associated with the spread of an IncF plasmid carrying Tn*4401d*.[6] Molecular analyses revealed that *E. coli* #40336 belonged to the ST167 phylogroup A, of the ST10 complex, also reported in US hospitals to spread bla_{KPC} genes.[7]

To the best of our knowledge, this is the first report of a KPC-producing Enterobacteriaceae isolated from filter-feeding molluscs, and more globally from a food product bought at a retail market, thereby posing a potential direct threat to public health. Considering the absence of carbapenem use in animals and the global epidemiology of bla_{KPC} plasmids and KPC-producing *E. coli* clones, this isolate is most likely of human origin. The mussels sampled here were grown in an area receiving hospital effluents, which is consistent with other data on KPC-producing *K. pneumoniae* reported from wastewater in Austria and Brazil.[8,9] Together with the very recent finding of KPC-2 in clinical isolates in Tunisia,[10,11] our data also support the spread of KPC-3 in humans in this country. Such an MDR KPC-producing *E. coli* strain in *M. galloprovincialis* bought at a retail market suggests that other filter-feeding mollusc species may be likely to concentrate carbapenem-resistant Enterobacteriaceae from the human reservoir. The question of whether the food chain has a role in transferring MDR organisms to humans has been raised repeatedly. Here we have shown that molluscs are likely to be an intermediate step disseminating carbapenem-hydrolysing β-lactamases most probably from hospital activities, and these enzymes could further spread back to the human community through food intake or handling.

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Transparency declarations

None to declare.

References

1 Chen L, Mathema B, Chavda KD *et al*. Carbapenemase-producing *Klebsiella pneumoniae:* molecular and genetic decoding. *Trends Microbiol* 2014; **22**: 686–96.

2 Hamza E, Dorgham SM, Hamza DA. Carbapenemase-producing *Klebsiella pneumoniae* in broiler poultry farming in Egypt. *J Glob Antimicrob Resist* 2016; **7**: 8–10.

3 Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; **18**: 1503–7.

4 Poirel L, Walsh TR, Cuvillier V *et al*. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; **70**: 119–23.

5 Naas T, Cuzon G, Villegas MV *et al*. Genetic structures at the origin of acquisition of the β-lactamase bla_{KPC} gene. *Antimicrob Agents Chemother* 2008; **52**: 1257–63.

6 Manageiro V, Ferreira E, Almeida J *et al*. Predominance of KPC-3 in a survey for carbapenemase-producing Enterobacteriaceae in Portugal. *Antimicrob Agents Chemother* 2015; **59**: 3588–92.

7 Chavda KD, Chen L, Jacobs MR *et al*. Molecular diversity and plasmid analysis of KPC-producing *Escherichia coli*. *Antimicrob Agents Chemother* 2016; **60**: 4073–81.

8 Chagas TP, Seki LM, da Silva DM *et al*. Occurrence of KPC-2-producing *Klebsiella pneumoniae* strains in hospital wastewater. *J Hosp Infect* 2011; **77**: 281.

9 Galler H, Feierl G, Petternel C *et al*. KPC-2 and OXA-48 carbapenemase-harbouring Enterobacteriaceae detected in an Austrian wastewater treatment plant. *Clin Microbiol Infect* 2014; **20**: O132–4.

10 Ben Tanfous F, Alonso CA, Achour W *et al*. First description of KPC-2-producing *Escherichia coli* and ST15 OXA-48-positive *Klebsiella pneumoniae* in Tunisia. *Microb Drug Resist* 2017; **23**: 365–75.

11 Battikh H, Harchay C, Dekhili A *et al*. Clonal spread of colistin-resistant *Klebsiella pneumoniae* coproducing KPC and VIM carbapenemases in neonates at a Tunisian university hospital. *Microb Drug Resist* 2016; doi: 10.1089/mdr.2016.0175.

