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Citrobacter braakii carrying plasmid-borne *mcr-1* colistin resistance gene from ready-to-eat food from a market in the Chaco region of Bolivia

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Sir,

Polymyxins (colistin and polymyxin B) have recently regained a role as last-resort antibiotics for treatment of infections caused by XDR Gram-negative bacteria.[1] Consequently, the emergence and diffusion of colistin resistance is of great concern.

Acquisition of colistin resistance is mainly associated with chromosomal mutations.[1] However, transferable resistance, mediated by the plasmid-borne *mcr*-type genes (encoding phosphoethanolamine transferases that modify lipid A), has recently been reported.[1] *mcr-1* was the first to be reported and, since its first identification in China,[2] it has been detected worldwide in Enterobacteriaceae (mostly *Escherichia coli*) isolated from animals, food, environments and humans.[1] The *mcr-1* gene has been associated with different plasmid replicons such as IncI2, IncHI1, IncHI2, IncP, IncFII, IncFIB and IncX4,[3] and was found only rarely to be chromosomally encoded.[4]

In Latin America, *E. coli* harbouring *mcr-1* have been described in Brazil, Ecuador, Venezuela and Argentina from food-producing animals and human clinical samples.[5–8] Moreover, a study reported intestinal colonization by *mcr-1*-positive *E. coli* in two unrelated travellers, who had visited Peru, Bolivia and Colombia, suggesting the presence of the gene in those countries.[9]

Here we report on the first detection of an *mcr-1*-positive *Citrobacter braakii* isolated in the Bolivian Chaco region from ready-to-eat food.

In 2013, a pilot study was performed in the local markets of Camiri (Cordillera Province, Santa Cruz Department) and Villa Montes (Gran Chaco Province, Tarija Department), with the aim of investigating the presence of antibiotic-resistant Enterobacteriaceae in samples of ready-to-eat food. A total of 100 food samples were analysed as follows. A sliver of each sample was incubated at 35°C in tryptic soy broth plus 2 mg/L vancomycin for 24 h, and then the broth (10 μL) was plated onto MacConkey agar. Overall, 83 food specimens yielded bacterial colonies on MacConkey agar, and these were successively pooled and stored at −80°C pending further analysis.

Screening for the presence of the *mcr-1* gene[2] identified one *mcr*-positive bacterial pool, obtained from a boiled potato. Plating the bacterial pool onto MacConkey agar plus 2 mg/L colistin allowed recovery of an isolate, named CA-26, identified as *Citrobacter freundii* by MALDI-TOF MS (bioMérieux Inc., Marcy-l'Étoile, France). Susceptibility testing performed by reference broth microdilution[10] and interpreted according to the EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints/) revealed that CA-26 was susceptible to all tested antibiotics (Table S1, available as Supplementary data at *JAC* Online) with the exception of colistin (MIC = 8 mg/L).

A conjugation experiment,[11] using *E. coli* J53 (F^- *met pro* Azi^r) as recipient and selection with colistin (2 mg/L) and sodium azide (150 mg/L) yielded J53 transconjugants at a frequency of $7\times10^{-5} \pm 2\times10^{-5}$ transconjugants per recipient. Transconjugants carried the *mcr* gene, as assessed by PCR, and were resistant to colistin (MIC = 4 mg/L).

Genomic DNA of CA-26 was extracted using a QIAsymphony automated station (Qiagen, Hilden, Germany) and subjected to WGS using a HiSeq Illumina platform (Illumina, San Diego, CA, USA). The draft genome assembly included 94 contigs, with an estimated genome size of 5 072 546 bp and an average coverage of 80×.

Analysis of 16S rRNA, *leuS*, *recN*, *rpoB*, *pyrG* and *fusA* gene sequences[12] unambiguously identified CA-26 as *C. braakii*. *In silico* analysis of the antimicrobial resistome confirmed the presence of a plasmid-borne *mcr-1* gene and of the chromosomal bla_{CMY-82} (accession no. KJ207203) AmpC-type β-lactamase gene.

