Nationwide Ubiquity of IncX4 Plasmids Carrying the Colistin Resistance Gene *mcr-1* in Enterobacteriaceae in Brazil

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Background: The emergence and rapid dissemination of the plasmid-mediated *mcr-1* gene among colistin-resistant Enterobacteriaceae has created an urgent need to elucidate the mechanisms involved in gene transfer. Using a WGS approach and, *in silico* analysis, we have investigated plasmids harbouring the *mcr-1*, isolated in Enterobacteriacae recovered from human, livestock, food and environmental (aquatic) samples, collected in different regions of Brazil. **Methods:** Thirty-eight colistin-resistant *E. coli* were sequenced using MiSeq and NextSeq platforms, whereas complete sequence of plasmids from *Enterobacteriaceae* were obatined from public database to a comparative analysis. Further plasmid transfer was evaluated by conjugation and/or transformation using *E. coli* C600 and TOP10 strains as receptor. Complete plasmid sequences were assemblies using Velvet and Spades, whereas whole-genome sequence data was analyzed using tools (i.e., PlasmidFinder, ResFinder, VirulenceFinder, MLST, and SerotypeFinder) provided by the Center of Genomic and Epidemiology. The sequences alignment were performed using Mauve/LastZ for comparing all *mcr-1*-harboring plasmids. **Results:** The presence of IncX4/33-kb (92%), HI2 (5%) and FII (3%) plasmids carrying the *mcr-1* gene was confirmed in all colistin-resistant *E. coli* strains belonging to ST10, ST46, ST48, ST101, ST206, ST224, ST393, ST479, ST533, ST537, ST1850, ST3024. While sixteen plasmids were successfully transferred by conjugation, 22 were transfer by transformation (electroporation). Eighteen plasmids presented a highly conserved IncX4 backbone, with replication, mobilization and

partition genes; however two plasmids were found truncation of the *mob*A gene due to the insertion of IS*1294,* which probably did not favor the conjugation of these plasmids. Comparative analysis of IncX4 plasmids recovered from animal, human, food and environmental Enterobacteriaceae displayed 95-100% nucleotide identity. In all plasmids, the insertion sequence IS*Apl1* was not present, whereas the presence of IS*26* was confirmed. **Conclusion:** The successful dissemination of colistin-resistant *E. coli* strains carrying *mcr-1* is supported by *E. coli* versatility (i.e., hosts and environment adaptation) and plasmid stability, where IncX4 plasmids are key vectors responsible for the dissemination of the *mcr-1* gene. On the other hand, identification of ST10 in different hosts and sources is a public health issue that deserve further investigation.