Spread of colistin resistance gene mcr-1 in Italy: characterization of the mcr-1.2 allelic variant in a colistin-resistant blood isolate of Escherichia coli

Serena Simoni, Gianluca Morroni, Andrea Benciani, Chiara Vincenzi, Oscar Cirioni, Sefora Castelletti, Pietro E. Varaldo, Eleonora Giovanetti, Marina Mingoia

PII: S0732-8893(17)30411-X
DOI: https://doi.org/10.1016/j.diagmicrobio.2017.12.015
Reference: DMB 14495

To appear in:

Received date: 14 September 2017
Revised date: 11 December 2017
Accepted date: 15 December 2017

Please cite this article as: Serena Simoni, Gianluca Morroni, Andrea Benciani, Chiara Vincenzi, Oscar Cirioni, Sefora Castelletti, Pietro E. Varaldo, Eleonora Giovanetti, Marina Mingoia, Spread of colistin resistance gene mcr-1 in Italy: characterization of the mcr-1.2 allelic variant in a colistin-resistant blood isolate of Escherichia coli. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Dmb(2017), https://doi.org/10.1016/j.diagmicrobio.2017.12.015

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Note (revised version of DMID-17-783)

Spread of colistin resistance gene *mcr-1* in Italy: characterization of the *mcr-1.2* allelic variant in a colistin-resistant blood isolate of *Escherichia coli*

Serena Simoni\(^a\), Gianluca Morroni\(^b\), Andrea Benciani\(^a\)*, Chiara Vincenzi\(^a\), Oscar Cirioni\(^b\), Sefora Castelletti\(^b\), Pietro E. Varaldo\(^a\), Eleonora Giovanetti\(^c\), Marina Mingoia\(^a\)

\(^a\) Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy
\(^b\) Unit of Infectious Diseases, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche Medical School, Ancona, Italy
\(^c\) Unit of Microbiology, Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

Running title: resistance gene *mcr-1.2* in *E. coli*, Italy

*Corresponding author:

Dr. Andrea Benciani, Ph.D.
Unit of Microbiology
Department of Biomedical Sciences and Public Health
Polytechnic University of Marche Medical School
Via Tronto 10/A
60126 Ancona, Italy
Telephone number: +39 071 2206299
Telefax number: +39 071 2206293
E-mail: a.benciani@univpm.it

\(^\dagger\) These authors contributed equally to the work.
Abstract

$mcr$-1.2, an allelic variant of the transferable colistin resistance gene $mcr$-1, was characterized in a colistin-resistant blood isolate of *Escherichia coli*. It was harbored by an IncX4-type plasmid (33,293 bp). Despite its low prevalence, the potentially worrying spread of the $mcr$-1 gene, particularly its $mcr$-1.2 variant, in Italy requires increasing surveillance.

Keywords
colistin resistance; $mcr$-1 gene; $mcr$-1.2 variant; *Escherichia coli*; blood isolates
The emergence of resistance to most classes of antibiotics and the shortage of new antimicrobials with activity against Gram-negative bacteria has prompted renewed interest in such old antibiotics as polymyxins: in particular, colistin and polymyxin B are currently regarded as possible last-resort agents against multidrug resistant gram-negatives (Poirel et al., 2017). Transferable colistin resistance, due to a plasmid-borne gene (mcr-1) which encodes an enzyme of the phosphoethanolamine transferase family, was first reported in China, in *Escherichia coli* isolates from animals, food and patients (Liu et al., 2016). In following screening and retrospective studies, the new gene disclosed broad distribution around the world since before its discovery (Schwarz and Johnson, 2016). Along with the prototype gene, nine allelic variants of mcr-1 have been reported lately: mcr-1.2, in Italy from a KPC-producing *Klebsiella pneumoniae* (accession no. KX236309), and in Switzerland from *E. coli* (accession no KY689634); mcr-1.3, mcr-1.4, mcr-1.6, and mcr-1.7 (accession nos. NG052861, KY041856, NG052893, and KY488488, respectively) in China; mcr-1.5 (accession no. KY283125) in Argentina; mcr-1.8 (accession no. KY683842) in Brunei; mcr-1.9 (accession no. KY964067) in Portugal, and mcr-1.10 (accession no. MF176238) in UK. mcr-1 is usually carried by conjugative plasmids — belonging to three major types, IncX4 (~33 kb), IncI2 (~60 kb), and IncHI2 (~210-280 kb) — which favor its horizontal transfer to different bacterial species (Li et al., 2016).

Here we report the detection and characterization of the mcr-1.2 variant, carried by an IncX4 conjugative plasmid, in colistin-resistant *E. coli* (a clinical isolate from blood culture), against a background of increasing circulation of the mcr-1-mediated colistin resistance in Italy.

A total of 263 *E. coli* — collected from blood samples between August 2015 and April 2017 at the University Hospital of Ancona, Italy, and identified using VITEK 2, bioMérieux, Marcy-l’Étoile, France — were tested
for colistin resistance by broth microdilution (EUCAST, 2017) and for the presence of the mcr-1 gene by PCR (Table S1). Two isolates (E. coli 277730 and E. coli 288328, recovered from the same patient with an interval of almost two months between each other) were found to share colistin resistance (MIC, 8 μg/ml for both) and positivity for mcr-1.

