First Report of the mcr-1 colistin Resistance Gene Identified in Escherichia coli Isolated from a Clinical Sample in Naples in 2020

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Keywords: Escherichia coli, colistin resistance, mcr-1, antimicrobial resistance, laboratory surveillance.

Posted Date: December 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-135159/v1>

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Abstract

Background: The emergence of a novel plasmid-mediated colistin resistance mechanism, encoded by the *mcr-1* gene, represents a major public health concern. The mechanism of resistance to colistin, mediated by plasmids, is a serious problem, both for its ability to be transferred to other species, and for infections caused by carbapenem-resistant Gram-negative, in which colistin is used as an antimicrobial drug of last line for the treatment of these infections. The present study highlights the first isolation and genetic evaluation of detecting plasmid-mediated resistance to colistin in a multidrug-resistant (MDR) *Escherichia coli* (*E. coli*) isolated from a clinical sample in the metropolitan city of Naples, Italy.

Results: Colistin-resistant *E. coli* isolate was identified in August 2020 from the blood culture of a male patient with multiple comorbidities. The minimum inhibitory concentration (MIC) of colistin was 8 mg/L. In addition to colistin, the isolate was resistant to third-generation cephalosporins (cefotaxime and ceftazidime), penicillin (amoxicillin and piperacillin), aminoglycosides (gentamicin and tobramycin), and fluoroquinolones (ciprofloxacin and levofloxacin). However, it showed susceptibility to carbapenems (ertapenem, imipenem, and meropenem), tetracyclines (tigecycline), and piperacillin-tazobactam. The results of the PCR confirmed the presence of the *mcr-1* resistance gene.

Conclusion: This study confirms the presence of resistance to colistin mediated by the *mcr-1* gene in a clinical isolate of *E. coli*. Although resistance to colistin caused by the *mcr-1* gene is not common in our region, it should not be ignored. Therefore, further surveillance studies are recommended to monitor the spread of plasmid-mediated colistin resistance genes in Gram-negative MDR bacteria.

Background

In recent years, the phenomenon of antibiotic resistance (AMR, Antimicrobial resistance) has increased significantly due to the inappropriate and extended use of antibiotics, both in human and veterinary medicine, causing the emergence, multiplication, and spread of strains resistant to more antibiotics [1]. It should be noted that this phenomenon often concerns healthcare-related infections, which develop and spread within hospitals and other health facilities [2, 3]. Unfortunately, the emergence of multidrug-resistance microorganisms progressively reduces the possibility of applying an antibiotic approach and reflects on effective and timely treatments. This leads to important implications in terms of clinical (increased morbidity, lethality, duration of the disease) as well as economic terms [4]. Carbapenems are among the first-line antibiotics used in the treatment of infections caused by multidrug-resistant bacteria (MDR). The direct consequence of a carbapenems wide use brought to an increase in resistance rates among Enterobacteriaceae (CRE), causing much public health concern [5]. In this scenario, the last chance for a patient with a carbapenem-resistant MDRs is represented by Colistin (polymyxin E) [6]. Colistin belongs to the class of Polymyxins, polycationic peptides that selectively act on the permeability of the cytoplasmic membrane of gram-negative bacteria, causing cell lysis [7]. Resistance to colistin is mainly caused by changes in the lipopolysaccharide molecules (LPS). Indeed, the addition of cationic groups to lipid A reduces the access of colistin to the plasma membrane [8]. Specifically, the resistance is

mediated by the enzymatic activity of the phosphoethanolamine transferase, encoded by the *mcr* gene, which transfers phosphoethanolamine to the lipid A, modifying the lipopolysaccharides of the membrane [9]. Resistance to colistin, mediated by the *mcr* gene, is readily transmissible through conjugation to other pathogenic species, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [10, 11]. Clinical isolates of colistin-resistant *E. coli* have been rarely reported. The first isolate of *E. coli* resistant to colistin mediated by plasmid (*mcr*-1) have been found in China in 2012 [12]. To date, in many countries of Europe and in many other areas of the world the *mcr*-1 gene has been identified in *E. coli* isolated from clinical samples [13–16]. In Italy, the *mcr*-1 gene was first described in 2016, found in eight clinical isolates of *E. coli*, in the clinical microbiology laboratories of two Italian hospitals (Florence, central Italy; Lecco, northern Italy). Two isolates positive for *mcr*-1 exhibited a multidrug-resistant phenotype, including expanded spectrum cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, and produced extended-spectrum β -lactamase activity [17]. More recently, three cases of sepsis caused by *E. coli* colistin resistant, which harbored the *mcr*-1 gene, have been described in patients admitted to the Policlinico San Matteo of Pavia in Italy, between August 2016 and January 2017 [18]. Despite the prevalence of *mcr* genes among CRE or the incidence of colistin resistance is still very rare, it raises concern among experts, being a last-line antibiotic used in the treatment of infection by carbapenem-resistant Gram-negative [19]. In this study, we reported a case of sepsis caused by colistin-resistant *E. coli* strain (*mcr*-1), isolated at the section of Microbiology and Virology, University Hospital “Luigi Vanvitelli”, Naples, Italy.

