

Plasmid-mediated *mcr-1* in carbapenem-susceptible *Escherichia coli* ST156 causing a blood infection: an unnoticeable spread of colistin resistance in Brazil?

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OBJECTIVE: We describe an IncX4 pHC891/16mcr plasmid carrying *mcr-1* in a colistin-resistant and carbapenem-susceptible *E. coli* isolate (HC891/16), ST156, which caused a blood infection in a Brazilian patient with gallbladder adenocarcinoma.

METHODS: Strain HC891/16 was subjected to whole genome sequencing using the MiSeq Platform (Illumina, Inc., USA). Assembly was performed using Mira and ABACAS.

RESULTS: The isolates showed resistance only to ciprofloxacin, ampicillin and ceftazidime, and whole-genome sequencing revealed the presence of *aac(6')Ib-cr* and *bla_{TEM1}*.

CONCLUSION: Our findings warn of the possible silent dissemination of colistin resistance by carbapenem-susceptible *mcr-1* producers, as colistin susceptibility is commonly tested only among carbapenem-resistant isolates.

KEYWORDS: Colistin Resistance; Polymyxin Resistance; *Escherichia Coli*; Carbapenem-Susceptible; ST156.

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Liu et al. (1) reported the emergence of an *mcr-1* plasmidial resistance mechanism to colistin in *E. coli* from China, which was followed by many reports worldwide (2-7). In Brazil, Fernandes et al. (8) reported an IncX4 plasmid carrying *mcr-1* in *E. coli* isolated from a soft tissue infection in a human. These authors also described evidence that *E. coli* harboring *mcr-1* has been occurring in food-producing animals in Brazil since at least 2012 (9). However, to date, there is no consensus in the scientific community regarding the clinical importance of this new colistin resistance mechanism, as it confers a low level of colistin resistance compared with chromosomal mechanisms and appears to have been present for a long time (10). Epidemiological data can be used to elucidate the impact of this plasmidial mechanism for the dissemination of colistin resistance. In this study, we describe a plasmid-mediated *mcr-1* in a colistin-resistant and

carbapenem-susceptible *E. coli* isolate that caused a blood infection in a cancer patient from Brazil.

ECF, a 75-year-old female with a gallbladder adenocarcinoma diagnosed in January 2016, was admitted to Hospital das Clínicas, São Paulo for investigation, and a hepatic drain was placed. Bile cultures were positive for *Enterococcus faecalis* susceptible to all drugs. One month later, she developed fever and presented to the emergency room (ER). Blood cultures were positive for *E. coli* phenotypically classified as an ESβL-producer that was susceptible to carbapenem and aminoglycosides, resistant to ciprofloxacin (MIC > 4 mg/L) and with a colistin MIC ≤ 0.5 mg/L. She received meropenem with good clinical evolution. One month later, she began ambulatory chemotherapy (cisplatin and gemcitabine). On April 11, 2016, she returned to the ER with a high fever, leukocytosis, and elevated CRP, and her bloodcultures were positive for *E. coli* with a different phenotypic profile. It was susceptible to amikacin (MIC, ≤ 2 mg/L), gentamicin (MIC, 2 mg/mL), cefuroxime (MIC, 4 mg/L), ceftazidime (MIC, ≤ 1 mg/L), ceftriaxone (MIC, ≤ 1 mg/L), cefepime (MIC, ≤ 1 mg/L), ertapenem (MIC, ≤ 0.5 mg/L), imipenem (MIC, ≤ 0.25 mg/L), meropenem (MIC, ≤ 0.25 mg/L), piperacillin/tazobactam (MIC, ≤ 4 mg/L) and tigecycline (MIC, ≤ 0.5 mg/L); intermediate to ceftazidime (MIC, 16 mg/L);

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and resistant to ampicillin (MIC, >32 mg/L) and ciprofloxacin (MIC, >4 mg/L). The colistin MIC was 4 mg/L. This strain was positive for *mcr-1* by PCR using specific primers as previously described (1) and was denominated HC891/16. The patient received antibiotics and support medication and was discharged with no infection. The identification and antimicrobial susceptibility tests of the isolates were performed by Vitek-MS and Vitek-2 (bioMérieux, France), respectively, and colistin susceptibility was confirmed by CLSI broth microdilution (11). Strain HC891/16 was subjected to whole genome sequencing using the MiSeq Platform (Illumina, Inc., USA). The sequencing library was prepared from 40 ng of HC891/16 strain total DNA using the Illumina Nextera DNA library preparation kit (Illumina, Inc., USA). After quantification of the resulting library using the KAPA Library Quantification Kit, a sequencing run was performed in the Illumina MiSeq to generate 250 bp paired-end reads. Assembly was performed using Mira (12) and ABACAS (13), resulting in 71 contigs encompassing the main chromosome (5,053,377 bp) and at least one plasmid (33,227 bp). Genome sequences were deposited in GenBank under accession number MRDN01000000. The presence of resistance genes was determined with ResFinder (14).

