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Coexistence of *mcr-1* and *bla*$_{NDM-1}$ in *Escherichia coli* from Venezuela

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Runnig Head: *mcr-1* and *bla*$_{NDM-1}$ in Venezuela

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Abstract

We studied the presence of the mobile colistin resistance gene *mcr-1* in human, animal and environmental *Enterobacteriaceae* from Cumana (Venezuela) collected in 2015. *mcr-1* was detected in 2/93 *Escherichia coli* from swine and human isolates resistant to colistin. Whole-genome sequencing and transformation experiments identified *mcr-1* on an IncI2 plasmid. One of the isolates also bore the widely spread carbapenemase NDM-1. A One Health approach is necessary to further elucidate the flux of these high-risk genes.

Carbapenem Resistant *Enterobacteriaceae* (CRE) are one of the most serious concerns to Public Health, since they are susceptible to very few antibiotics, which convert remaining compounds into last resort agents (1).

One last resort antibiotic against CRE is colistin (polymyxin E) (2). It has been used in veterinary medicine since its discovery in 1949, mainly for the treatment of intestinal tract infections, although it was initially restricted to ophthalmic and topical use in humans, due to its toxicity (3). As a result of the limited therapeutic alternatives, in 2012 the WHO included colistin on the list of critically important agents for human medicine (2).

Until recently, resistance to polymyxins had only been identified as chromosomally mediated mutations, which cannot be transferred between bacteria (3). However, in November 2015, a new plasmid-mediated colistin resistance mechanism, called MCR-1was discovered (4). Since its first identification, *mcr-1* has been widely reported from human, animal, food and environmental origins (5-7). Coexistence of *mcr-1* with a carbapenemase is especially worrisome, as therapeutic options in these cases are very limited. Currently,

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48 the carbapenemase NDM-1 (New Delhi metallo-β-lactamase) is broadly disseminated
49 worldwide (8), although it has been scarcely described in South America (9, 10).
50 In this work, we have detected *mcr-1* positive isolates of different origins in Venezuela, as
51 well as the coexistence of this resistance gene with $bla_{\text{NDM-1}}$.
52 Ninety-three samples from Cumana, Venezuela were selected for their capacity to grow on
53 MacConkey agar (Oxoid Ltd., Basingstoke, United Kingdom). These isolates were
54 collected in August 2015 from human faecal clinical samples (16), faeces of dogs (8
55 samples), swine (17 samples) and poultry (16 samples) and from sewage (36 samples) in
56 different locations of Cumana. The presence of *mcr-1* was screened for by PCR and Sanger
57 sequencing (Secugen S. L. Madrid, Spain) using primers and conditions previously
58 described (4). The two positive isolates (2.1%), identified as *E. coli* by MALDI-TOF MS
59 (Bruker), were collected from faecal samples of a 43 year old man and a swine, BB1290
60 and BB1291 respectively, and displayed 100% identity to *mcr-1* (4). Antimicrobial
61 resistance was determined by Minimal Inhibitory Concentration using broth microdilutions
62 in microtiter plates (Sensititre EUVSEC; Trek Diagnostics, Inc., Westlake, OH) and
63 interpreted following the EUCAST guidelines (11). BB1290 and BB1291 exhibited a
64 multidrug resistant profile (Table).
65 BB1290 and BB1291 were sequenced (MiSeq, Illumina, San Diego, CA, USA) producing
66 100-bp single-end reads with 36× coverage (Life sequencing S.L., Valencia, Spain,
67 GenBank accession nr SRR3745274 for BB1290 and SRR3745275 for BB1291). Assembly
68 was performed with SPADES version 3.6.2, which produced 2,408 and 888 contigs
69 respectively. The data were used to characterize the strains according to antibiotic
70 resistance genes, pathogenicity, serotype and plasmid incompatibility groups, through the

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website of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Moreover, the strains were typed by assigning alleles and sequence types (STs) from the MLST Institute Pasteur website (http://bigsdb.web.pasteur.fr/ecoli/ecoli.html).

