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Emergence of the Plasmid-Mediated *mcr-1* Gene in Clinical KPC-2-Producing *Klebsiella pneumoniae* Sequence Type 392 in Brazil

Caio Augusto Martins Aires,[a] Orlando Carlos da Conceição-Neto,[a] Thamirys Rachel Tavares e Oliveira,[a] Carolina Frizzera Dias,[b] Lara Feital Montezzi,[c] Renata Cristina Picão,[c] Rodolpho Mattos Albano,[d] Marise Dutra Asensi,[a] Ana Paula D'Alincourt Carvalho-Assef[a]

Laboratório de Pesquisa em Infecção Hospitalar (LAPIH), Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil[a]; Comissão de Controle de Infecção Hospitalar, Hospital Santa Casa da Misericórdia de Vitória, Vitória, Brazil[b]; Laboratório de Investigação em Microbiologia Médica (LIMM), Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil[c]; Departamento de Bioquímica, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil[d]

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Since the first report of the plasmid-mediated colistin resistance *mcr-1* gene in *Escherichia coli* and *Klebsiella pneumoniae* isolates from China (1), *mcr-1* has already spread to most continents, being detected in different species from several sources, including carbapenemase-producing clinical isolates (2). In Brazil, *mcr-1* has been identified in *E. coli* isolates from food-producing animals (3), migratory birds (4), and a human clinical sample (5). In this study, we report the detection of *mcr-1* in KPC-2-producing *K. pneumoniae* from a human clinical specimen in Brazil.

In September 2016, a 61-year-old man diagnosed with thrombotic thrombocytopenic purpura was admitted to the intensive care unit of a hospital in Vitória, Espírito Santo (southern Brazil), with ischemic stroke, bicytopenia, and pulmonary focus sepsis. After mechanical ventilation, bladder catheterization, and multiple catheter punctures, he developed a urinary tract infection caused by a *K. pneumoniae* strain (CCBH24080) resistant to polymyxin B and imipenem by Etest (bioMérieux, France). Interestingly, the patient responded well to therapy with polymyxin B (500,000 IU every 12 h) and meropenem (1 g every 8 h). The patient remained in isolation and died in November due to a severe hemorrhage.

Bacterial identification was confirmed by matrix-assisted laser desorption ionization (Bruker Daltonics, Germany). The MICs of colistin (16 μg/ml) and imipenem (>64 μg/ml) were confirmed by microdilution with cation-adjusted Mueller-Hinton broth (6), while for the other drugs, testing was performed by Vitek 2 (bioMérieux). Antimicrobial susceptibility was interpreted according to CLSI guidelines (7), except for tigecycline and colistin, for which the EUCAST criteria were used (8) (Table 1).

Whole-genome sequencing of CCBH24080 (GenBank accession no. NBOS00000000) was performed on the MiSeq platform (Illumina, USA). Genome assembly was carried out with the A5 assembly pipeline (9), and annotation was performed on RAST v.2.0 (http://rast.nmpdr.org). Multilocus sequence typing and searching for resistance genes were done with the Center for Genomic Epidemiology platform (www.genomicepidemiology.org). Virulence genes and plasmids were searched for by manual curation with Geneious v.6.1.8 (Biomatters Ltd., New Zealand) and the BLAST

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Address correspondence to Ana Paula D'Alincourt Carvalho-Assef, anapdca@ioc.fiocruz.br.

TABLE 1 Molecular and phenotypic characterization of MCR-1-producing *K. pneumoniae* strain CCBH24080 and its transconjugants

