



HAL
open science

mcr-1 Colistin Resistance in ESBL-Producing *Klebsiella pneumoniae* , France

Yvan Caspar, Mylène Maillet, Patricia Pavese, Gilles Francony, Jean-Paul Brion, Marie-Reine Mallaret, Richard Bonnet, Frédéric Robin, Racha Beyrouthy, Max Maurin

► **To cite this version:**

Yvan Caspar, Mylène Maillet, Patricia Pavese, Gilles Francony, Jean-Paul Brion, et al.. mcr-1 Colistin Resistance in ESBL-Producing *Klebsiella pneumoniae* , France. *Emerging Infectious Diseases*, 2017, 23 (5), pp.874 - 876. 10.3201/eid2305.161942 . hal-01639760

HAL Id: hal-01639760

<https://uca.hal.science/hal-01639760v1>

Submitted on 26 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

tests from Panama City versus other areas of Panama and might result in a sampling bias. Despite these limitations, the recent Zika outbreak has shown the speed at which vectorborne diseases can spread and highlights the importance of detecting emerging viruses like PTVs.

Acknowledgments

We thank staff in the Department of Research in Virology and Biotechnology at the Gorgas Memorial Institute for Health Studies in Panama and in the Ministry of Health National Epidemiology Department for the surveillance data and outbreak response during 2009. We also thank Meghan Tipre for help creating the epidemiologic map.

This work was done in compliance with the Gorgas Bioethics Committee (1010/CBI/ICGES/15).

Funding was provided by the Panama Ministry of Economy and Finance (09.044.051 to S.L.-V. and 09.044.050 to J.M.P.); the Secretaría Nacional de Ciencia, Tecnología, e Innovación (SENACYT; FID-09-103 to J.-P.C); and Gorgas Memorial Institute, University of Alabama at Birmingham (to N.D.G.). J.M.P. and S.L.-V. are members of the Sistema Nacional de Investigación (SNI) of SENACYT in Panama.

Dr. Gundacker is an infectious disease fellow at the University of Alabama at Birmingham. His primary interest are the clinical description of febrile tropical infectious diseases, laboratory differential diagnosis of these diseases, and host–pathogen interactions. Mr. Carrera is an epidemiologist and virologist at Gorgas Memorial Institute. His primary research interests are ecology, evolution, and epidemiology of arthropodborne and zoonotic viruses.

References

1. Arminen B, Pascale JM, Munoz C, Lee SJ, Choi KL, Avila M, et al. Incidence rate for hantavirus infections without pulmonary syndrome, Panama. *Emerg Infect Dis*. 2011;17:1936–9. <http://dx.doi.org/10.3201/eid1710.101717>
2. Fauci AS, Morens DM. Zika virus in the Americas—yet another arbovirus threat. *N Engl J Med*. 2016;374:601–4. <http://dx.doi.org/10.1056/NEJMp1600297>
3. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM. Ecology of viruses isolated from Panamanian phlebotomine sandflies. *Am J Trop Med Hyg*. 1974;23:258–69.
4. Palacios G, Wiley MR, Travassos da Rosa AP, Guzman H, Quiroz E, Savji N, et al. Characterization of the Punta Toro species complex (genus *Phlebotomus*, family *Buniviridae*). *J Gen Virol*. 2015;96:2079–85. <http://dx.doi.org/10.1099/vir.0.000170>
5. Xu F, Chen H, Travassos da Rosa AP, Tesh RB, Xiao SY. Phylogenetic relationships among sandfly fever group viruses (*Phlebotomus*: *Buniviridae*) based on the small genome segment. *J Gen Virol*. 2007;88:2312–9. <http://dx.doi.org/10.1099/vir.0.82860-0>
6. Liu DY, Tesh RB, Travassos Da Rosa AP, Peters CJ, Yang Z, Guzman H, et al. Phylogenetic relationships among members of the genus *Phlebotomus* (*Buniviridae*) based on partial M segment sequence analyses. *J Gen Virol*. 2003;84:465–73. <http://dx.doi.org/10.1099/vir.0.18765-0>
7. Sánchez-Seco MP, Echevarría JM, Hernández L, Estévez D, Navarro-Marí JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT–nested-PCR assays with degenerated primers. *J Med Virol*. 2003;71:140–9. <http://dx.doi.org/10.1002/jmv.10465>
8. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33:1870–4. <http://dx.doi.org/10.1093/molbev/msw054>
9. Valderrama A, Tavares MG, Andrade Filho JD. Anthropogenic influence on the distribution, abundance and diversity of sandfly species (Diptera: Phlebotominae: Psychodidae), vectors of cutaneous leishmaniasis in Panama. *Mem Inst Oswaldo Cruz*. 2011; 106:1024–31. <http://dx.doi.org/10.1590/S0074-02762011000800021>