The human isolate BB1290 bore, in addition to *mcr-1*, a plethora of genes conferring resistance to beta-lactams ($bla_{\text{NDM-1}}$, $bla_{\text{TEM-1}}$, $bla_{\text{ACT-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-15}}$), aminoglycosides (*aadA5*, *aph(3')-IIa*, *aacA4*, *aac(3)-IIa*, *strA*, *aadA15* and *strB*), fluoroquinolones (*aac(6')Ib-cr* and *qnrB1*), macrolides (*mph(A)* and *erm(B)*), phenicols (*catB3*, *catA1* and *floR*), sulphonamides (*sul1*, *sul2*, *sul3*), tetracycline (*tet*(B)) and trimethoprim (*dfrA1*, *dfrA12*, *dfrA14* and *dfrA17*), which is in line with the extremely drug resistant profile of this isolate. Incompatibility typing detected the presence of the replicons IncHI2, IncHI2A, ColBS512, IncI2 and IncFII. The strain belongs to ST19 and was identified as the O100:H25 serotype, which is related to human enteropathogenic *E. coli* strains (EPEC) (12).

The animal sample harboured *mcr-1*, *aadA1*, *aph(4)-Ia*, *aac(3)-VIa*, $bla_{\text{CTX-M-2}}$, *oqxB*, *sul1*, *tet*(A) and *dfrA14*. Incompatibility group analysis showed the presence of the replicons IncFIB, IncI2, ColpVC and Col8282. Remarkably, IncI2 is the only replicon shared by both isolates. Furthermore, BB1291 belongs to a novel ST, ST452. *In silico* analysis assigned the isolate to serotype O17/O44:H34 and identified 684 pathogenic protein families, predicting the isolate as a human pathogen.

The 100-bp single-end reads were then mapped against the Chinese plasmid pHNSHP45 bearing *mcr-1* (4) (Geneious, version 8.1.7 [http://www.geneious.com] (13)). The results showed that BB1290 and BB1291 harbour a plasmid with 98% and 97% identity,

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93 respectively, to pHNSHP45, albeit lacking the IS*Apl1* mobile element upstream *mcr-1*.
94 Absence of IS*Apl1*was further confirmed by PCR from IS*Apl1* to *mcr-1* (data not shown).
95 Plasmid DNA extractions from BB1290 and BB1291 (QIAprep, Qiagen Inc., Chatswoth,
96 CA.) were transformed into *E. coli* HST08 (Stellar™ Competent Cells, Clontech
97 Laboratories Inc-Takara BioInc, Otsu, Japan) following the manufacturer's protocol and
98 selected on BHI agar containing colistin (2 mg/L). BB1290T and BB1291T transformants
99 obtained from the wild-type strains were both positive for the *mcr-1* gene and IncI2 plasmid
100 incompatibility group (PBRT KIT-PCR-based replicon typing, DIATHEVA). Resistance
101 profiles of the transformants showed that the plasmids only conferred resistance to colistin
102 (Table).
103 To the best of our knowledge, the two colistin resistant *E. coli* isolates from Venezuela
104 constitute the first detection of *mcr-1* in this country. The patient had no direct contact with
105 animals, had not been treated with colistin and had not recently travelled to other countries.
106 However, just a very small number of samples have been tested, and no significant
107 statements regarding transmission routes can be made.
108 One of the *mcr-1* positive *E. coli*, BB1290, also harboured $bla_{\text{NDM-1}}$, further revealing the
109 coexistence of these two genes in the same isolate. This combination in a human pathogen
110 is worrying as it impedes the use of most last resort antibiotics (14). In our case, BB1290
111 was still susceptible to tigecycline.
112 Control of *mcr-1*, its genetic platforms and the bacteria implicated in its dissemination is
113 essential. The collection of surveillance data from developing countries where the
114 information is scarce, such as Venezuela, is crucial in order to establish accurate measures
115 that eventually safeguard the effectiveness of last resort antibiotics.

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References

1. **Rossolini GM.** 2015. Extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: an emerging challenge for clinicians and healthcare systems. J Intern Med **277:**528-531.

2. **WHO.** 2011. Critically important antimicrobial agents for human medicine, 3rd edn. World Health Organization, Geneve.

3. **Catry B, Cavaleri M, Baptiste K, Grave K, Grein K, Holm A, Jukes H, Liebana E, Lopez Navas A, Mackay D, Magiorakos AP, Moreno Romo MA, Moulin G, Muñoz Madero C, Matias Ferreira Pomba MC, Powell M, Pyörälä S, Rantala M, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Törneke K, van Duijkeren E, Torren Edo J.** 2015. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. Int J Antimicrob Agents **46:**297-306.

4. **Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu**

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JH, Shen J. 2016. 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 **16:**161-168.

5. **Skov RL, Monnet DL.** 2016. Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds. Euro Surveill **21**.

