

# Dissemination of the mcr-1 colistin resistance gene

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In their Comment on the Article by Yi-Yun Liu and colleagues about the emergence of plasmid-mediated colistin resistance involving the *mcr-1* gene from bacteria isolated in China,<sup>1</sup> David Paterson and Patrick Harris<sup>2</sup> referred to our finding of colistin resistance in two *Escherichia coli* isolates from a pig and a human being in Laos that were indistinguishable by pulsed-field gel electrophoresis.<sup>3</sup> Our results, suggested animal to human transmission for which no known chromosomally encoded colistin resistance mechanisms were identified, raising the question of a similar mechanism of colistin resistance to that identified by Liu and colleagues. We screened for the presence of the *mcr-1* gene in these isolates along with other colistin-resistant *E coli* isolates from several countries (Laos, Thailand, France, and Nigeria) from human beings and pigs (only in Laos)<sup>4</sup> and in poultry from Algeria using PCR and sequencing as described by Liu and colleagues (table).<sup>1</sup> We included as controls a colistin-susceptible revertant *E coli* strain (obtained at the 18th passage in colistin-free medium) from a pig<sup>3</sup> and 12 colistin-susceptible *E coli* strains isolated from the same

stool samples from which the colistin-resistant *E coli* strains were isolated. 12 (63%) of 19 colistin-resistant *E coli* strains tested were positive for the *mcr-1* gene and sequences were 100% identical to that of *mcr-1* gene sequence reported by Liu and colleagues (table).<sup>1</sup> These include *E coli* strains from Laos (from asymptomatic people and pigs including the strain transferred from a pig to a farmer), Thailand (asymptomatic people), and from Algeria (chickens). None of the 12 colistin-susceptible *E coli* strains and the colistin-susceptible revertant strain (the parent resistant strain was positive for *mcr-1*) were positive for *mcr-1*. The susceptible revertant isolate has probably lost the resistance by loss of a plasmid that is under investigation. We clearly show that plasmid-mediated colistin resistance has spread beyond China to the neighbouring southeast Asian countries and even further to Africa where colistin is widely used in animal production. We anticipate that with the extensive use of colistin in animal production, including in Europe,<sup>5</sup> plasmid-mediated colistin resistance has already spread worldwide, and this calls for prompt international action to restrict or ban the use of colistin in agriculture to avoid further spread of resistance, as occurred with NDM-1 5 years ago.

We declare no competing interests.

Abiola Olumuyiwa Olaitan,  
Selma Chabou, Liliane Okdah,  
Serge Morand, \*Jean-Marc Rolain  
[jean-marc.rolain@univ-amu.fr](mailto:jean-marc.rolain@univ-amu.fr)

Aix-Marseille Université, URMITE, 13385 Marseille Cedex 5, France (AOO, SC, LO, J-MR); Institut des Sciences de l'Evolution, CNRS-IRD-UM2, CC065, Montpellier, France (SM); and CNRS-CIRAD, Centre d'Infectiologie Christophe Mérieux du Laos, Vientiane, Lao PDR (SM)

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	Colistin minimum inhibitory concentration (mg/L)	Year	Source	Country	<i>mcr-1</i>
LH30	6	2012	Human	Laos	+
LH57	8	2012	Human	Laos	+
LH1	6	2012	Human	Laos	+
LH121	16	2012	Human	Laos	+
LH140	12	2012	Human	Laos	+
LH257	12	2012	Human	Laos	+
P10	6	2012	Pig	Laos	+
P6	6	2012	Pig	Laos	+
P17	4	2012	Pig	Laos	+
P7	4	2012	Pig	Laos	-
TH176	6	2012	Human	Thailand	-
TH214	6	2012	Human	Thailand	+
TH99	4	2012	Human	Thailand	+
FHM19*	12	2012	Human	France	-
FHA102†	12	2012	Human	France	-
FHA113‡	12	2012	Human	France	-
NH94§	12	2012	Human	Nigeria	-
235	4	2015	Chicken	Algeria	+
249	3	2015	Chicken	Algeria	-

Mutations in PmrB sensor kinase of the two-component system: \*Pro7\_Gln12del (deletion of 6 aminoacids); †Ala159Val; ‡Thr156Lys; and §Iso92 ins (insertion of isoleucine at position 92).

**Table: Colistin-resistant *Escherichia coli* with the associated *mcr-1* gene isolated from different sources**

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Plasmid-mediated transferable colistin resistance encoded by the *mcr-1* gene was described in *Escherichia coli* and *Klebsiella pneumoniae* isolates from pigs and chicken at a prevalence of around 20%, and in clinical isolates from human beings at a prevalence of around 1% in China.<sup>1</sup> The prevalence of the *mcr-1* gene in Enterobacteriaceae in other countries and in the community is unknown.