Isolate	Resistance determinants[a]	Virulence genes[b]	MIC (μg/ml)[c]													
			TZP	FOX	CXM	CRO	CAZ	FEP	AMK	GEN	CIP	ETP	MEM	IPM	TGC	CST
CCBH24080	*mcr-1*, bla_{KPC-2}, *aac(6′)Ib-cr*, *aac(3)-IIa*, *strA*, *strB*, bla_{TEM-1}, bla_{OXA-1}, bla_{SHV-11}, $bla_{CTX-M-15}$, *oqxAB*, *qnrB66*, *gyrA* mutation N87D, *parC* mutation S80I, *fosA*, *catB3*, *sul2*, *tet*(A), *dfrA14*	*entB*, *fimH*, *iutA*, *kpn*, *mrkD*, *traT*, *uge*, *ureA*, *wabG*, *ycfM*	≥128	≥64	≥64	≥64	≥64	≥64	16	≥16	≥4	≥8	≥16	>64	2	16
TC-*mcr*[d]	*mcr-1*	ND[e]	≤4	≤4	4	≤1	≤1	≤1	≤2	≤1	≤0.25	≤0.5	≤0.25	0.25	≤0.5	8
TC-*mcr*/bla_{KPC}[d]	*mcr-1*, bla_{KPC-2}	ND	≥128	≥4	≥64	2	2	≤1	≤2	≤1	≤0.25	0.5	1	2	≤0.5	8
J53	NA[f]	NA	≤4	≤4	4	≤1	≤1	≤1	≤2	≤1	≤0.25	≤0.5	≤0.25	0.25	≤0.5	<0.125

[a]Resistance determinants were detected by whole-genome sequencing for CCBH24080 and by PCR for the transconjugants.
[b]Virulence determinants were associated with the production of adhesins (*fimH*, *mrkD*, *kpn*), lipopolysaccharides (*wabG*, *uge*, *ycfM*), siderophores (*iutA*, *entB*), urease (*ureA*), and serum resistance (*traT*).
[c]Abbreviations: TZP, piperacillin-tazobactam; FOX, cefoxitin; CXM, cefuroxime; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; ETP, ertapenem; MEM, meropenem; IPM, imipenem; TGC, tigecycline; CST, colistin.
[d]TC, transconjugant.
[e]ND, not determined.
[f]NA, not applicable.

tool (https://www.ncbi.nlm.nih.gov). CCBH24080 belongs to sequence type 392, a member of internationally successful clonal group 147 (10). In addition, the isolate presented a wide variety of resistance and virulence genes (Table 1). In Brazil, polymyxin B resistance in *K. pneumoniae* has been associated with *mgrB* mutations (11), but no mutations in the *mgrB*, *pmrAB*, *phoPQ*, and *crrAB* sequences were detected in CCBH24080.

By reference mapping, we were able to identify an IncX4 plasmid of 33.3 kb carrying the *mcr-1* gene that is identical to an *E. coli* plasmid from Brazil (GenBank accession no. CP015977.1) (5) with 100% coverage. The bla_{KPC-2}-bearing plasmid was very similar to an IncN plasmid detected in São Paulo (GenBank accession no. CP004367.2), except for a 2.250-bp deletion in the CCBH24080 plasmid. Analysis by S1 pulsed-field gel electrophoresis, followed by Southern blotting, confirmed that *mcr-1* and bla_{KPC-2} were located on plasmids of ~33 and ~44 kb, respectively. Mobilization of both plasmids was successfully assayed by mating donor cells with *E. coli* J53, and transconjugants were selected in 300 μg/ml sodium azide Mueller-Hinton agar containing colistin (2 μg/ml) or imipenem (1 μg/ml). Colistin was found to select transconjugants carrying either both plasmids or the *mcr-1*-carrying plasmid only. Nevertheless, imipenem selected only transconjugants carrying both plasmids (Table 1). The presence of the HicBA toxin/antitoxin system encoded by the IncX4 plasmid may explain this phenomenon (12).

Isolates of *K. pneumoniae* harboring *mcr* variants have been reported in Asia and Europe (1, 13–17), including carbapenemase (NDM-5 and KPC-3)-producing ones (15, 17). To our knowledge, this is the first description of *mcr-1* in a human KPC-2-producing *K. pneumoniae* isolate. This finding raises a major concern, since KPC-producing *K. pneumoniae* is disseminated worldwide, and highlights the potential for the dissemination of *mcr-1* associated with multidrug-resistant international clones. Furthermore, the possibility of the simultaneous transfer of these genes poses a threat to infection control strategies and clinical therapy.

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