

TITLE: ENDOPHYTIC LIFESTYLE OF CLINICALLY SIGNIFICANT CTX-M-15-PRODUCING ENTEROBACTERIACEAE USE COMMERCIAL VEGETABLES AS A TROJAN HORSE

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ABSTRACT:

β -lactam antibiotics are the most commonly used antimicrobials in clinical practice. One of the most important mechanisms of antibiotic resistance in enterobacteria is the production of extended-spectrum β -lactamases (ESBLs). Although the environmental dissemination of ESBL-producing bacteria belonging to high-risk clones has been described, the colonization and persistence of these pathogens within plants has not been reported, so far. Endophytic bacteria are resistant to superficial disinfection methods, and so, can colonize hosts that use vegetables in the diet. Using a WGS approach, the present study aimed to identify and characterize ESBL-producing endophytic enterobacteria in fresh vegetables marketed in the state of São Paulo. Initially, the samples had the surface sterilized, were ground, and then aliquots were transferred to MacConkey agar supplemented with ceftriaxone. One *Klebsiella pneumoniae* and one *Enterobacter cloacae* isolated from lettuce and spinach, respectively, and two *Escherichia coli* from cabbage and celery produced CTX-M-15-type ESBL and were resistant to β -lactams antibiotics, as well as to others drugs. Transfer of plasmid-mediated ESBL was performed by conjugation or transformation assays using *E. coli* TOP10 as receptor. In addition, the endophytic lifestyle capacity was confirmed by the inoculation of the isolates in sprouted bean grains (*Phaseolus vulgaris*) and subsequent re-isolation from root and aerial parts of plants grown aseptically for 10 to 15 days. The genomes of the isolates were sequenced and are being analyzed. A ~150-kb IncFIB(K) plasmid (pKP301cro) was identified in the endophytic *K. pneumoniae* ST198, and this plasmid carried resistance genes to β -lactam (*bla*_{CTX-M-15}, *bla*_{TEM-1B}), aminoglycosides (*strA*, *strB*), fosfomycin (*fosA*), sulphonamides (*sul2*), and heavy metals (*cusSRCFBA*, *copABCDRSE*, *arsRDABC*). The coexistence of antibiotic and heavy metal resistance genes on the same plasmid suggests that the presence of heavy metals in agricultural crops, possibly from inorganic fertilizers and pesticides, may explain the persistence of resistance genes to multiple antibiotics in endophytic bacteria. In summary, the presence of plasmid-mediated ESBL (CTX-M-15)-producing endophytic bacteria is reported for the first time, and its occurrence in vegetables marketed for consumption constitutes a source of dissemination to humans and other animals, similarly to a Trojan horse mechanism, seriously compromising food security.

Keywords: endophytic, multidrug-resistant, CTX-M-15, heavy metals, food security

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