We did a prospective study of acquisition of fecal colonisation and carriage with extended-spectrum



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	Traveller with isolate 1	Traveller with isolate 2	Traveller with isolate 3	Traveller with isolate 4	Traveller with isolate 5	Traveller with isolate 6
Travel destination	Thailand, Vietnam, Cambodia, Laos	Tunisia	Peru, Bolivia, Colombia	China	China	Peru, Bolivia
Travel duration (days)	21	8	40	14	23	22
Age (years)	56	55	25	54	62	26
Sex	Female	Female	Female	Male	Female	Male
ESBL gene (ESBL group)	CTX-M-14 (CTX-M group 9)	CTX-M-1 (CTX-M group 1)	CTX-M-15 (CTX-M group 1)	CTX-M-65 (CTX-M group 9)	CTX-M-55 (CTX-M group 1)	CTX-M-55 (CTX-M group 1)
Minimum inhibitory concentration of antimicrobial drug (mg/L)*						
Amoxicillin-clavulanic acid	16	8	>16	16	8	4
Piperacillin-tazobactam	8	≤4	8	≤4	≤4	≤4
Cefotaxime	16	8	32	16	>32	>32
Cefoxitin	16	≤4	8	≤4	≤4	≤4
Ceftazidime	≤1	≤1	16	≤1	4	4
Cefepime	2	2	2	≤1	2	2
Imipenem	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Meropenem	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Gentamicin	>8	≤1	>8	≤1	>8	≤1
Tobramycin	8	≤1	>8	8	8	≤1
Nitrofurantoin	256	≤16	128	32	64	≤16
Co-trimoxazole	>8	>8	>8	≤1	>8	>8
Norfloxacin	>8	8	>8	2	>8	>8
Ciprofloxacin	>2	>2	>2	1	>2	>2
Colistin	4	4	4	4	4	8

ESBL=extended-spectrum β-lactamase. \*Determined using Vitek-2, except for colistin for which E-test results are provided.

**Table: Characteristics of travellers and acquired fecal *Escherichia coli* isolates carrying the *mcr-1* gene**

β-lactamase (ESBL)-producing Enterobacteriaceae in 2001 Dutch travellers (the COMBAT study),<sup>2</sup> from November, 2012, to November, 2013. Acquisition was defined as the absence of ESBL-producing Enterobacteriaceae in a fecal swab sample taken immediately before travel and detection of ESBL-producing Enterobacteriaceae in a sample taken within 1–2 weeks after return to the Netherlands. Of 1847 travellers at risk, 633 (34%) acquired ESBL-producing Enterobacteriaceae. Nine of these 633 travellers acquired ESBL-producing *Escherichia coli* with a colistin minimum inhibitory concentration of 4–8 mg/L (EUCAST clinical breakpoint for resistance >2 mg/L) as detected using Vitek-2 and confirmed by E-test. After publication of the report by Yi-Yun Liu and colleagues,<sup>1</sup> these nine isolates were tested by PCR<sup>1</sup> for the presence of the *mcr-1* gene. The gene was detected in six of

nine isolates and sequencing of the amplicons showed a 100% homology over the length of the fragments with the published sequence.<sup>1</sup> Three ESBL-producing *E coli* were *mcr-1* PCR negative, suggesting colistin resistance due to other mechanisms.<sup>3</sup> Analysis of ESBL genes by microarray,<sup>4</sup> PCR, and sequencing showed that the *mcr-1* positive ESBL-producing *E coli* carried ESBL genes belonging to multiple groups (table). Of the six travellers who acquired ESBL-producing *E coli* carrying the *mcr-1* gene, two unrelated travellers visited Peru and Bolivia, two unrelated travellers visited China, one visited Tunisia, and one visited multiple countries in southeast Asia (Thailand, Vietnam, Laos, and Cambodia). The duration of travel ranged between 8 and 40 days (mean 21.3 days). None of the travellers had accessed medical care and none had used antimicrobial drugs during travel, while five had experienced

traveller's diarrhoea. Analysis of subsequent fecal samples collected at 1, 3, 6, and 12 months after return to the Netherlands did not show ESBL-producing *E coli*, suggesting short-term colonisation with colistin resistant ESBL-producing *E coli* or loss of plasmids carrying ESBL and potentially *mcr-1* genes.