The strain HC891/16 belongs to sequence type (ST) 156. *E. coli* ST156 harboring the *mcr-1* gene was previously detected in one duck sample, from China, showing a carbapenem-resistant profile (15). The *mcr-1* gene is in pHC891/16mcr, a 33.2-kb plasmid, which is classified as incompatibility group IncX4. This plasmid shows 100% nucleotide identity to pICBEC7Pmcr (CP017246.1), and it is found in animal samples from Latin America. Furthermore, 99% nucleotide identity was observed when this plasmid was compared with the following previously described conjugative plasmids harboring the *mcr-1* gene: pOW3E1 (KX129783.1), detected in environmental and animal samples; pMCR1.2-IT (KX236309.1), harboring a *mcr-1.2* variant of *K. pneumoniae* from a human infection in Italy; p*mcr1*_IncX4 (KU761327.1), found in clinical isolates from China; pMCR1-NJ-IncX4 (KX447768.1), found in a carbapenem-resistant *E. coli* isolate harboring *mcr-1* and *bla*_{NDM-5}, causing a complicated urinary tract infection in the USA (16-19). The IncX4 plasmid appears to be globally disseminated among environmental, food and human *mcr-1* producer strains, including KPC-2 and a *mcr-1* producer-*K. pneumoniae* strain that have been recently reported in Brazil (20). In addition to *mcr-1*, we also detected the presence of genes *aac(6)'Ib-cr* and *bla*_{TEM1} in the HC891/16 strain.

In a previous study from our institution, carbapenem susceptibility was observed in 21.5% of all colistin-resistant Gram-negative isolates examined in the last five years (21). It is important to highlight that the carbapenem-susceptible and *mcr-1*-positive phenotype may easily go unnoticed because many clinical laboratories in Brazil only test or release colistin susceptibility to clinicians when the isolates are carbapenem-resistant. Olaitan et al. (22) state that clinicians and microbiologists should be vigilant for the possible existence of colistin-resistant bacteria not only in patients undergoing colistin therapy but also in patients who are not receiving the drug, as resistant bacteria could later be selected if colistin is used. In Brazil, colistin has been used as a first-line drug in many critical care units due to high rates of carbapenem-resistance among the OXA-23-producer *Acinetobacter baumannii* and KPC-producer *K. pneumoniae* (23,24).

The dissemination of colistin resistance is a serious problem for infection control, as this antimicrobial is one of the last therapeutic options for infections caused by multidrug-resistant Gram-negative isolates. At this time, attention should be paid to this carbapenem-susceptible-*mcr-1* phenotype, as these isolates may be silently contributing to the spread of colistin resistance.

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AUTHOR CONTRIBUTIONS

Rossi F and Girardello R were responsible for the data analysis and preparation of the manuscript. Morais C, Martins LF, Silva AM, Setubal JC were responsible for the bioinformatics analysis. Cury AP was responsible for the data analysis. Abdala E was responsible for the description of clinical case. Duarte AJ was responsible for the preparation of the manuscript.

REFERENCES

- Liu YY, Wang Y, Walsh TR, Yi LX, 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(2):161-8. [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7).
- Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill.* 2015;20(49). <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085>.
- Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the *mcr-1* colistin resistance gene in carbapenem-resistant Enterobacteriaceae. *Lancet Infect Dis.* 2016;16(3):287-8. [http://dx.doi.org/10.1016/S1473-3099\(16\)00056-6](http://dx.doi.org/10.1016/S1473-3099(16)00056-6).
- Falgenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Käsböhrer A, Roesler U, et al. Colistin resistance gene *mcr-1* in extended-spectrum β -lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. *Lancet Infect Dis.* 2016;16(3):282-3. [http://dx.doi.org/10.1016/S1473-3099\(16\)00009-8](http://dx.doi.org/10.1016/S1473-3099(16)00009-8).
- Olaitan AO, Chabou S, Okdah L, Morand S, Rolain JM. Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis.* 2016;16(2):147. [http://dx.doi.org/10.1016/S1473-3099\(15\)00540-X](http://dx.doi.org/10.1016/S1473-3099(15)00540-X).
- Stoesser N, Mathers AJ, Moore CE, Day NP, Crook DW. Colistin resistance gene *mcr-1* and pHNSHP45 plasmid in human isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Lancet Infect Dis.* 2016;16(3):285-6. [http://dx.doi.org/10.1016/S1473-3099\(16\)00010-4](http://dx.doi.org/10.1016/S1473-3099(16)00010-4).
- Rapoport M, Faccione D, Pasteran F, Ceriana P, Albornoz E, Petroni A, et al. First Description of *mcr-1*-Mediated Colistin Resistance in Human Infections Caused by *Escherichia coli* in Latin America. *Antimicrob Agents Chemother.* 2016;60(7):4412-3. <http://dx.doi.org/10.1128/AAC.00573-16>.
- Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, et al. First Report of the Globally Disseminated IncX4 Plasmid Carrying the *mcr-1* Gene in a Colistin-Resistant *Escherichia coli* Sequence Type 101 Isolate from a Human Infection in Brazil. *Antimicrob Agents Chemother.* 2016;60(10):6415-7. <http://dx.doi.org/10.1128/AAC.01325-16>.
- Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, et al. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene. *Euro Surveill.* 2016;21(17). <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.17.30214>.
- Skov RL, Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. *Euro Surveill.* 2016;21(9):30155. <http://dx.doi.org/10.2807/1560-7917.ES.2016.21.9.30155>.
- CLSI. Clinical Laboratory Standard Institute Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard— Document M07-A9. Wayne, Pa. 2012. <http://dx.doi.org/10.1016/j.jpha.2015.11.005>.
- Chevreaux B, Wetter T, Suhai S. Genome Sequence Assembly Using Trace Signals and Additional Sequence Information. *Computer Science and Biology: Proceedings of the German Conference on Bioinformatics (GCB).* 1999;99:45-56.
- Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. ABACAS: algorithm-based automatic contiguation of assembled sequences. *Bioinformatics.* 2009;25(15):1968-9. <http://dx.doi.org/10.1093/bioinformatics/btp347>.