6. **Rapoport M, Faccone D, Pasteran F, Ceriana P, Albornoz E, Petroni A, Corso A, MCR-Group.** 2016. *mcr-1*-mediated colistin resistance in human infections caused by *Escherichia coli*: First description in Latin America. Antimicrob Agents Chemother **60:**4412-4413.

7. **Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, Lopes R, Otutumi LK, Gonçalves DD, Dropa M, Matté MH, Monte DF, Landgraf M, Francisco GR, Bueno MF, de Oliveira Garcia D, Knöbl T, Moreno AM, Lincopan N.** 2016. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene. Euro Surveill **21**.

8. **Wei WJ, Yang HF, Ye Y, Li JB.** 2015. New Delhi Metallo-β-Lactamase-Mediated Carbapenem Resistance: Origin, Diagnosis, Treatment and Public Health Concern. Chin Med J (Engl) **128:**1969-1976.

9. **Seija V, Medina Presentado JC, Bado I, Papa Ezdra R, Batista N, Gutierrez C, Guirado M, Vidal M, Nin M, Vignoli R.** 2015. Sepsis caused by New Delhi metallo-β-lactamase (bla_{NDM-1}) and qnrD-producing *Morganella morganii*, treated successfully with fosfomycin and meropenem: case report and literature review. Int J Infect Dis **30:**20-26.

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10. **Kazmierczak KM, Rabine S, Hackel M, McLaughlin RE, Biedenbach DJ, Bouchillon SK, Sahm DF, Bradford PA.** 2016. Multiyear, Multinational Survey of the Incidence and Global Distribution of Metallo-β-Lactamase-Producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother **60:**1067-1078.

11. **EUCAST.** 2016. Breakpoint tables for interpretation of MICs and zone diameters, 6 ed. European Committee on Antimicrobial Susceptibility Testing.

12. **Delannoy S, Beutin L, Fach P.** 2013. Towards a molecular definition of enterohemorrhagic *Escherichia coli* (EHEC): detection of genes located on O island 57 as markers to distinguish EHEC from closely related enteropathogenic *E. coli* strains. J Clin Microbiol **51:**1083-1088.

13. **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A.** 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics **28:**1647-1649.

14. **Yu H, Qu F, Shan B, Huang B, Jia W, Chen C, Li A, Miao M, Zhang X, Bao C, Xu Y, Chavda KD, Tang YW, Kreiswirth BN, Du H, Chen L.** 2016. Detection of *mcr-1* colistin resistance gene in carbapenem-resistant *Enterobacteriaceae* (CRE) from different hospitals in China. Antimicrob Agents Chemother. doi:10.1128/AAC.00440-16.

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183 Table. Data and MICs (mg/L) of the two *mcr-1* positive *E.coli*, their transformants and the recipient strain.

Isolate	Country	Date of isolation	Source	MLST	MICs[a,b]											
					AMP	GEN	CIP	NAL	TMP	MEM	TET	CTX	CHL	TGC	CAZ	CST
BB1290	Venezuela	August 2015	Human faeces	ST19	**>64**	**>32**	**>8**	**>128**	**>32**	**8**	**>64**	**>4**	**>128**	1	**>8**	**4**
BB1290T[c]	-	-	Laboratory	-	4	<0.5	0.06	**64**	<0.25	<0.03	<2	<0.25	<8	<0.25	<0.5	**4**
BB1291	Venezuela	August 2015	Swine faeces	ST452	**>64**	**>32**	**>8**	**>128**	**>32**	<0.03	**>64**	**>4**	**>128**	<0.25	4	**4**
BB1291T[c]	-	-	Laboratory	-	4	<0.5	0.06	**64**	<0.25	<0.03	<2	≤0.25	≤8	<0.25	<0.5	**4**
E. coli HST08	-	-	Laboratory	-	4	<0.5	0.03	**64**	<0.25	<0.03	<2	<0.25	<8	<0.25	<0.5	<1

184 [a] AMP, ampicillin; GEN, gentamicin; CIP, ciprofloxacin; NAL, nalidixic acid; TMP, trimethoprim; MEM, meropenem; TET, tetracycline; CTX, cefotaxime;
185 CHL, chloramphenicol; TGC, Tigecycline; CAZ, ceftazidime; CST, colistin.
186 [b] Resistance is highlighted in bold.
187 [c] *E. coli* HST08 transformed with *mcr-1*-bearing plasmid.