Address for correspondence: Sandra López-Vergès, Gorgas Memorial Institute for Health Studies, Department of Research in Virology and Biotechnology, Ave Justo Arosemena and St 35, No. 0816-02593, Panama City, Panama; email: slopez@gorgas.gob.pa

***mcr-1* Colistin Resistance in ESBL-Producing *Klebsiella pneumoniae*, France**

Yvan Caspar, Mylène Maillet, Patricia Pavese, Gilles Francony, Jean-Paul Brion, Marie-Reine Mallaret, Richard Bonnet, Frédéric Robin, Racha Beyrouthy, Max Maurin

Author affiliations: Centre Hospitalier Universitaire Grenoble-Alpes, Grenoble, France (Y. Caspar, M. Maillet, P. Pavese, G. Francony, J.-P. Brion, M.-R. Mallaret, M. Maurin); University Grenoble Alpes, CNRS, Grenoble; Centre Hospitalier Universitaire Clermont-Ferrand, Clermont-Ferrand, France (R. Bonnet, F. Robin, R. Beyrouthy); Centre National de Référence de la Résistance aux Antibiotiques, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Université Clermont Auvergne, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Institut National de la Santé et de la Recherche Médicale, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy); Institut National de la Recherche Agronomique, Clermont-Ferrand (R. Bonnet, F. Robin, R. Beyrouthy)

DOI: <http://dx.doi.org/10.3201/eid2305.161942>

We report intestinal carriage of an extended-spectrum β-lactamase–producing *Klebsiella pneumoniae* strain with high-level resistance to colistin (MIC 24 mg/L) in a patient in France who had been hospitalized for fungal meningitis. The strain had the *mcr-1* plasmid gene and an inactivated *mgrB* gene, which are associated with colistin resistance.

Resistance to colistin in gram-negative bacteria stems mainly from structural modifications of bacterial lipopolysaccharide. These modifications include addition of 4-amino-4-deoxy-L-arabinose or phosphoethanolamine caused by chromosomal mutations in genes encoding the 2-component systems PhoPQ and PmrAB, or mutations in the *mgrB* gene, a negative regulator of PhoPQ (1).

The recent discovery of a horizontally transferable plasmid-mediated *mcr-1* gene encoding a phosphoethanolamine transferase is a cause for concern, but few *mcr-1*-positive clinical strains of *Klebsiella pneumoniae* have been reported so far in Europe (2). Colocalization of carbapenemases or extended-spectrum β -lactamase (ESBL) genes and the *mcr-1* gene on the same plasmids is of concern because it might lead to pandrug resistance (1,3). We report *mcr-1* colistin resistance in ESBL-producing *K. pneumoniae* isolated from a patient in France.

The patient was a 38-year-old man who had chronic granulomatous disease that was diagnosed when he was 8 months old. Since then, he has had several minor and major diseases and conditions, including primitive femoral osteitis, hepatic abscesses, disseminated candidiasis, and bacteremia, which required several treatments with antimicrobial drugs. However, the patient was never given colistin.

In April 2016, he was hospitalized for surgical removal of a thyroid abscess. Fungal cultures of the abscess grew *Aspergillus fumigatus*. Despite antifungal treatment with amphotericin B and flucytosine, fungal meningitis, cerebral arterial vasospasm at the Willis polygon, and hydrocephalus developed. The patient also received immunosuppressive therapy (methylprednisolone and anakinra) and empiric antimicrobial drug therapy, including cotrimoxazole, clindamycin, meropenem, and vancomycin successively.