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Co-occurrence of *mcr-1* and bla_{KPC-2} in a clinical isolate of *Escherichia coli* in Brazil

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Table 1.. MICs (mg/L) of several antibiotics for *E. coli* 3431F, transconjugant 3431T1 (IncX4 *mcr-1*), transconjugant 3431T2 (IncX4 *mcr-1* and IncFIB bla_{KPC-2}) and *E. coli* J53

Antibiotic	*E. coli* 3431F	Transconjugant 3431FT1 (IncX4 *mcr-1*)	Transconjugant 3431FT2 (IncX4 *mcr-1* and IncFIB bla_{KPC-2})	*E. coli* J53
Ertapenem	32	0.002	0.125	0.004
Meropenem	32	0.012	0.125	0.023
Imipenem	≥32	0.12	0.5	0.12
Ciprofloxacin	4	≤0.125	≤0.125	≤0.125
Amikacin	2	1	1	≤0.5
Gentamicin	1	0.5	0.5	≤0.125
Tigecycline	0.25	0.25	0.25	0.13
Colistin	4	4	2	≤0.125

MICs were determined by broth microdilution, except those of ertapenem, imipenem and meropenem, which were determined by Etest.

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Sir,

Polymyxins are the last resort for the treatment of infections caused by carbapenem-resistant Enterobacteriaceae (CRE). In November 2015, Liu *et al.*[1] described for the first time a colistin resistance mechanism mediated by a new gene (*mcr-1*), which was present in a transferable plasmid. Since then, several reports have indicated that *mcr-1* has silently spread worldwide since 1980.[2] Of particular concern is the dissemination of the *mcr-1* gene in CRE, potentially leading to pan-drug-resistant isolates. There are only scattered reports of co-occurrence of bla_{KPC} and *mcr-1* genes in the same isolate.[3,4] This study evaluates the characteristics of the first clinical isolate of *Escherichia coli* harbouring both *mcr-1* and bla_{KPC-2} genes in Latin America.

Among 1883 CRE clinical isolates screened, 1 KPC-2-producing *E. coli* was also positive for the *mcr-1* gene (isolate 3431F). This isolate was obtained from a rectal swab of a patient hospitalized, in September 2014, at an emergency room of a general hospital in Porto Alegre city, Rio Grande do Sul, the southernmost Brazilian state.

The bla_{KPC-2}/*mcr-1*-positive clinical isolate carried at least two distinct plasmids, which were successfully transferred to *E. coli* J53 by conjugation. Two transconjugants were further evaluated: the transconjugant 3431FT1, which carried only one plasmid and was positive for *mcr-1*, and the transconjugant 3431FT2, which carried two plasmids and was positive for both *mcr-1* and bla_{KPC-2} (Table 1). The 3431F clinical isolate presented high MICs (≥32 mg/L) of the carbapenems and low-level resistance to colistin (MIC 4 mg/L); the transconjugants 3431FT1 and 3431FT2 presented significant increases in the MIC of colistin in comparison with *E. coli* J53 and the transconjugant 3431FT2 also presented increased MICs of the carbapenems (Table 1).

The assembled WGS of the clinical isolate 3431F produced 67 scaffolds, which resulted in an estimated draft genome 4891834 bp in length, with a G + C content of 50.7% and a total of 4819 genomic features. The *in silico* analyses of the data indicated that *E. coli* 3431F belongs to ST744, an *E. coli* lineage usually associated with resistance genes, including a clinical isolate harbouring *mcr-1* and ESBL genes in Denmark.[5]

The WGS data confirmed the presence of the bla_{KPC-2} and *mcr-1* genes, as well as several other resistance genes, such as genes encoding aminoglycoside-modifying enzymes [*strA*, *strB* and *aph(3′)-Ia*], a β-lactamase-encoding gene (bla_{TEM-1}) and genes related to resistance to macrolides [*mph*(A) and *erm*(42)], phenicols (*floR* and *catA1*), sulphonamides (*sul2*) and tetracycline [*tet*(A) and *tet*(B)].

