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Title: Identification, enumeration and antimicrobial susceptibility testing of the epiphytic microbial population of leafy vegetables of Cyprus

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In the last few years, leafy vegetables have increased in consumption due to their well-known role in our daily nutrition. Their contamination though by microbes originating from soil, manure and handlers microflora is of increasing interest from the public health perspective. Food safety regulations declare that foodstuffs should not contain micro-organisms in quantities that present an unacceptable risk for human health. In addition to their microbial load, it is of increasing concern the microorganisms resistance to antibiotics due to increasing usage of antibiotics in agriculture and other related fields. The aim of this study was the enumeration and identification of leafy vegetables (lettuce, rucola, spinach, purslane) microflora in Cyprus and the percentage estimation of antibiotic resistant microorganisms in ampicillin (Aminopenicillins), cefotaxime (Cephalosporins), gentamicin (Aminoglycosides) and vancomycin (Glycopeptides). The results showed high number of the total microflora and Enterobacteriaceae with a range of 6.31-7.85 and 5.86-6.7 log cfu/g respectively. More than 95% of total microflora and 87% of Enterobacteriaceae were resistant to ampicillin. Lower was the rate of resistance to cefotaxime (24-63%) and gentamicin (16-27%) for Enterobacteriaceae from all samples except purslane and higher for the total microflora (75-91% and 49-91% respectively) from all samples. Rucola and spinach were the samples with positive results for *Escherichia coli*, with rucola be the only sample with antibiotic resistant isolates (~3 cfu/g) in cefotaxime. High population numbers were also found for Enterococcus, but antibiotic resistance was found only in ampicillin (~10 cfu/g). Samples had similar population numbers for yeasts and moulds (4.04-5.67 log cfu/g). Sequencing of antibiotic resistant Enterobacteriaceae showed many species on the surface of leafy vegetables. As a result, current study showed that leafy vegetables can be a great source of pathogenic microorganisms and antibiotic resistant isolates. Thus, proper hygiene practices during production and handling are good ways for minimizing the microbial load. Washing during packaging shows to negatively affect the epiphytic microflora without disregarding the washing before consumption that leads to significant

improvement of leafy vegetables safety, and should be regarded as an inviolable rule of good hygiene practice.