TITLE: MULTIDRUG-RESISTANT EPIPHYTIC AND ENDOPHYTIC GRAM-NEGATIVE PATHOGENS FROM READY-TO-EAT VEGETABLES

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ABSTRACT:

The global dissemination of antibiotic-resistant bacteria and their resistance genes is a critical issue that is no longer restricted to hospital facilities, but also represents a growing problem involving environmental and food safety. In the present study, a microbiological investigation of multidrug-resistant bacteria (MDR) was performed, focusing on the screening of epiphytic and endophytic Gram-negative bacteria in fresh organic and non-organic vegetables (*i.e*., arugula, *n*= 8; lettuce, *n*= 7; salad mix, *n*= 2) sold unpacked and ready-to-eat (RTE), in São Paulo, Brazil. While epiphytic bacteria were isolated after direct incubation of vegetables in buffered peptone water, endophytic bacteria were isolated from washed and disinfected vegetables submitted to maceration. All vegetable samples were plated on MacConkey agar supplemented with ceftriaxone (2 µg/mL), and bacterial species were identified by MALDI-TOF/MS, with antimicrobial susceptibility profiles being determined by disk diffusion method. Twenty-seven bacterial species were identified from all vegetables, of which 8 were endophytic (*Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., *Aeromonas* spp., and *Stenotrophomonas maltophilia*), and 19 were epiphytic (*Acinetobacter* spp., *Escherichia coli*, *Pseudomonas* spp., and *Stenotrophomonas maltophilia*). Noteworthy, three epiphytic and one endophytic *P. putida* group, isolated from arugula or salad mix, were resistant to fluoroquinolones (levofloxacin, ciprofloxacin, enrofloxacin), carbapenems (meropenem), cephalosporin (cefepime, ceftazidime) or monobactam (aztreonam); whereas two *E. coli* strains isolated from two different samples of arugula, displayed resistance to cephalosporins, carbapenems, fluoroquinolones, sulfamethoxazole-trimethoprim and tetracycline. The presence of MDR bacteria exhibiting a endophytic/epiphytic lifestyle support a wide potential for their dissemination, even through hygienized vegetables. Finally, the presence of Enterobacterales, *Aeromonas* and non-fermentative bacteria from ready-to-eat vegetables reveals that hygienization by the manufacturer not guarantee a bacteria-free product. While, genomic analysis allows elucidate the genetic background (antibiotics, heavy metals and disinfectant resistome, virulome, and clonal relatedness) of these MDR species, evaluation of physical/chemical methods for disinfection of food vegetables will be investigated.

Keywords: ready-to-eat vegetables, antibiotic resistance, multidrug-resistant bacteria, epiphytic, endophytic.