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Plasmid-mediated colistin resistance (*mcr-1*) in *Escherichia coli* from non-imported fresh vegetables for human consumption

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Background: Colistin constitutes one of the few therapeutic options available for the treatment of human infectious diseases caused by Gram negative multidrug-resistant (MDR) isolates. Moreover, the detection of mobile colistin resistance determinants in the food chain raises significant food safety concerns. Hence, it is crucial to preserve this antibiotic and carefully monitor the dissemination of the recently identified plasmid-mediated colistin resistance gene *mcr-type*.

Material/methods: In the scope of the analysis of the colistin nonsusceptibility of MDR *Escherichia coli* and *Klebsiella pneumoniae* isolates recovered from human clinical samples (n=47) and vegetables (n=25), we identified the presence of the *mcr-1* gene in two *E. coli* isolates, recovered from lettuces produced and marketed in Portugal in May of 2013. MICs were obtained by microdilution broth method, according to EUCAST guidelines. Direct transfer of the *mcr-1* gene was attempted by mating-out assays, with *E. coli* J53 as receptor strain (160 mg/L sodium azide and 2 mg/L colistin). To understand the genetic background of INSali25 we performed whole genome sequencing (Myseq, Illumina). A set of bioinformatic web tools were used to estimate the presence of pathogenicity determinants, antibiotic resistance (AR) genes, and clinically relevant mobile genetic elements.

Results: *E. coli* isolates (INSali25 and INSali204) were non-wild-type to colistin (16 mg/L and 8 mg/L, respectively). These isolates were also nonsusceptible to penicillins, quinolones, aminoglycosides and phenicols, consistent with MDR phenotype. Transfer experiments did not yield any transconjugants containing *mcr-1*-harboring plasmids. Directed bioinformatics analysis identified the *mcr-1* gene in a 36,263 bp length contig with an upstream 1,537 bp region harboring a partial Tn3 that included the antibiotic resistance gene *bla*_{TEM-1}, and a downstream 32,099 bp region showing >99% identity with

previously described *mcr-1*-harboring IncHI1 plasmids. Indeed, the absence of a successful conjugation may be due to thermosensitivity of IncHI1 transfer, which makes these plasmids potential vectors for resistance dissemination among environmental bacteria. Further *in silico* analysis showed the presence of additional antibiotic resistance genes, such as *aph(4)-Ia*, *sat2*, *strA*, *strB*, *sul2*, and *bacA* among others involved in efflux, transport, and permeability. Multilocus Sequence Typing assigned the isolate to ST1716.

Conclusions: Lettuce is a vegetable that is commonly consumed fresh and not subjected to any cooking process, which might amplify the human food safety risks involved, posing a serious health risk to humans, which may result in sporadic cases of gastroenteritis, outbreaks and permanent colonization. Our findings highlight that although *mcr-1* has already been described in humans and in imported vegetables, the occurrence of plasmid-mediated colistin resistance in a non imported fresh produce, acquired at a large retail store may reflect an already widespread distribution.