In August 2016, systematic culture of a rectal swab specimen showed an ESBL-producing strain of *K. pneumoniae*; 2 previous rectal screenings showed negative results. The strain was resistant to colistin (MIC >4 mg/L). Resistance was determined by using a broth microdilution assay (BD Phoenix Instrument; Becton Dickinson, Franklin Lakes, NJ, USA) (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/23/5/16-1942-Techapp1.pdf>). An MIC of 24 mg/L was found for polymyxin B by using

the E-test method (Polymyxin B E-test strip; bioMérieux, Marcy L'Etoile, France).

The strain was sent to the French National Reference Center for Antibiotic Resistance in *Enterobacteriaceae* (Hôpital Gabriel Montpied, Clermont-Ferrand, France), which confirmed phenotypic resistance to colistin and identified the *mcr-1* gene by using PCR and previously described primers (2). Whole-genome sequencing showed that the *K. pneumoniae* strain had genotype ST15 and confirmed the presence of the *mcr-1* gene on a 33,303-kb transferable plasmid of incompatibility group IncX4 (online Technical Appendix). This plasmid differed by only 4 mutations from *mcr-1.2*-encoding plasmid pMCR-1.2.IT (GenBank accession no. KX236309) previously characterized in Italy (4). Conjugation of the plasmid into *Escherichia coli* K12 conferred colistin resistance (MIC increased from 0.25 mg/L to 4 mg/L) to the *E. coli* strain.

Other resistance genes were also identified (Table), including the ESBL-encoding gene *bla*_{SHV106} (online Technical Appendix). None of them were localized with the *mcr-1* gene on the IncX4 plasmid. Moreover, insertion of mobile element *IS5* in the *mgrB* gene was detected, which is also associated with colistin resistance (5). No mutations were found in the *prmA*, *prmB*, *phoP*, and *phoQ* genes.

There is currently no commercial medium to screen gram-negative bacteria harboring the *mcr-1* gene. Nordmann et al. (6) described an in-house SuperPolymyxin medium composed of eosin methylene blue agar, 3.5 mg/L of colistin sulfate, 10 mg/L of daptomycin, and 5 mg/L amphotericin B, which showed excellent sensitivity and specificity. Colistin resistance can be confirmed within 2 h by using an in-house rapid polymyxin Nordmann-Poirel test (7). The *mcr-1* gene can be rapidly detected by real-time PCR of DNA extracts obtained from bacterial strains or directly from stool samples (2,8,9).

We obtained subcultures of the strain from the patient on Columbia CNA agar containing 10 mg/L of colistin and 15 mg/L of nalidixic acid and 5% sheep blood (CNA⁺; bioMérieux) but not on Thayer-Martin agar medium containing unknown concentrations of vancomycin, colistin, amphotericin B, and trimethoprim (VCA3; bioMérieux). Lack of growth on this medium might be related to a high colistin concentration or the presence of vancomycin, which can potentiate colistin activity (6). Further investigations using

Table. Resistance genes identified by whole-genome sequencing of an ESBL-producing *mcr-1*-positive *Klebsiella pneumoniae* strain isolated from a 38-year-old man, France*

Resistance gene	Target antimicrobial drug
<i>mcr-1</i> and inactivation of <i>mgrB</i> by <i>IS5</i> insertion	Colistin
<i>bla</i> _{SHV-106}	β -lactams
<i>aac</i> (3)-IId and <i>aadA</i> 16-like	Aminoglycoside
<i>aac</i> (6')Ib-cr	Quinolone and aminoglycoside
<i>fosA5</i>	Fosfomycin
<i>sull</i> and <i>folP</i>	Sulfonamide
<i>dfpA27</i>	Trimethoprim
<i>tetD</i>	Tetracycline

*ESBL, extended-spectrum β -lactamase.

CNA⁺ medium did not identify intestinal carriage of ESBL-negative but *mcr-1*-positive enterobacteria in the index case-patient. On the basis of these results, rectal screening of 39 contacts was performed by using an ESBL-screening medium (BLSE agar [MacConkey agar and Drigalski agar]; bioMérieux). All of the tests showed negative results.

The origin of the *mcr-1* strain remains unknown. Nosocomial acquisition cannot be ruled out because colistin-resistant strains harboring the *mcr-1* gene might have been isolated in the hospital but not identified because this resistance mechanism was initially reported in February 2016. Food might also be incriminated (1); one study identified a 21% *mcr-1* prevalence among ESBL-producing *E. coli* in calves in France (10).