The *in silico* analyses also allowed identification of the following plasmid incompatibility types in the 3431F isolate: IncN, IncFIB, IncQ1 and IncX4. Detailed analyses indicated that bla_{KPC-2} was located in the IncFIB plasmid and *mcr-1* was located in the IncX4 plasmid. bla_{KPC-2} was located on a Tn*4401* transposon, isoform *b*. The scaffold bearing bla_{KPC-2} was 85492 bp in length and no other resistance gene was found in this scaffold.

Detailed analysis of the scaffold bearing the *mcr-1* gene suggested that it was a complete plasmid (a possible circular molecule 33511 bp in length). For this reason, primers were designed to close the gaps in this scaffold and PCRs, followed by Sanger sequencing, confirmed the circular plasmid molecule. This plasmid, which was termed pMCR1poa (GenBank MTJV00000000), presented >99% overall identity as compared with pICBEC72H (GenBank CP015977),[6] pESTMCR (GenBank KU743383),[7] pMCR1.2-IT (GenBank KX236309)[3] and pMCR1-IncX4 (GenBank KU761327),[8] and 96% identity with pAf48 (GenBank KX032520).[9] pMCR1poa harbours an IS*Apl1* insertion and a *pap2* gene upstream and downstream of the *mcr-1* gene, respectively, as also observed in other *mcr-1*-bearing plasmids described in other countries.[1,3,6–9]

The IncX4-type plasmid carrying the *mcr-1* gene, identified in this study, was previously described in several isolates carrying the *mcr-1* gene, including a clinical isolate obtained from a hospitalized patient in north-eastern Brazil.[6] Isolates of *E. coli* harbouring the IncX4-type plasmid containing the *mcr-1* gene have

also been identified in poultry from southern Brazil by our group (data not shown).

In summary, we report the occurrence of the *mcr-1* gene in a bla_{KPC-2}-positive *E. coli* clinical isolate from Brazil. To the best of our knowledge this is the first report of a clinical isolate with both genes in Latin America. In addition, our findings underscore the broad intercontinental distribution of the IncX4 *mcr-1*-bearing plasmid. Considering the low prevalence of the *mcr-1* gene among clinical isolates of CRE observed in our study, it seems that while carbapenem resistance is high, plasmid-mediated colistin resistance is still rare and sporadic among CRE clinical isolates.

Accession number

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MTJV00000000. The version described in this paper is version MTJV01000000.

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Transparency declarations

None to declare.

References

1 Liu YY, Wang Y, Walsh TR *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**: 161–8.

2 Shen Z, Wang Y, Shen Y *et al*. Early emergence of *mcr-1* in *Escherichia coli* from food-producing animals. *Lancet Infect Dis* 2016; **16**: 293.

3 Di Pilato V, Arena F, Tascini C *et al*. MCR-1.2: a new MCR variant encoded by a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* of sequence type 512. *Antimicrob Agents Chemother* 2016; **60**: 5612–5.

4 Falgenhauer L, Waezsada SE, Yao Y *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**: 282–3.

5 Hasman H, Hammerum AM, Hansen F *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**: pii=30085.

6 Fernandes MR, McCulloch JA, Vianello MA *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**: 6415–7.

7 Brauer A, Telling K, Laht M *et al*. Plasmid with colistin resistance gene *mcr-1* in extended-spectrum-β-lactamase-producing *Escherichia coli* strains isolated from pig slurry in Estonia. *Antimicrob Agents Chemother* 2016; **60**: 6933–6.

8 Li A, Yang Y, Miao M *et al*. Complete sequences of *mcr-1*-harboring plasmids from extended-spectrum-β-lactamase- and carbapenemase-producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2016; **60**: 4351–4.

9 Poirel L, Kiefer N, Brink D *et al*. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. *Antimicrob Agents Chemother* 2016; **60**: 4394.