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ORIGINAL RESEARCH

# Multicenter prospective study on the prevalence of colistin resistance in *Escherichia coli*: relevance of *mcr-1*-positive clinical isolates in Lombardy, Northern Italy

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**Background:** The emergence of the plasmid-mediated colistin resistance mechanism in *Escherichia coli* has raised concern among public health experts as colistin is a last-line antimicrobial resort. The primary aim of the study was to investigate the prevalence of this resistance trait in *E. coli* isolates circulating in the Lombardy region, Northern Italy. The presence of *mcr*-type genes and their genetic relationship were also studied.

**Materials and methods:** A prospective study was performed during a 4-month period (May to August, 2016) in six acute care Hospitals. Consecutive nonduplicate clinical isolates of *E. coli* from any type of clinical specimen, with the exception of rectal swabs, were included in the study. Isolates that exhibited MIC values for colistin >2 mg/L were further investigated. Bacterial identification was obtained by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Amplification of *mcr*-type genes (−1 to −5 variants) and microarray analysis were accomplished. Repetitive sequence-based PCR (Rep-PCR) and multilocus sequence typing (MLST) analysis were used for genotyping.

**Results:** Overall, 3,902 consecutive *E. coli* isolates (2,342 from outpatients, 1,560 from inpatients) were evaluated during the study period. Of them, 18/3,902 (0.5%), collected from 4/6 centers, showed resistance to colistin. These isolates were mostly obtained from urine of both outpatients (n=12) and inpatients (n=6). Colistin MIC values ranged from 4 to 8 mg/L. The *mcr-1* gene was detected in 10/18 isolates (7 from outpatients, 3 from inpatients). Rep-PCR and MLST analysis revealed the presence of nine different clusters. Further *mcr*-type genes were not detected.

**Conclusion:** Resistance to colistin in *E. coli* clinical isolates appears low in our geographic area. With regard to *mcr-1*-positive isolates, they accounted for approximately 50% of colistin-resistant *E. coli* isolates, thus representing a relevant resistance mechanism in this context. Although overall limited, the presence of *mcr-1* determinant in our region should not be ignored and great concern should be given to the continuous surveillance.

**Keywords:** MCR-1, colistin, *Escherichia coli*, prevalence, surveillance, epidemiology

## Introduction

The increasing role of colistin in humans as a last antimicrobial resort in the treatment of infections caused by carbapenem-resistant Enterobacteriaceae has prompted more accurate and careful monitoring of resistance to this polypeptide.[1] To this regard, the recent emergence of the plasmid-mediated colistin resistance encoded by *mcr-1* in *Escherichia coli* has raised concern among public health experts worldwide.[2] Due to its ability to transfer itself among bacterial strains and species by mobile genetic elements, the *mcr-1* determinant could make real the nightmare of bacterial isolates

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resistant to all classes of antibiotics. In line with this worrisome prospect, *mcr-1* gene has been also detected in *Klebsiella pneumoniae*, *Salmonella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Shigella* spp., and sometimes associated with carbapenemase or extended-spectrum beta-lactamase (ESBL) producers.[1,3–5]

After its first description in People's Republic of China (2015) during the routine surveillance of food animals, the *mcr-1* gene has been reported (often retrospectively) across a wide geographic area, comprising 39 countries, in human, animal, and food-related samples.[4,6] The first known MCR-1-producing isolate was from the 1980s and was detected in *E. coli* in People's Republic of China from animal source, while in humans it was isolated in 2012 from the blood.[7,8] In Europe, the first MCR-1-producing strain was an *E. coli* isolated in France from animal sources in 2005.[9] Subsequently, the *mcr-2* plasmid-mediated colistin resistance gene was detected from porcine and bovine *E. coli* in Belgium,[10] and a variant of the *mcr-1* determinant (named *mcr-1.2*) was isolated from the rectal swab of an Italian child in *K. pneumoniae*.[11] A third mobile colistin resistance gene, *mcr-3*, has been reported in *E. coli*, *Aeromonas* spp., and *Salmonella* spp. isolates from human and animal samples,[12–16] whereas the *mcr-4* and *mcr-5* genes were detected in *Salmonella* spp. and *E. coli* isolates, but only from animal sources.[17–19] In summary, plasmid-mediated resistance to colistin had been around for more than 25 years, but without being detected until 2015.