14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother.* 2012;67(11):2640-4, <http://dx.doi.org/10.1093/jac/dks261>.
15. Yang RS, Feng Y, Lv XY, Duan JH, Chen J, Fang LX, et al. Emergence of NDM-5- and MCR-1-Producing *Escherichia coli* Clones ST648 and ST156 from a Single Muscovy Duck (*Cairinamoschata*). *Antimicrob Agents Chemother.* 2016;60(11):6899-6902, <http://dx.doi.org/10.1128/AAC.01365-16>.
16. Zurfluh K, Klumpp J, Nüesch-Inderbinen M, Stephan R. Full-Length Nucleotide Sequences of *mcr-1*-Harboring Plasmids Isolated from Extended-Spectrum- β -Lactamase-Producing *Escherichia coli* Isolates of Different Origins. *Antimicrob Agents Chemother.* 2016;60(9):5589-91, <http://dx.doi.org/10.1128/AAC.00935-16>.
17. 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(9):5612-5, <http://dx.doi.org/10.1128/AAC.01075-16>.
18. Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X, et al. Complete Sequences of *mcr-1*-Harboring plasmids from Extended-Spectrum- β -Lactamase- and Carbapenemase-Producing Enterobacteriaceae. *Antimicrob Agents Chemother.* 2016;60(7):4351-4, <http://dx.doi.org/10.1128/AAC.00550-16>.
19. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, et al. Colistin- and Carbapenem-Resistant *Escherichia coli* Harboring *mcr-1* and *bla*NDM-5, Causing a Complicated Urinary Tract Infection in a Patient from the United States. *MBio.* 2016;7(4).pii:e01191-16, <http://dx.doi.org/10.1128/mBio.01191-16>.
20. Aires CAM, da Conceição-Neto OC, Tavares E Oliveira TR, Dias CF, Montezzi LF, Picão RC, et al. Emergence of the Plasmid-Mediated *mcr-1* Gene in Clinical KPC-2-Producing *Klebsiella pneumoniae* Sequence Type 392 in Brazil. *Antimicrob Agents Chemother.* 2017;61(7).pii:e00317-17, <http://dx.doi.org/10.1128/AAC.00317-17>.
21. Rossi F, Girardello R, Cury AP, Di Gioia TS, Almeida JN Jr, Duarte AJ. Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years. *Braz J Infect Dis.* 2017;21(1):98-101, <http://dx.doi.org/10.1016/j.bjid.2016.09.011>.
22. 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, <http://dx.doi.org/10.1016/j.ijantimicag.2015.11.009>.
23. Agência Nacional de Vigilância Sanitária (ANVISA). Boletim Informativo: Segurança e qualidade em serviço de saúde nº 12: Relatório da resistência microbiana em infecções primárias de corrente sanguínea confirmadas laboratorialmente relacionadas ao uso de cateter venoso central em unidades de terapia intensiva (2014). Acessado em 05 de agosto 2016. Disponível em: <http://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoes/item/12>
24. Vasconcelos AT, Barth AL, Zavascki AP, Gales AC, Levin AS, Lucarevski BR, et al. The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. *Diagn Microbiol Infect Dis.* 2015;83(4):382-5, <http://dx.doi.org/10.1016/j.diagmicrobio.2015.08.006>.