The sequence of the *mcr-1*-bearing plasmid, named pCA-26, was assembled in two contigs and the complete sequence was achieved using PCR and Sanger sequencing. Plasmid pCA-26 was 62 214 bp long, with an average GC content of 42.7%, and belonged to the IncI2 lineage. Its overall structure was similar to that

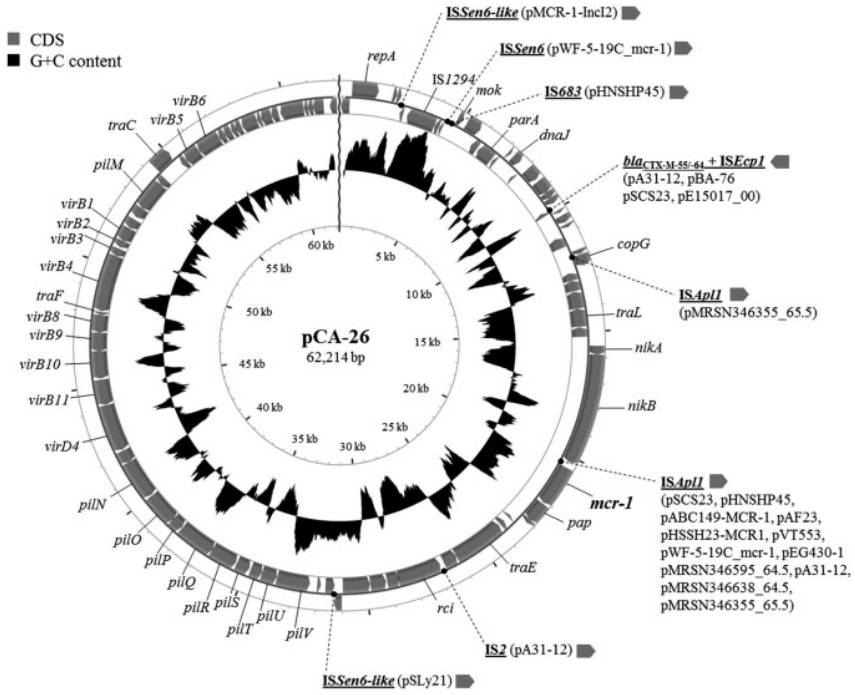

Figure 1. Circular map of plasmid pCA-26. The circles, from the outermost to the innermost, show: (i) coding DNA sequences (CDSs) encoded on the plus and minus DNA strands (annotation is reported for known genes only); and (ii) the G + C content, shown as deviation from the average G + C content of the entire molecule. Main differences against other *mcr-1*-carrying IncI2 plasmids are indicated by dotted lines and bold and underlined text. CDSs are shown in the two outer circles and the GC content is shown in the circle inside these.

of other IncI2 plasmids carrying *mcr-1*, previously described from other areas (99% nucleotide identity over a sequence coverage ranging from 85% to 96%; Table S2). The main differences consisted of the presence of additional IS elements or of an IS*Ecp1*-$bla_{\text{CTX-M-55/64}}$ resistance module in some of the other plasmids (Figure 1). The *mcr-1-pap* cassette in pCA-26 was inserted in a conserved position (i.e. downstream of the *nikB* locus) compared with other IncI2-type plasmids, and was not associated with any flanking mobile element (e.g. IS*Apl1*). An SNP-based phylogenetic analysis identified pA31-12, an *mcr-1*- and $bla_{\text{CTX-M-55}}$-carrying plasmid from an *E. coli* isolated from chicken in China in 2012, as the closest relative to pCA-26 (Table S2). The similarity of the *mcr-1* genetic support to that from isolates from China suggests a possible epidemiological link between China and Bolivia, as also previously suggested.[11] Nevertheless, a possible spread at the food, environmental and human level among Latin American countries could not be excluded. In fact, a recent report,[5] showing the presence of the *mcr-1* gene in Brazilian livestock, dated the presence of *mcr-1* in Latin America back to 2012, suggesting that this resistance determinant has been circulating in Latin America for at least 5 years.