The history of plasmid-mediated resistance to colistin had a very important veterinary component. Although colistin has been used in clinical settings in a limited manner in the past, due to its nephrotoxicity, its use in veterinary medicine has been carried on for decades (as it was so far a cheap antibiotic).[1,20] The main indications for colistin use in veterinary setting are the prevention and treatment of infections caused by Enterobacteriaceae (especially gastrointestinal disorders), but it has been used as growth promoter in terrestrial and aquatic animals.[20,21] Data regarding colistin resistance in bacteria from animals and food of animal origin are relatively scarce. Prevalence of colistin resistance in *E. coli* from animals (pigs, ruminants, poultries, and companion animals) shows wide differences ranging from 0% to 52.4%, with highest resistance percentages reported from Asia.[20] It has been reported in several studies that the *E. coli* colistin resistance rate is higher in pigs compared with other animal productions.[6,21–23] Not all studies recognized the colistin resistance mechanism, and so the real prevalence of *mcr-1* determinant in the veterinary setting remains still largely underestimated.[20] In this scenario, due to the high rate of colistin-resistant (CR) *E. coli* carrying the *mcr-1* gene isolated from food animals compared with humans, livestock production was pinpointed as the greatest cause of colistin resistance amplification and spread, also in humans.[6,21] This source of infection led to consider MCR-1-producing *E. coli* mostly as a community-associated microorganism, being isolated especially in outpatient samples. In this context, several publications have reported the detection of CR *E. coli* from healthy individuals without prior colistin usage.[21,24–27] The observation of colistin resistance in humans without prior colistin exposure is of particular clinical importance and concern, because an antimicrobial stewardship program based on preservation of colistin in the hospital context could not be enough. However, *mcr-1*-positive *E. coli* has been almost never associated to hospital epidemic events, giving the reason to think to multi-variegate source of infection outside the hospital setting.

In Italy, data regarding the diffusion of *E. coli* clinical isolates harboring plasmid-mediated resistance to colistin are very scarce. The *mcr-1* determinant was firstly described in 2016 in eight *E. coli* isolates collected from clinical specimens during the period 2013–2015 in two hospitals.[28] Later, another study reported the presence of three *E. coli* isolates producing both MCR-1 and CTX-M-type ESBL enzymes as intestinal carriage in long-term care facilities residents, during a point prevalence survey on ESBL-producing Enterobacteriaceae.[29] More recently, three cases of bloodstream infections caused by MCR-1-producing *E. coli* were reported among oncologic patients,[30] whereas 37 out of 51 (72.5%) CR *E. coli* isolates from pigs were positive for *mcr-1* gene.[31] Finally, the *mcr-1* determinant was detected in *S. enterica* isolates obtained from human and animals in the period 2012–2015,[32] and the more recent *mcr-4* gene was detected in *S. enterica* serovar Typhimurium (collected in 2013 and retrospectively studied) from an animal source.[17] The aim of our study was to investigate 1) the prevalence of this resistance trait in *E. coli* isolates from clinical samples, 2) the presence of *mcr*-type genes, and 3) their genetic relationship. Our work represents the first evaluation of the diffusion of clinical *mcr*-1-positive *E. coli* in a specific defined area in our country.

## Materials and methods

### Study design and participating centers

Bacterial isolates were obtained during a multicenter prospective study that involved six clinical microbiology laboratories located in the Lombardy region (Northern Italy). The following centers were included: Busto Arsizio, Lecco, Legnano, Lodi, Rozzano, and Vimercate (Figure 1). Participating hospitals