Results

Based on the microbial identification of characteristic protein fingerprints of bacteria, we evaluated the *E. coli* isolate by MALDI-TOF MS. Among all the output results, the only microorganism with a valid score over 2.0 were taken into consideration. Figure 1 shows one of the spectra obtained from the MALDI-TOF analysis, which refers to the obtained *E.coli*.

After the bacterial isolation, an antibiogram evaluation was performed. The antibiotic panel resistances are summarized in Table 1. Indeed, the isolate was not susceptible to third-generation cephalosporins (cefotaxime and ceftazidime) due to ESBL (extended-spectrum beta-lactamase) production. Also, it showed resistance to penicillin (amoxicillin and piperacillin), aminoglycosides (gentamicin and tobramycin), and fluoroquinolones (ciprofloxacin and levofloxacin). However, it was still able to be inhibited by carbapenems (ertapenem, imipenem, and meropenem), tetracyclines (tigecycline), and piperacillin-tazobactam.

Table 1
MIC of antimicrobials against mcr-1 positive *E.coli*.

Antibiotics	MIC	SIR
Amikacin	>16	R
Amoxicillin-clavulanate	>32/2	R
Ampicillin	> 8	R
Cefepime	> 8	R
Cefotaxime	> 4	R
Ceftazidime	> 8	R
Cefuroxime	> 8	R
Ciprofloxacin	> 1	R
Ertapenem	≤0,25	S
Fosfomycin c/G6P	≤16	S
Gentamicin	> 4	R
Imipenem	≤0,25	S
Levofloxacin	> 2	R
Meropenem	≤0,125	S
Piperacillin	>16	R
Piperacillin-tazobactam	8/4	S
Tigecycline	≤0,5	S
Tobramycin	> 4	R
Trimethoprim-sulfamethoxazole	>4/76	R

In order to confirm the colistin resistance, we performed a PCR for the detection of the mcr-1 and mcr-2 genes, responsible for the colistin resistance. The PCR results confirmed the presence, in the *E. coli* isolate, of the mcr-1 gene, and the absence of the mcr-2 gene.

Discussion

This study aimed to highlight the first evidence of colistin-resistant *E. coli* strain, isolated in the metropolitan area of Naples, Italy. This strain was collected on August 29, 2020, from a blood culture sample of a 63-year-old male who had a urinary tract infection and high white blood cell count, admitted to the A.O.U. “Luigi Vanvitelli” of Naples, Italy. The isolate was identified with a minimal inhibitory concentration (MIC) to colistin of 8 mg/mL using a BD Phoenix system (BD Diagnostics, Sparks, MD,

USA). The same MIC to colistin was confirmed by broth microdilution, as recommended by EUCAST and ECDC guidelines. Conventional PCR to detect *mcr-1* and *mcr-2* genes was performed on DNA extracted from colistin-resistant *E.coli* colony. Results demonstrated that the *E. coli* isolate was positive for *mcr-1* gene transport. This strain was resistant to third-generation cephalosporins (ESBL), aminoglycosides and fluoroquinolones, but remained sensitive to fosfomycin, carbapenems, tigecycline, and piperacillin/tazobactam, similar to previous reports of colistin resistance isolated in Italy [17]. The isolated strain was negative for carbapenem resistance genes *bla*NDM, *bla*KPC, *bla*VIM, *bla*IMP-1, and *bla*OXA-48 as reported by the Cepheid Xpert1 Carba-R assay PCR (Cepheid, Sunnyvale, CA, USA). The patient had unprecedented treatments with colistin, recorded in the medical history. No colistin therapy was used in the provincial ward of the clinical isolate. The patient had been admitted to other hospitals before admission to this ward hospital, where the colistin-resistant isolate was isolated.

Conclusion

This report should serve as a public health warning signal, however, to intensify surveillance efforts and identify *mcr-1* tanks in sensitive populations. Environmental, food and clinical monitoring are essential in order to reduce the transfer of multi-strain resistant bacteria between animals and humans Finally, increased vigilance and the implementation of antimicrobial management programs are necessary and important in the health sector to slow the spread of antimicrobial resistance [22–25].