To the best of our knowledge this is the first description of the *mcr-1* gene in *C. braakii*, and also the first direct report of *mcr-1* from Bolivia. This finding underlines that the *mcr-1* determinant could be widespread throughout Latin America, and further underscores its ability to horizontally disseminate among different enterobacterial species.

Nucleotide sequences

The draft genome of *C. braakii* CA-26 and the complete sequence of plasmid pCA-26 have been deposited under GenBank accession numbers MTJW00000000 and KY624633, respectively.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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IMI-2 carbapenemase in a clinical *Klebsiella variicola* isolated in the UK

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Sir,

IMI carbapenemases, named based on their ability to hydrolyse imipenem, are Ambler class A enzymes. bla_{IMI-1} was first identified on the chromosome of two *Enterobacter cloacae* isolates from the USA in 1984,[1] and subsequently small numbers of IMI-positive isolates have been identified in China, Finland, France, Ireland, Norway, Singapore, Tahiti (French Polynesia) and the USA.[2,3] In contrast, IMI-2 and IMI-3 have been described as plasmid-mediated enzymes in *Enterobacter asburiae* and *E. cloacae*, respectively.[4,5] Twelve IMI variants have now been identified (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047) and to date the only report of an IMI carbapenemase identified outside of the genus *Enterobacter* has been in a single isolate of *Escherichia coli* from Spain.[6] Here we report an IMI-2 carbapenemase in a *Klebsiella variicola* strain isolated in the UK.

The strain was initially isolated from an intensive therapy unit patient in 2011 from a soft tissue infection of the buttock and referred to the Specialist Antimicrobial Chemotherapy Unit, Public Health Wales, for investigation of carbapenem resistance. The patient had no known recent travel history. The strain was identified as a *Klebsiella pneumoniae* by BD Phoenix™ (BD Diagnostics, Oxford, UK). MICs of ertapenem, meropenem and imipenem were >32 mg/L, with susceptibility to cefotaxime and ceftazidime (MICs ≤0.25 mg/L) as determined by Liofilchem® MIC test strips (Launch Diagnostics, Longfield, UK). Disc inhibition tests (BioConnections, Knypersley, UK) gave positive results for class A and AmpC enzyme activity, but the isolate was negative for KPC, OXA-48 and OXA-23 carbapenemase and plasmid-mediated AmpC genes by in-house PCR. The isolate was subsequently referred to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for further investigation, where MICs were determined against AMRHAI's standard panel of Gram-negative antibiotics, which includes ertapenem, meropenem and imipenem, using agar dilution, and interpreted using EUCAST criteria. Identification as a *K. pneumoniae* was confirmed by MALDI-TOF MS (Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany).

To investigate the underlying carbapenem resistance mechanism(s) the strain was used as a plasmid donor in electrotransformation to *E. coli* Alpha-Select cells (Bioline, London, UK), with transformants selected on LB agar containing 100 mg/L ampicillin onto which a 10 μg ertapenem disc was placed. WGS of the clinical isolate and transformant was performed using a HiSeq sequencer (Illumina) and the resulting data were analysed using an in-house bioinformatics pipeline, as previously described.[7] Transformant reads were assembled into contigs using SPAdes 3.5.0 (http://cab.spbu.ru/software/spades/). Plasmid contigs were extracted from the *E. coli* Alpha-Select genome by *in silico* subtraction and assembled; replicon typing was performed using PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/).

The strain was resistant to the carbapenems (MICs: ertapenem >16 mg/L, meropenem >32 mg/L and imipenem >128 mg/L), but fully susceptible to the cephalosporins (MICs: cefotaxime