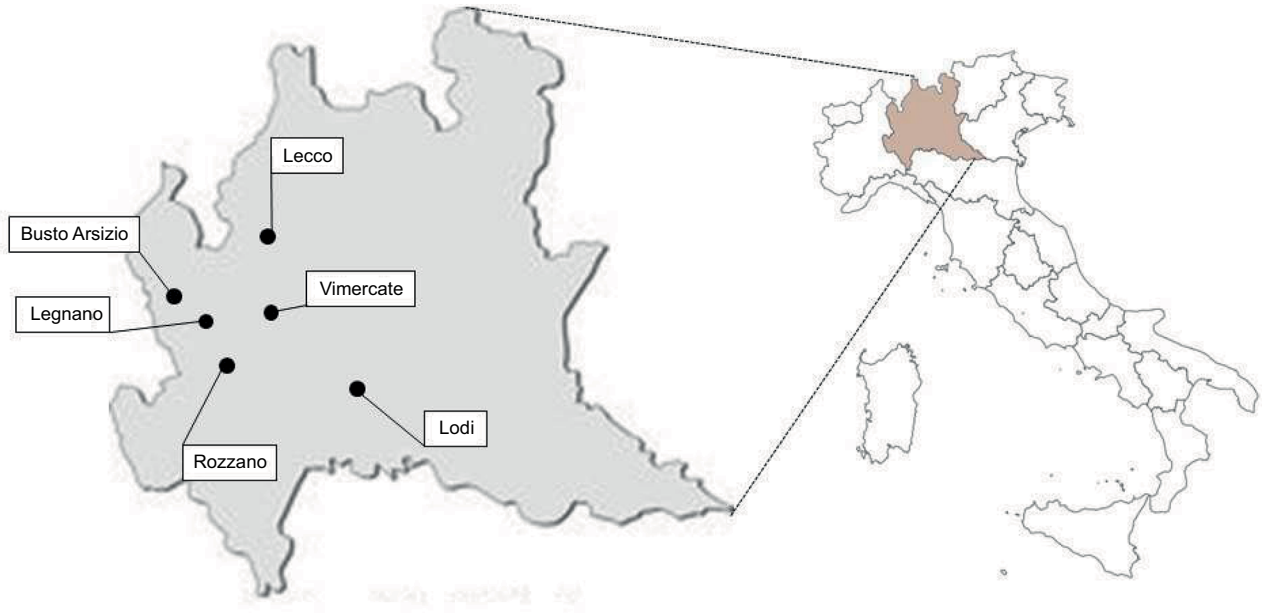


**Figure 1** Participating centers in the Lombardy region, Italy.

had approximately 4,000 beds and served 2,400,000 people. The survey was conducted over a 4-month period, starting in May 2016. Consecutive nonduplicate clinical isolates of *E. coli* from any type of clinical specimen, with the exception of rectal swabs, were included in the study. Isolates that exhibited MIC values for colistin >2 mg/L were further investigated. Bacterial identification and antimicrobial susceptibility testing were routinely carried out by the collecting laboratories using either the Phoenix automated system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) or the Vitek2 system (bioMérieux, Marcy l'Etoile, France). Both inpatients and outpatients were included in the study. Outpatients were defined as patients not hospitalized at the time of specimen collection. For each isolate, information on the clinical specimen and type of ward (in the case of isolates from inpatients) was included. Moreover, each participating laboratory provided information on the total number of consecutive nonduplicate clinical isolates of *E. coli* observed during the collection period. The collected isolates were sent to reference laboratories for confirmation of both species identification and antimicrobial resistance. Characterization of the colistin resistance mechanism(s) and analysis of clonal relatedness were also carried out.

## Characterization of bacterial isolates

Bacterial identification of collected isolates was assessed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (Vitek MS; bioMérieux). Antimicrobial susceptibility for colistin was evaluated by the reference broth microdilution method using a dedicated TREK panel (DKMGN; Thermo Fisher Diagnostics, Milan, Italy). This panel also provided MIC values for amoxicillin-clavulanate, piperacillin-tazobactam, cefotaxime, ceftazidime, aztreonam, ertapenem, imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, trimethoprim-sulfamethoxazole, ceftolozane-tazobactam, ceftazidime-avibactam, and tigecycline. All collected isolates confirmed to be resistant to colistin according to EUCAST breakpoints[33] (MIC value >2 mg/L) were evaluated for the presence of *mcr*-type genes.

## Characterization of antimicrobial resistance determinants

The presence of the *mcr*-type determinants (−1 to −5 variants) was investigated by PCR using specific primers and conditions, as previously described.[6,10,12,17,18]

The content of the entire beta-lactamase resistance determinants of the *mcr*-type-positive isolates was tested by the Check-MDR CT103XL array (Check-Points Health B.V., Wageningen, The Netherlands).

## Molecular typing

Repetitive sequence-based PCR (rep-PCR) was performed with the Diversilab (DL) System (bioMérieux), according to the manufacturer's instructions. DNA extraction was performed with the UltraClean Microbial DNA isolation kit (Mo Bio Laboratories Inc). Analysis of the PCR amplicons was performed using a 2100 Bioanalyzer (Agilent Technologies ,

Santa Clara, CA, USA). DL fingerprints were analyzed with the DL software 3.4, using the Pearson correlation statistical method to determine clonal relationships.

Multilocus sequence typing (MLST) of *mcr-1*-positive *E. coli* isolates was carried out according to the protocol of Wirth et al. (2006).[34] Allelic profiling and sequence-type (ST) determination were performed using the *E. coli* MLST scheme from the website of the University of Warwick (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Phylogenetic groups were determined by a 2-step triplex PCR as described by Clermont et al.[35]

Plasmid incompatibility groups of *mcr*-1-positive strains were determined by the PCR-based replicon typing (PBRT) method using the commercially available PBRT kit (Diatheva),[36] according to the manufacturer's instructions. Specific primers and protocol were used for the amplification of IncX4 replicon.[37]

## Ethics approval and consent to participate

Ethics approval and consent to participate were not required. Samples were taken from six different institutions as part of the standard patient care and used anonymously.

# Results

## Bacterial isolates and epidemiological data

A total of 3,902 consecutive nonduplicate *E. coli* clinical isolates (outpatients, n=2,342; inpatients, n=1,560) were evaluated during the collection period. Of note, *E. coli* isolates obtained from patients admitted to nursing homes (included in the outpatients group) accounted for 5.8% (n=135) of study isolates. Clinical isolates were mostly obtained from urine samples (n=3070, 78.7%), followed by skin and soft tissues (n=316, 8.1%), and blood cultures (n=301, 7.7%).

Overall, 18 out of 3,902 (0.5%) isolates, collected from 4/6 centers, were confirmed as CR (MIC>2 mg/L). In particular, 6/18 were from inpatients and 12/18 from outpatients (no one from nursing homes). Thus, the prevalence of colistin resistance was 0.5% (6/1560) and 0.4% (12/2342) among inpatients and outpatients, respectively. Particularly, CR isolates recovered from hospitalized patients came from medical (n=3), rehabilitation (n=2), and surgical (n=1) wards. Overall, CR isolates were obtained from patients (male, n=8; female, n=10) aging from 52 to 94 years, mostly from urine samples (n=16), while the remaining isolates were from blood cultures (n=2).