Multiple antimicrobial drug therapy for this patient might have selected for this multidrug-resistant bacteria. The presence of a plasmid containing the *mcr-1* and ESBL or other resistance genes in the same strain might be involved in selection of colistin-resistant strains during administration of any ineffective antimicrobial drug (3). Development of efficient tools for rapid detection of *mcr-1*-harboring strains should be a priority to prevent dissemination of these strains in hospital settings.

Dr. Caspar is a clinical microbiologist in the Bacteriology Laboratory, Grenoble-Alpes University Hospital, and a probationary lecturer in the Medicine Faculty, Grenoble-Alpes University, Grenoble, France. His primary research interests are bacterial resistance or treatment failures for tularemia and evaluation of novel antimicrobial compounds.

References

1. Baron S, Hadjadj L, Rolain J-M, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. *Int J Antimicrob Agents*. 2016;48:583–91. <http://dx.doi.org/10.1016/j.ijantimicag.2016.06.023>
2. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, 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;16:161–8. [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7)
3. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother*. 2016;71:2066–70. <http://dx.doi.org/10.1093/jac/dkw274>
4. Di Pilato V, Arena F, Tascini C, Cannatelli A, Henrici De Angelis L, Fortunato S, 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;60:5612–5. <http://dx.doi.org/10.1128/AAC.01075-16>
5. Cannatelli A, D'Andrea MM, Giani T, Di Pilato V, Arena F, Ambretti S, et al. *In vivo* emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP *mgrB* regulator. *Antimicrob Agents Chemother*. 2013;57:5521–6. <http://dx.doi.org/10.1128/AAC.01480-13>
6. Nordmann P, Jayol A, Poirel L. A universal culture medium for screening polymyxin-resistant gram-negative isolates. *J Clin Microbiol*. 2016;54:1395–9. <http://dx.doi.org/10.1128/JCM.00446-16>
7. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. *Emerg Infect Dis*. 2016;22:1038–43. <http://dx.doi.org/10.3201/eid2206.151840>
8. Nijhuis RH, Veldman KT, Schelfaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, et al. Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay. *J Antimicrob Chemother*. 2016;71:2344–6. <http://dx.doi.org/10.1093/jac/dkw192>
9. Bontron S, Poirel L, Nordmann P. Real-time PCR for detection of plasmid-mediated polymyxin resistance (*mcr-1*) from cultured bacteria and stools. *J Antimicrob Chemother*. 2016;71:2318–20. <http://dx.doi.org/10.1093/jac/dkw139>
10. Haenni M, Métayer V, Gay E, Madec J-Y. Increasing trends in *mcr-1* prevalence among ESBL-producing *E. coli* in French calves despite decreasing exposure to colistin. *Antimicrob Agents Chemother*. 2016;60:6433–4. <http://dx.doi.org/10.1128/AAC.01147-16>

Address for correspondence: Yvan Caspar, Laboratoire de Bactériologie, Institut de Biologie et Pathologie, Centre Hospitalier Universitaire Grenoble Alpes, CS10217, 38043 Grenoble CEDEX 9, France; email: ycaspar@chu-grenoble.fr

Chromosomal 16S Ribosomal RNA Methyltransferase RmtE1 in *Escherichia coli* Sequence Type 448

Bin Li, Marissa P. Pacey, Yohei Doi

Author affiliations: Fujian Medical University Union Hospital, Fuzhou, China (B. Li); University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA (B. Li, M.P. Pacey, Y. Doi)

DOI: <http://dx.doi.org/10.3201/eid2305.162000>

We identified *rmtE1*, an uncommon 16S ribosomal methyltransferase gene, in an aminoglycoside- and cephalosporin-resistant *Escherichia coli* sequence type 448 clinical strain co-harboring *bla*_{CMY-2}. Long-read sequencing revealed insertion of a 101,257-bp fragment carrying both resistance genes to the chromosome. Our findings underscore *E. coli* sequence type 448 as a potential high-risk multidrug-resistant clone.

RmtE (RmtE1 and its variant RmtE2) is an uncommon plasmid-mediated 16S rRNA methyltransferase (16S RMTase) found in gram-negative bacteria; only 4 strains have been reported to produce RmtE, all *Escherichia coli*, including 1 from the University of Pittsburgh Medical