The patient was a 58-year-old female suffering from hairy cell leukemia and neutropenia, with a clinical history of type 2 diabetes, breast cancer, inactive HBsAg carrier state. She was admitted to the Infectious Diseases Clinic of the University Hospital in late January 2016, with fever and other symptoms of sepsis. Blood culture upon admission yielded E. coli (strain 277730) resistant to colistin, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole (Table S2). Meropenem therapy (2 g x 3/die) was followed by prompt disappearance of fever and of colistin-resistant E. coli in blood cultures. Fever reappeared four weeks later, when blood culture yielded β-lactam-resistant, multidrug-resistant Enterococcus faecium and meropenem was substituted with teicoplanin. However, meropenem discontinuation was punctually followed by recurrence of colistin-resistant E. coli in the blood culture (strain 288328, isolated in mid-March 2016). In spite of reintroduction of meropenem, the patient’s condition further deteriorated, and she died in early April 2016. Remarkably, she had not received previous treatment with colistin and had no recent history of travel or contact with farm animals.

The two colistin-resistant E. coli isolates proved indistinguishable — identical XbaI PFGE profile, same sequence type (ST354) (Wirth et al., 2006) — and only one (E. coli 288328) was investigated further for molecular traits. mcr-1 sequencing demonstrated that it was identical to the allelic variant mcr-1.2 recently described in Italy in K. pneumoniae (Di Pilato et al., 2016). To verify the transferability, mating experiments, using rifampin (50 μg/ml)
and colistin (2 μg/ml) for transconjugant selection, were performed. mcr-1.2 was successfully transferred to E. coli DH5α and K. pneumoniae ATCC 700603 (at a frequency of 7.1×10⁻⁸ and 5.3×10⁻⁹ per recipient cell, respectively), but not to Acinetobacter baumannii ATCC 19606 and Pseudomonas aeruginosa ATCC 27853. In vitro growth curves displayed similar rates for the E. coli and the K. pneumoniae recipients and for the respective transconjugants (Figure 1), indicating a negligible impact of mcr-1.2 acquisition on fitness. S1-PFGE showed three plasmid bands (ranging from ~33 to ~100 kb) in the E. coli 288328 donor, while only one ~33-kb band was observed in all transconjugants. Southern blot followed by hybridization (performed in intraspecific matings) revealed that mcr-1.2 was located on the ~33-kb plasmid (Figure S1). Since mcr-1 plasmids of ~33 kb share highly conserved nucleotide sequences (Li et al., 2016), we used several primers (Table S1), designed from the DNA sequence of pMCR1.2-IT (Di Pilato et al., 2016), to complete the sequence of our mcr-1.2-carrying plasmid, that we designated pMCR1.2-IT-Ec (accession no. MF093645). It was an IncX4-type plasmid of 33,293 bp and carried no antibiotic resistance genes besides mcr-1.2. Its sequence was almost identical (100% coverage, 99% nucleotide identity) to those of IncX4-type plasmids pMCR1.2-IT and p31349 (accession nos. KX236309 and KY689634, respectively).

This study is consistent with other data indicating a low prevalence, so far, of the mcr-1 gene in clinical isolates (Cannatelli et al., 2016; Schwarz and Johnson, 2016; Skov and Monnet, 2016). Compared to the only other report — in Switzerland of late — of mcr-1.2 in E. coli (Donà et al., 2017), our isolate differs, besides in the clinical source (blood vs. feces), in the sequence type (ST354, belonging to ST354 Cplx, vs. ST5, belonging to ST13 Cplx). In Italy, the mcr-1.2 variant has till now been identified only in K. pneumoniae on an almost identical plasmid (Di Pilato et al., 2016). On the other hand, a polyclonal diffusion of mcr-1-harboring E. coli has been
reported in Italy lately (Cannatelli et al., 2016; Giuffrè et al., 2016; Corbella et al., 2017), but in the absence of specific investigations it cannot be excluded that some of such \textit{mcr-1} genes were, in fact, \textit{mcr-1.2}. This ongoing spread of \textit{mcr-1}-mediated colistin resistance, in addition to the low fitness cost of the gene acquisition and the finding of colistin resistance in patients not treated with the drug, denote a potentially worrying situation in our country and emphasize the importance of surveillance for \textit{mcr-1} among colistin-non-susceptible or colistin-resistant enterobacteria in clinical and nonclinical settings.

\textbf{Funding}

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
References


FIGURE 1
Growth rates, determined to compare bacterial fitness, of the recipient (●) and a randomly chosen transconjugant (○) of *E. coli* (A) and *K. pneumoniae* (B).
Revised version of manuscript DMID-17-783. **Highlights**

**Highlight 1**
Transferable colistin resistance is mediated by the plasmid-borne gene *mcr-1*

**Highlight 2**
Though reported worldwide, *mcr-1* is currently being increasingly detected in Italy

**Highlight 3**
Its allelic variant *mcr-1.2* has been described in Italy, in *K. pneumoniae*

**Highlight 4**
Here we report the detection of *mcr-1.2* in a bloodstream isolate of *E. coli*

**Highlight 5**
*mcr-1.2* was the sole antibiotic resistance gene carried by a 33,293-bp IncX4 plasmid