## Molecular characterization and genetic relationship among *mcr-1*-positive *E. coli* clinical isolates

PCR analysis detected the *mcr-1* gene in 10/18 CR isolates, all of which were from urine samples (seven from outpatients and three from inpatients). Isolates were uniformly negative for other *mcr*-type genes. Genetic relationship among *mcr-1*-positive isolates was investigated using different methods. The Rep-PCR technique showed the presence of nine different clusters (data not shown). These data agreed with MLST analysis that revealed nine different STs, with a new one consisting of the following allelic profile: 6–23–5–8–24–18–6. The phylogenetic group analysis showed high heterogeneity among isolates: four belonged to groups A and D, respectively, whereas the remaining two strains were from groups B1 and B2. Seven *mcr-1*-positive isolates harbored a plasmid of IncX4 group; in three cases, the IncHI2 incompatibility group was found. Details are reported in Table 1.

## Antimicrobial susceptibility of CR isolates and associated resistance mechanisms

As shown in Table 2, *mcr-1*-positive isolates showed MIC values for colistin ranging from 4 to 8 mg/L. These isolates were frequently resistant to co-trimoxazole (8/10) and ciprofloxacin (8/10), and sometimes also to gentamicin (3/10) and tobramycin (3/10). Notably, two of them (both positive for the SHV-12 determinant) were not susceptible to third-generation cephalosporins (cefotaxime and ceftazidime). In all cases, however, carbapenems (ertapenem, imipenem, and meropenem), amikacin, ceftazidime/avibactam, ceftolozane/tazobactam, and tigecycline maintained their activity. As assessed by microarray analysis, six isolates co-harbored the $bla_{\text{TEM-1}}$ gene (Table 1).

Similarly to *mcr-1*-positive isolates, *mcr*-type-negative isolates had MIC values for colistin ranging from 4 to 8 mg/L (Table 3). With the exception of ciprofloxacin (4/8 isolates), resistance to other antimicrobials was overall rare, even though three of them were not susceptible to third-generation cephalosporins (cefotaxime and ceftazidime) due to ESBL production.

# Discussion

Colistin is increasingly used as one of the last available treatment options for patients with severe infections caused by carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.[2,38] Colistin resistance follows the increasing trend in consumption of colistin

**Table 1** Characteristics of *mcr-1*-positive *E. coli* clinical isolates

| Center | Strain code | Date of isolation | Patient data (sex, age [years]) | Hospital service/per ward | Colistin MIC value (mg/L) | Other BL resistance determinants | ESBL profile | Sequence type | Rep-PCR profile | Inc-type | Phylogroup |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Busto A. | 01-EC01 | Oct. 2016 | M, 65 | Outpatient | 4 | - | Neg | ST354 | 1 | IncX4 | D |
| Lecco | 02-EC08 | Aug. 2016 | M, 52 | Rehabilitation | 8 | TEM-1 | Neg | ST617 | 6 | IncX4 | A |
| Lecco | 02-EC09 | Aug. 2016 | F, 87 | General surgery | 4 | TEM-1 | Neg | ST93 | 8 | IncHI2 | A |
| Lodi | 04-EC16 | Jun. 2016 | M, 83 | Outpatient | 4 | TEM-1, SHV-12 | Pos | ST88 | 2 | IncX4 | A |
| Lodi | 04-EC17 | Jun. 2016 | F, 83 | Outpatient | 8 | TEM-1 | Neg | ST428 | 3 | IncHI2 | B2 |
| Lodi | 04-EC18 | Jul. 2016 | F, 94 | Rehabilitation | 8 | TEM-1 | Neg | ST117 | 5 | IncHI2 | D |
| Lodi | 04-EC21 | Aug. 2016 | M, 74 | Outpatient | 4 | - | Neg | new ST | 7 | IncX4 | A |
| Lodi | 04-EC22 | Aug. 2016 | M, 77 | Outpatient | 8 | TEM-1 | Neg | ST359 | 9 | IncX4 | B1 |
| Vimercate | 06-EC05 | Jun. 2016 | F, 77 | Outpatient | 4 | SHV-12 | Pos | ST69 | 4 | IncX4 | D |
| Vimercate | 06-EC07 | Aug. 2016 | F, 73 | Outpatient | 8 | - | Neg | ST117 | 5 | IncX4 | D |

**Abbreviations:** BL, beta-lactam; ESBL, extended-spectrum beta-lactamase; F, female; M, male; Neg, negative; Pos, positive; Rep-PCR, repetitive sequence-based PCR; ST, sequence type; Aug, August; Jun, June; Oct, October.

