

TITLE: SUCCESSFUL DISSEMINATION OF *KLEBSIELLA PNEUMONIAE* CC258 (ST11, ST437 AND ST340) IN BRAZIL IS SUPPORTED BY AMR GENES, UBIQUITY, PLASMID STABILITY AND VIRULENT BEHAVIOR

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ABSTRACT:

In Brazil, *K. pneumoniae* isolates belonging to the CC258 (ST11, ST340 and ST437) have spread beyond the hospital, becoming identified in human, animal and aquatic environments, in metropolitan areas. Particularly, the presence of *Klebsiella pneumoniae* ST340 carrying yersiniabactin is worrisome, representing an important public health problem. Using a WGS approach [Illumina NextSeq and MiSeq, *de novo* assemblies (Velvet and Spades), annotations (NCBI Prokka and ISFinder)] we have performed a comparative analysis of the full resistome of clones ST11, ST340 and ST437 isolated from 3 different ecosystems (human, food-producing animal and urban rivers), in Brazil. MLST, plasmid replicons, antimicrobial resistance genes, and virulence genes were identified using multiple databases available from the Center for Genomic Epidemiology (<http://genomicepidemiology.org/>); whereas, *qac* genes, QRDR mutations and heavy metal genes were screened using an in-house script. Transfer of plasmid carrying *bla*_{KPC-2} was evaluated by conjugation and/or transformation using *E. coli* C600 and TOP10 strains as receptor. *In vivo* assays were performed using *Galleria mellonella* and bean plants as animal and endophytic model of colonization/infection, respectively. The presence of conjugative *bla*_{KPC-2}–harboring IncN plasmids (~50-kb) was confirmed in eight carbapenem-resistant *K. pneumoniae* strains belonging to ST340, ST11, ST437, recovered from human and aquatic environment samples, sharing 95-100% nucleotide identity. *Tn4401b* was confirmed in most isolates, but presence of a novel *Tn4401b*-like array was found. On the other hand, a IncX3 plasmid carried a NTE_{KPC}. The resistome revealed that environmental isolates (ST11, ST437 and ST340) carried further QAC and heavy metal genes, whereas the animal strain (ST340) harbored heavy metal (i.e. cooper, mercury and silver) genes. *In vivo*, ST340 lineages carrying yersinoabactin system were more virulent than ST11 and ST437. Indeed, larvae infected with lineages ST340 showed ≤40% survival at 48h infection (1x10⁶ UFC/ml). On the other hand, independent of ST, only environmental- and animal-borne isolates showed endophytic capacity. In Brazil, the successful dissemination of CC258 is supported by *K. pneumoniae* versatility (i.e., hosts and environment adaptation), antimicrobial resistance genes, genomic plasticity, plasmid stability, where ST340 is highlighted by a superior virulence behavior.

Keywords: *Klebsiella pneumoniae*, CG258, ST340, WGS, KPC-2

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