Materials And Methods

Sample collection and preparation:

A volume of 5–10 mL of blood, obtained from peripheral vein sampling, was inoculated in blood culture bottles (aerobic bottle and anaerobic bottle). Blood culture bottles were incubated in the automated blood culture monitoring BACTEC 9240 blood culture system (Becton Dickinson Diagnostic Instrument Systems). Positive blood cultures were subjected to Gram staining and subcultured on Columbia Blood Agar, Chocolate agar, CNA Agar with 5% Sheep Blood (with the addition of colistin and nalidixic acid), MacConkey Agar and Sabouraud Dextrose Agar (Becton, Dickinson and Company, Germany) and incubated at 37 °C overnight. After 24–48 hours of incubation, each plate was examined and bacterial identification and antimicrobial susceptibility test were performed.

Identification and antimicrobial susceptibility test:

Bacterial identification was performed by MALDI-TOF mass spectrometry (Matrix-Assisted Laser Desorption Ionization time-off light) (Bruker Daltonics GmbH, Bremen, Germany). The isolate was tested for antimicrobial susceptibility by Phoenix BD (Becton Dickinson, United States). As recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the European Centre for

Disease Prevention and Control (ECDC), minimal inhibitory concentration (MIC) of colistin was determined by broth microdilution (ComASP® Colistin, LIOFILCHEM S.r.l., Teramo, Italy).

DNA Extraction and Primer Sequence:

Genomic DNA was extracted using GF-1 Bacterial DNA Extraction Kit ChargeSwitch™ gDNA Mini Bacteria Kit (Invitrogen, California, USA), following the manufacturer's recommendations. The primers used for the identification of *E. coli* species were selected based on previous studies [17]. PCR analysis was conducted for colistin resistance genes *mcr-1* (MCR-1F: 5'-CTCATGATGCAGCATACTTC-3' and MCR-1R: 5'-CGAATGGAGTGTGCGGTG-3') and *mcr-2* (MCR-2F: 5'-TGTTGCTTGTGCCGATTGGA-3' and MCR-2R: 5'-AGATGGTATTGTTGGTTGCTG-3') and for *E. coli* genes of the family (Pho) used as positive control (Pho-F: 5'-GTGACAAAAGCCCGGACACCATAAATGC-3' and Pho-R: 5'-TACACTGTCATTACGTTGCGGGATTGCGG-3'). The primers were obtained from Eurofins Genomics (Italy).

Polymerase Chain Reaction (PCR) Amplification:

PCRs were performed employing the Kodaq 2X PCR MasterMix Kit (Abm, Canada). Two microliters of bacterial DNA were amplified in a 50 µL reaction volume with 10 µM of each primer, using an annealing temperature of 62 °C and 38 cycles. Analyses of the PCR products were conducted on 1,8% agarose gel and detected using Gel Doc™ EZ Imager (Bio-Rad, USA).

Declarations

Ethics approval and consent to participate:

The study was designed and conducted in accordance with the Helsinki declaration. This study was performed in accordance with the National Guidelines System (SNLG), for the use of biological samples. We declare that the following work was carried out following standard hospital protocols, approved by the Ethics Committee of the University of Campania "Luigi Vanvitelli" - A.O.U. "Luigi Vanvitelli". The need for written informed consent was waived because de-identified retrospective data were used. The permission of the corresponding author was required to access the raw data/samples.

Consent for publication:

Not applicable.

Availability of data and materials:

All data generated or analysed during this study are included in this published article

Conflict of interest:

All the authors declare to have not conflict of interests in the publication of this paper.

Funding:

This study did not receive any funding from public or private agency.

Author Contributions: Conceptualization, B.S.; writing—review and editing, B.S. and C.Z.; supervision, G.F. and C.Z.; funding acquisition, G.B. and G.F.; Visualization, C.D.V., A.C., V.F., M.G., A.F., F.M., A.P.P., F.F, F.C., and Massimiliano Galdiero; All authors have read and agreed to the published version of the manuscript.

Acknowledgments:

The authors would like to thank the staff of the U.O.C University Hospital of Campania “Luigi Vanvitelli” in Naples, Italy and the staff of University Hospital "San Giovanni di Dio e Ruggi d’Aragona", for their contributions.

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Figures

Figure 1

Mass spectrum of colistin-resistant *E. coli* obtained with a spectrometer MALDI / TOF MS. On the Cartesian axes are reported, the charge mass ratio (m/z) and relative abundance of the molecular species found in the sample, are reported..