in human medicine, especially in countries with high rates of carbapenem-resistant gram-negative bacilli, including Italy.[39] Chromosomally mediated resistance, often generated by mutations in the *mgrB* gene and upregulation of *PhoP*/*PhoQ* system, seems to be related to this trend and mostly associated with *K. pneumoniae* in the hospital setting.[40–43] A mutation in the *pmrB* gene has been also recently described in *E. coli*.[44] On the contrary, the plasmid-mediated colistin resistance (mostly due to the *mcr-1* determinant) has been especially found in *E. coli*, a common cause of urinary tract infections in healthy individuals in the community setting without prior colistin usage.[21,24–27]

Prevalence data on colistin resistance are overall scarce. In particular, data regarding the plasmid-mediated resistance to colistin among clinical isolates of *E. coli* are lacking in Italy. This prospective multicenter study represents the first evaluation on the dissemination of clinical isolates of *mcr-1*-positive *E. coli* in Lombardy, the most inhabited Italian region, accounting for about 10 million of residents. Our results show that resistance to colistin in *E. coli* clinical isolates is almost low in this area (0.5%), with similar percentages among both inpatients and outpatients (0.5% and 0.4%, respectively). Notably, considering only outpatients, resistance to colistin was not detected in nursing home patients, thus enforcing the theory of a major risk source outside the health-care setting.[1,3,4,20,21,24,38]

As a limitation of the study, however, it should be taken into account that some methodological difficulties affect automated systems in determining the correct MIC value for colistin, especially when it ranges from 1 to 2 mg/L. This issue could lead to a possible underestimation of colistin resistance.

With regard to *mcr-1*-positive isolates, they accounted for approximately 50% of CR *E. coli* isolates, thus representing a relevant mechanism in the context of colistin resistance. Overall, however, these isolates represented a low rate (10 isolates, 0.2%) of total isolates studied in the survey. These results are similar to the previously published prevalence data, ranging from 0.05% to 1%.[6,45–52] The aforementioned studies included isolates from infected or colonized patients and showed higher prevalence rates in Asian countries compared with those reported from Europe, thus highlighting a major concern toward *mcr*-related colistin resistance in that geographic area. This issue is reinforced by a high prevalence value of 3.5% described in a report including colonized patients from People's Republic of China.[53]

These data, showing a low prevalence of *mcr-1*-positive isolates, are mostly reassuring since *mcr-1* appears as a

**Table 2** Susceptibility profile of *mcr-1*-positive *E. coli* clinical isolates, as assessed by broth microdilution method

| Strain code | AMC | TZP | AZT | CTX | CAZ | CFT | CZA | ERT | IMP | MEM | CIP | SXT | AMK | GEN | TOB | TIG | COL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01-EC01 | ≤4/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | >8/152 | ≤4 | >8 | 8 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (R) | (R) | (S) | (R) |
| 02-EC08 | 8/2 | 8/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | ≤1/19 | ≤4 | 1 | ≤1 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (S) | (R) |
| 02-EC09 | 8/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | 1 | >8/152 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (R) |
| 04-EC16 | >64/2 | >32/4 | >32 | 2 | >16 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | >8/152 | ≤4 | 8 | 8 | ≤0.25 | 4 |
| | (R) | (R) | (R) | (I) | (R) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (R) | (R) | (S) | (R) |
| 04-EC17 | 8/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | 0,25 | >8/152 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (R) |
| 04-EC18 | >64/2 | 4/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | >8/152 | ≤4 | 1 | ≤1 | ≤0.25 | 8 |
| | (R) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (R) |
| 04-EC21 | 32/2 | 2/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≥2 | >8/152 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (R) |
| 04-EC22 | 16/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≥2 | >8/152 | ≤4 | >8 | 8 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (R) | (R) | (S) | (R) |
| 06-EC05 | 8/2 | ≤1/4 | 16 | >8 | 4 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | 0,25 | >8/152 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (R) | (R) | (I) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (R) |
| 06-EC07 | ≤4/2 | 2/4 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≥2 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (S) | (R) |

**Abbreviations:** AMC, amoxicillin-clavulanate; AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; CFT, ceftolozane-tazobactam; CIP, ciprofloxacin; COL, colistin; CTX, cefotaxime; CZA, ceftazidime-avibactam; *E. coli*, *Escherichia coli*; ERT, ertapenem; GEN, gentamicin; IMP, imipenem; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; TIG, tigecycline; TOB, tobramycin; TZP, piperacillin-tazobactam; S, susceptible; R, resistant.