Colistin is used as an ultimate refuge antimicrobial drug in the treatment of infections caused by multidrug resistant Gram-negative microorganisms.<sup>5</sup> Our data suggest a worrisome spread of the *mcr-1* gene in *E coli* in the community across at least three continents. The diversity of ESBL genes present in *mcr-1* positive isolates suggests that the *mcr-1* gene might be carried on multiple plasmid backbones.

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†Maris S Arcilla, †Jarne M van Hattem, Sebastien Matamoros, Damian C Melles, John Penders, Menno D de Jong, \*Constance Schultsz, for the COMBAT consortium ‡schultsz@gmail.com

†Contributed equally

‡Members listed in the appendix

Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Centre, Netherlands (MSA, DCM); Department of Medical Microbiology (JMvH, SM, MDdJ, CS) and Department of Global Health-Amsterdam Institute for Global Health and Development (CS), Academic Medical Centre, Amsterdam, Netherlands; and Department of Medical Microbiology (JP), Maastricht University Medical Centre, School for Nutrition, Toxicology and Metabolism and School for Public Health and Primary Care, Maastricht, Netherlands

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## Plasmid-mediated colistin resistance mechanisms: is it time to revise our approach to selective digestive decontamination?

In November, 2015, Yi-Yun Liu and colleagues<sup>1</sup> published their observations of the emergence of a plasmid-mediated mechanism for polymyxin E (colistin) resistance, MCR-1. They reported its identification in both human and animal

Enterobacteriaceae isolates and documented its high in-vitro horizontal transfer rate ( $10^{-1}$  to  $10^{-3}$ ). Although the detection rate in human Enterobacteriaceae samples remained low (1.4% of *Escherichia coli* and 0.7% *Klebsiella pneumoniae*), their prevalence was high in Chinese livestock monitored within this study.<sup>1</sup> With the potential threat of pan-resistant Gram-negative organisms, the authors support WHO's consideration of colistin as an antimicrobial of crucial importance and emphasise the potential Darwinian selection pressures that have arisen from the growing use of polymyxins as growth promoters in agriculture.<sup>1</sup>

The threat of potentiating the emergence of colistin resistant Gram-negative organisms raises several concerns. One of these surrounds the use of colistin as an oral or enteral antimicrobial for selective digestive decontamination, predominantly in the intensive care setting.<sup>2-4</sup> Selective digestive decontamination in the intensive care unit combines oral or enteral decontamination of the gastrointestinal tract (usually with colistin, tobramycin, and amphotericin B) with a short course, intravenous broad-spectrum antimicrobial (such as a third generation cephalosporin).<sup>2</sup> This intervention has been shown through meta-analysis of randomised control trials to reduce both mortality (at 28 days) and the occurrence of respiratory tract infections in patients admitted to the intensive care unit.<sup>2-4</sup>

Although the short-term benefits of prophylactic antimicrobial chemotherapy in patients in the intensive care unit have been shown for mortality and respiratory tract infections, data for the long-term effects of this approach to therapy in terms of development of antimicrobial resistance is scarce. This remains a major concern in the literature and has meant that selective digestive decontamination remains controversial and not widely

accepted in clinical practice within the UK.<sup>2,4,5</sup> With the emergence in human beings of plasmid-mediated resistance mechanisms for antimicrobials of crucial importance, such as colistin, consideration of the increased selection pressures created by exposure to these antimicrobials in prophylactic regimes must be deemed a priority.

To achieve this, engagement with all clinical specialties to promote clinical leadership of antimicrobial stewardship is crucial.<sup>6</sup> Effective antimicrobial stewardship must consider the future costs (both financial and therapeutic) of the selection of MCR-1 producing organisms on modern medicine. Although the use of selective digestive decontamination in the short-term might be of benefit for the individual patient in intensive care, we call for greater collaboration between clinical specialties to consider the long-term effects of using colistin as part of this regimen. Moreover, we suggest that the role of surveillance for the development of resistance reservoirs in individuals receiving selective digestive decontamination must be considered as a routine to help with the early detection of the unintended effects of this therapy.<sup>7,8</sup> Although there is a potential place for the use of selective digestive decontamination, investigation of modified regimens devoid of important antimicrobials is required.

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\*Timothy Miles Rawson,  
Luke Stephen Prockter Moore,  
James Christopher Hatcher,  
Hugo Donaldson,  
Alison Helen Holmes  
[timothy.rawson07@ic.ac.uk](mailto:timothy.rawson07@ic.ac.uk)

National Institute for Health Research, Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Campus, London W12 0NN, UK (TMR, LSPM, AHH); and Imperial College Healthcare NHS Trust, London, UK (JCH, HD, AHH)

See Online for appendix