolate agar plates, selective plates and into Schaedler agar and 5% sheep blood plates (bioMérieux SA, Marcy-l'Étoile, France) anaerobically and incubated at 37°C overnight: the organisms were identified by Matrix-Assisted Laser Desorption Ionization time-of-flight (MALDI-TOF) (Bruker Daltonics GmbH, Bremen, Germany).

The isolates were tested for antimicrobial susceptibility using Phoenix 100 (BD) automated system N-MIC panel. Isolates flagged positive for colistin resistance by the system were further tested according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (version 6.0) [9]. As recommended by EUCAST [10] and the European Centre for Disease Prevention and Control (ECDC) [3], minimal inhibitory concentration (MIC) of colistin was determined by broth microdilution (UMIC colistine ARNIKA SRL Diagnostic Line, Milano, Italy). The MIC of colistin was 4 mg/L for all the isolates (≤2 susceptible, >2 resistant).

Only blood isolates were further tested: unfortunately, the urine ones, which had a similar resistance profile to the isolates from blood, were not available for investigation.

Whole genome DNA was extracted from each isolate using a QIAamp DNA minikit (Qiagen) following the manufacturer's instructions, and sequenced using an Illumina Miseq platform with a 2 by 250 paired-end run after Nextera XT paired-end library preparation.

Genome assembly was performed using SPAdes-3.10.1 software. The genomic sequences were submitted to the European Nucleotide Archive (ENA) (accession

numbers pending). Multilocus sequence typing (MLST) profiles were obtained in silico by analysing appropriate gene variants for each genome, using an in-house Perl script, based on the Achtman MLST scheme [11]. Analysis of the 7-gene MLST showed that the three isolates belong to three different sequence types: ST131, ST3941 and ST1851 respectively.

All three *E. coli* isolates obtained from blood were colistin-resistant. We thus searched the genomes for the presence of *mcr-1* and *mcr-2* genes as both these genes can confer plasmid-mediated resistance to colistin. A Basic Local Alignment Search Tool (BLAST) searches of *mcr-1* (plasmid accession number KP347127) and *mcr-2* (LT598652) against the three assemblies were performed. For each genome we obtained a best hit for *mcr-1* gene with a very low number of nucleotide (nt) differences (2, 0 and 1 nt differences for the ST131, ST3941 and ST1851 strains, respectively) and no best hit for *mcr-2*. Considering that *mcr-1* and *mcr-2* gene sequences used in the analysis are very different at nt level (375 nt differences), the results show that all three genomes harboured *mcr-1* and none harboured *mcr-2*.

Discussion

The occurrence of colistin resistance based on the plasmid-encoded *mcr-1* gene in *Enterobacteriaceae* has been described in different European countries since it was first reported in November 2015 [3-8].

To our knowledge, our data show the first three bloodstream infections mediated by *mcr-1*-encoding *E. coli* in Italy [12,13]. A limitation of our report is that we did not perform a systematic investigation of all bloodstream infections over a certain period in our hospital and we are thus lacking denominator data that would provide better insight into the frequency of the problem. A study is planned to retrospectively analyse all *E. coli* strains obtained in the past year in our hospital to complete our data.

All three patients in our case series had underlying oncological diseases with different degrees of severity which would put them at higher risk of sepsis, however not at higher risk of exposure to *mcr-1*-positive pathogens. None of the patients was previously exposed to colistin. Except for being hospitalised at the same institution at different points in time over a 6-month period, no other epidemiological link could be determined between them. Moreover, the three *mcr-1*-positive isolates belonged to three different STs, indicating the presence of different colistin-resistant strains.

The first of the three strains belonged to ST131. This ST has been described by Overvest et al. as an *E. coli* clone associated with extended-spectrum beta-lactamase (ESBL) production that can colonise patients for prolonged periods, with an estimated half-life of 13 months [14] and by Wang et al. as a prevalent ST in China probably associated with a high risk

of dissemination among *E. coli* [6]. In our patient, the ST131 *E. coli* did not appear to be more pathogenic.

The ST1851 is a new ST, and the draft genome assembly of the respective *E. coli*-1851 strain has been deposited at ENA (accession numbers pending).

All three isolates described here showed a favourable susceptibility profile to other classes of antimicrobials and the three bloodstream infections were rapidly resolved with the chosen empirical therapy. In a recently published article, Poirel et al. [15] describe how not all plasmids with an *mcr-1* gene carry other genes encoding resistance to clinically relevant antibiotics, such as β -lactams, aminoglycosides, quinolones, fosfomycin, sulfonamides, and tetracyclines. In 2016, Bernasconi et al. [16] and Prim et al. [17] also reported occurrence of the *mcr-1* gene in pathogens without presence of further genes conferring resistance for example to extended-spectrum cephalosporins or carbapenemase. This fact may account for the positive outcome of the infections described here but on the other side further highlights the importance of horizontal dissemination of *mcr-1* gene-related colistin resistance in non-multidrug-resistant (MDR) *E. coli* isolates of human origin.

The identification of three patients with bloodstream infections caused by different strains of *mcr-1*-positive *E. coli* detected within 6 months in a single hospital suggests an important and widespread problem [3]. Furthermore, our findings suggest that the dissemination of *mcr-1*-positive *E. coli* in Italy could be underestimated because isolates may be susceptible to other tested antibiotics and screening is often focused on carbapenem-resistant strains only. Moreover, in Italy, where the MDR bacteria are endemic, the acquisition of *mcr-1* plasmid-mediated genes by other MDR *Enterobacteriaceae* could lead to a severe public health concern because it seriously limits treatment options as already reported in 2016 by Di Pilato et al. [18]. Our data further highlight the need of strict surveillance of colistin resistance even in multi-susceptible isolates.

Ethics statement

The study was designed and conducted in accordance with the Helsinki declaration. This study was performed according to the guidelines of the Fondazione IRCCS Policlinico San Matteo Institutional Review Board for the use of biological specimens for scientific purposes in keeping with Italian law (art.13 D.Lgs 196/2003).

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: MC, BM; performed laboratory investigations: PC, MC, BM; genome sequencing and sequences analysis: ES, CF, FC; revised the manuscript: PC, PM.

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