**Table 3** Susceptibility profile of *mcr*-type-negative *E. coli* clinical isolates, as assessed by broth microdilution method

| Strain code | AZT | AMC | TZP | CTX | CAZ | CFT | CZA | ERT | IMP | MEM | CIP | SXT | AMK | GEN | TOB | TIG | COL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 02-EC05 | ≤0.5 | ≤4/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≤0.06 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) |
| 02-EC06 | ≤0.5 | ≤4/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≤0.06 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) |
| 04-EC10 | ≤0.5 | 32/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (S) | (R) |
| 04-EC19 | >32 | 64/2 | 2/4 | >8 | >16 | 1/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0,125 | >2 | >8/152 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 8 |
| | (R) | (R) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (R) |
| 04-EC20 | ≤0.5 | ≤4/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0,125 | ≤0.06 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 8 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) |
| 04-EC23 | 16 | 32/2 | ≤1/4 | >8 | 8 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (R) | (S) | (S) | (R) | (R) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (S) | (R) |
| 04-EC24 | 16 | 32/2 | ≤1/4 | >8 | 4 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | >2 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (R) | (S) | (S) | (R) | (I) | (S) | (S) | (S) | (S) | (S) | (R) | (S) | (S) | (S) | (S) | (S) | (R) |
| 06-EC06 | ≤0.5 | ≤4/2 | ≤1/4 | ≤0.5 | ≤0.5 | ≤0.5/4 | ≤0.5/4 | ≤0.125 | ≤0.5 | ≤0.125 | ≤0.06 | ≤1/19 | ≤4 | ≤0.5 | ≤1 | ≤0.25 | 4 |
| | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (S) | (R) |

**Abbreviations:** AMC, amoxicillin-clavulanate; AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; CFT, ceftolozane-tazobactam; CIP, ciprofloxacin; COL, colistin; CTX, cefotaxime; CZA, ceftazidime-avibactam; *E. coli*, *Escherichia coli*; ERT, ertapenem; GEN, gentamicin; IMP, imipenem; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; TIG, tigecycline; TOB, tobramycin; TZP, piperacillin-tazobactam; S, susceptible; R, resistant.

transferable resistance determinant capable of limited propensity to spread so far. To date, it was never associated with epidemic events, even though association of *mcr*-type determinants with high-risk clones (e.g., *E. coli* ST131) capable of large diffusion has been described.[16,28] Moreover, other resistance determinants (including those responsible for carbapenemase and ESBL production) have been already reported in association with *mcr* genes, mainly limiting therapeutic options really effective against these strains.[54,55]

As previously reported, *mcr-1*-positive isolates usually show a multi-susceptible profile.[1,6,28] In our study, resistance to co-trimoxazole (8/10 isolates) and ciprofloxacin (8/10 isolates) was common. Interestingly, *mcr-1*-positive isolates were detected only in urine samples. Furthermore, 2/10

isolates were resistant to third-generation cephalosporins (cefotaxime and ceftazidime) due to ESBL production. This worrisome finding could essentially reflect Italian epidemiology for ESBL production in *E. coli* isolates circulating among both inpatients and outpatients.[56]

All *mcr-1*-positive isolates were genetically unrelated, as demonstrated by molecular typing. Both Rep-PCR and MLST revealed nine different clusters, giving the reason to assess a multi-variegate source of infection. Only one couple of isolates was genetically related despite these isolates had been collected from different centers and had no obvious epidemiological link.

In conclusion, we can speculate that the prevalence of CR *E. coli* isolates is low in our region, and the diffusion of *mcr-1* determinant is very limited among clinical isolates. No epidemic events caused by CR *E. coli* are so far described in Italy in the hospital setting, thus highlighting the community origin of these isolates. Accordingly, in our experience, *mcr-1*-positive strains were not genetically related and were mostly isolated from outpatients, evidencing their different sources and the low-level diffusion in the community. Although limited, the presence of *mcr-1* determinant in our region should not be ignored. Great concern should be given to continuous surveillance, improving prevalence data in both human and veterinary settings in our country.

## Ethics approval and consent to participate

Ethics approval and consent to participate were not required. Samples were taken from six different institutions as part of the standard patient care and used anonymously.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Nordmann P, Poirel L. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. *Clin Microbiol Infect*. 2016;22(5):398–400.
2. Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. *Clin Microbiol Rev*. 2017;30(2):557–596.
3. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother*. 2016;71(8):2066–2070.
4. Al-Tawfiq JA, Laxminarayan R, Mendelson M. How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *Int J Infect Dis*. 2017;54:77–84.
5. Sennati S, Di Pilato V, Riccobono E, et al. *Citrobacter braakii* carrying plasmid-borne mcr-1 colistin resistance gene from ready-to-eat food from a market in the Chaco region of Bolivia. *J Antimicrob Chemother*. 2017;72(7):2127–2129.
6. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16(2):161–168.
7. Shen Z, Wang Y, Shen Y, Shen J, Wu C. Early emergence of *mcr-1* in *Escherichia coli* from food-producing animals. *Lancet Infect Dis*. 2016;16(3):293.
8. Rapoport M, Faccone D, Pasteran F, Ceriana P, Albornoz E, Petroni A; MCR Group, Corso A. First description of *mcr-1*-mediated colistin resistance in human infections caused by *Escherichia coli* in Latin America. *Antimicrob Agents Chemother*. 2016;60(7):4412–4413.
9. Haenni M, Poirel L, Kieffer N, et al. Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids. *Lancet Infect Dis*. 2016;16(3):281–282.
10. Xavier BB, Lammens C, Ruhal R, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill*. 2016;21(27):30280.
11. Di Pilato V, Arena F, Tascini C, et al. *mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512. *Antimicrob Agents Chemother*. 2016;60(9):5612–5615.
12. Yin W, Li H, Shen Y, et al. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBio*. 2017;8(4):e00543-17.
13. Ling Z, Yin W, Li H, et al. Chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat. *Antimicrob Agents Chemother*. 2017;61(11):e01272-17.
14. Hernández M, Iglesias MR, Rodríguez-Lázaro D, et al. Co-occurrence of colistin-resistance genes *mcr-1* and *mcr-3* among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. *Euro Surveill*. 2017;22(31):30586.
15. Litrup E, Kiil K, Hammerum AM, Roer L, Nielsen EM, Torpdahl M. Plasmid-borne colistin resistance gene *mcr-3* in *Salmonella* isolates from human infections, Denmark, 2009-2017. *Euro Surveill*. 2017;22(31):30587.
16. Roer L, Hansen F, Stegger M, Sönksen UW, Hasman H, Hammerum AM. Novel *mcr-3* variant, encoding mobile colistin resistance, in an ST131 *Escherichia coli* isolate from bloodstream infection, Denmark, 2014. *Euro Surveill*. 2017;22(31):22846.
17. Carattoli A, Villa L, Feudi C, et al. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill*. 2017;22(31):30589.
18. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in *d*-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. *J Antimicrob Chemother*. 2017;72(12):3317–3324.
19. Fukuda A, Sato T, Shinagawa M, et al. High prevalence of *mcr-1*, *mcr-3* and *mcr-5* in *Escherichia coli* derived from diseased pigs in Japan. *Int J Antimicrob Agents*. 2018;51(1):163–164.
20. Kempf I, Jouy E, Chauvin C. Colistin use and colistin resistance in bacteria from animals. *Int J Antimicrob Agents*. 2016;48(6):598–606.
21. Rhouma M, Beaudry F, Letellier A. Resistance to colistin: what is the fate for this antibiotic in pig production? *Int J Antimicrob Agents*. 2016;48(2):119–126.
22. de Jong A, Thomas V, Simjee S, et al. Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother*. 2012;67(3):638–651.

23. Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Butaye P, Goossens H. Colistin resistance gene *mcr-1* harboured on a multidrug resistant plasmid. *Lancet Infect Dis*. 2016;16(3):283–284.
24. Olaitan AO, Morand S, Rolain JM. Emergence of colistin-resistant bacteria in humans without colistin usage: a new worry and cause for vigilance. *Int J Antimicrob Agents*. 2016;47(1):1–3.
25. Prim N, Rivera A, Español M, Mirelis B, Coll P. In vivo adaptive resistance to colistin in *Escherichia coli* isolates. *Clin Infect Dis*. 2015;61(10):1628–1629.
26. Olaitan AO, Thongmalayvong B, Akkhavong K, et al. Clonal transmission of a colistin-resistant *Escherichia coli* from a domesticated pig to a human in Laos. *J Antimicrob Chemother*. 2015;70(12):3402–3404.
27. Urban C, Tiruvury H, Mariano N, Colon-Urban R, Rahal JJ. Polymyxin-resistant clinical isolates of *Escherichia coli*. *Antimicrob Agents Chemother*. 2011;55(1):388–389.
28. Cannatelli A, Giani T, Antonelli A, Principe L, Luzzaro F, Rossolini GM. First detection of the *mcr-1* colistin resistance gene in *Escherichia coli* in Italy. *Antimicrob Agents Chemother*. 2016;60(5):3257–3258.
29. Giufrè M, Monaco M, Accogli M, Pantosti A, Cerquetti M; PAMURSA Study Group. Emergence of the colistin resistance *mcr-1* determinant in commensal *Escherichia coli* from residents of long-term-care facilities in Italy. *J Antimicrob Chemother*. 2016;71(8):2329–2331.
30. Corbella M, Mariani B, Ferrari C, et al. Three cases of *mcr-1*-positive colistin-resistant *Escherichia coli* bloodstream infections in Italy, August 2016 to January 2017. *Euro Surveill*. 2017;22(16):30517.
31. Curcio L, Luppi A, Bonilauri P, et al. Detection of the colistin resistance gene *mcr-1* in pathogenic *Escherichia coli* from pigs affected by post-weaning diarrhoea in Italy. *J Glob Antimicrob Resist*. 2017;10:80–83.
32. Carnevali C, Morganti M, Scaltriti E, Bolzoni L, Pongolini S, Casadei G. Occurrence of *mcr-1* in colistin-resistant *Salmonella enterica* isolates recovered from humans and animals in Italy, 2012 to 2015. *Antimicrob Agents Chemother*. 2016;60(12):7532–7534.
33. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, 2017. Available from: http://www.eucast.org. Accessed December 15, 2017.
34. Wirth T, Falush D, Lan R, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*. 2006;60(5):1136–1151.
35. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol*. 2000;66(10):4555–4558.
36. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods*. 2005;63(3):219–228.
37. Johnson TJ, Bielak EM, Fortini D, et al. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. *Plasmid*. 2012;68(1):43–50.
38. Catry B, Cavaleri M, Baptiste K, et al. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *Int J Antimicrob Agents*. 2015;46(3):297–306.
39. European Centre for Disease Prevention and Control (ECDC). Plasmid-mediated colistin resistance in *Enterobacteriaceae*. Stockholm: ECDC; 2016. Available from https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/enterobacteriaceae-risk-assessment-diseases-caused-by-antimicrobial-resistant-microorganisms-europe-june-2016.pdf.
40. Giacobbe DR, Del Bono V, Trecarichi EM, et al; ISGRI-SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia Antinfettiva). Risk factors for bloodstream infections due to colistin-resistant KPC-producing *Klebsiella pneumoniae*: results from a multicenter case-control-control study. *Clin Microbiol Infect*. 2015;21(12):1106.
41. Giani T, Arena F, Vaggelli G, et al. Large nosocomial outbreak of colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae* traced to clonal expansion of an *mgrB* deletion mutant. *J Clin Microbiol*. 2015;53(10):3341–3344.
42. Monaco M, Giani T, Raffone M, et al. Colistin resistance superimposed to endemic carbapenem-resistant *Klebsiella pneumoniae*: a rapidly evolving problem in Italy, November 2013 to April 2014. *Euro Surveill*. 2014;19(42):20939.
43. Poirel L, Jayol A, Bontron S, et al. The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. *J Antimicrob Chemother*. 2015;70(1):75–80.
44. Cannatelli A, Giani T, Aiezza N, et al. An allelic variant of the PmrB sensor kinase responsible for colistin resistance in an *Escherichia coli* strain of clinical origin. *Sci Rep*. 2017;7(1):5071.
45. Terveer EM, Nijhuis RHT, Crobach MJT, et al. Prevalence of colistin resistance gene (*mcr-1*) containing *Enterobacteriaceae* in feces of patients attending a tertiary care hospital and detection of a *mcr-1* containing, colistin susceptible *E. coli*. *PLoS One*. 2017;12(6):e0178598.
46. Zhong LL, Phan HTT, Shen C, et al. High rates of human fecal carriage of *mcr-1*-positive multi-drug resistant *Enterobacteriaceae* isolates emerge in China in association with successful plasmid families. *Clin Infect Dis*. Epub 2017 Oct 10.
47. Wang Y, Tian GB, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. *Lancet Infect Dis*. 2017;17(4):390–399.
48. Quan J, Li X, Chen Y, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. *Lancet Infect Dis*. 2017;17(4):400–410.
49. Heras-Cañas VJ, López-Cerero L, Díaz de-Alba P, Pascual Á. Low prevalence of *mcr-1* positive *Enterobacteriaceae* isolates in a health area. *Enferm Infecc Microbiol Clin*. 2017;35(7):467–468.
50. Kuo SC, Huang WC, Wang HY, Shiau YR, Cheng MF, Lauderdale TL. Colistin resistance gene *mcr-1* in *Escherichia coli* isolates from humans and retail meats, Taiwan. *J Antimicrob Chemother*. 2016;71(8):2327–2329.
51. Liassine N, Assouvie L, Descombes MC, et al. Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract *Enterobacteriaceae* in Switzerland. *Int J Infect Dis*. 2016;51:4–5.
52. Prim N, Rivera A, Rodríguez-Navarro J, et al. Detection of *mcr-1* colistin resistance gene in polyclonal *Escherichia coli* isolates in Barcelona, Spain, 2012 to 2015. *Euro Surveill*. 2016;21(13):30183.
53. Bi Z, Berglund B, Sun Q, et al. Prevalence of the *mcr-1* colistin resistance gene in extended-spectrum β-lactamase-producing *Escherichia coli* from human faecal samples collected in 2012 in rural villages in Shandong Province, China. *Int J Antimicrob Agents*. 2017;49(4):493–497.
54. Beyrouthy R, Robin F, Lessene A, et al. MCR-1 and OXA-48 in vivo acquisition in KPC-producing *Escherichia coli* after colistin treatment. *Antimicrob Agents Chemother*. 2017;61(8):e02540-16.
55. Savov E, Todorova I, Politi L, et al. Colistin resistance in KPC-2- and SHV-5-producing *Klebsiella pneumoniae* clinical isolates in Bulgaria. *Chemotherapy*. 2017;62(6):339–342.
56. Giani T, Antonelli A, Caltagirone M, et al. Evolving beta-lactamase epidemiology in Enterobacteriaceae from Italian nationwide surveillance, October 2013: KPC-carbapenemase spreading among outpatients. *Euro Surveill*. 2017;22(31):30583.