

Detection of Antibiotic Resistance Genes and Antibiotic Residues in Plant-based Products

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Abstract : Vegetables represent an integral part of a healthy diet due to their valuable nutritional properties and the growth in consumer demand in recent years is particularly remarkable for a diet rich in vitamins and micronutrients. However, plant-based products are involved in several food outbreaks connected to various sources of contamination and quite often, bacteria responsible for side effects showed high resistance to antibiotics. The abuse of antibiotics can be one of the main mechanisms responsible for increasing antibiotic resistance (AR). Plants grown for food use can be contaminated directly by spraying antibiotics on crops or indirectly by treatments with antibiotics due to the use of manure, which may contain both antibiotics and genes of antibiotic resistance (ARG). Antibiotic residues could represent a potential way of human health risk due to exposure through the consumption of plant-based foods. The presence of antibiotic-resistant bacteria might pose a particular risk to consumers. The present work aims to investigate through a multidisciplinary approach the occurrence of ARG by means of a biomolecular approach (PCR) and the prevalence of antibiotic residues using a multi residues LC-MS/MS method, both in different plant-based products. During the period from July 2020 to October 2021, a total of 74 plant samples (33 lettuces and 41 tomatoes) were collected from 57 farms located throughout the Piedmont area, and 18 out of 74 samples (11 lettuces and 7 tomatoes) were selected to LC-MS/MS analyses. DNA extracted (ExtractME, Blirt, Poland) from plants used on crops and isolated bacteria were analyzed with 6 sets of end-point multiplex PCR (Qiagen, Germany) to detect the presence of resistance genes of the main antibiotic families, such as tet genes (tetracyclines), bla (β -lactams) and mcr (colistin). Simultaneous detection of 43 molecules of antibiotics belonging to 10 different classes (tetracyclines, sulphonamides, quinolones, penicillins, amphenicols, macrolides, pleuromotilines, lincosamides, diaminopyrimidines) was performed using Exion LC system AB SCIEX coupled to a triple quadrupole mass spectrometer QTRAP 5500 from AB SCIEX. The PCR assays showed the presence of ARG in 57% (n=42): tetB (4.8%; n=2), tetA (9.5%; n=4), tetE (2.4%; n=1), tetL (12%; n=5), tetM (26%; n=11), blaSHV (21.5%; n=9), blaTEM (4.8%; n=2) and blaCTX-M (19%; n=8). In none of the analyzed samples was the mcr gene responsible for colistin resistance detected. Results obtained from LC-MS/MS analyses showed that none of the tested antibiotics appear to exceed the LOQ (100 ppb). Data obtained confirmed the presence of bacterial populations containing antibiotic resistance determinants such as tet gene (tetracycline) and bla genes (beta-lactams), widely used in human medicine, which can join the food chain and represent a risk for consumers, especially with raw products. The presence of traces of antibiotic residues in vegetables, in concentration below the LOQ of the LC-MS/MS method applied, cannot be excluded. In conclusion, traces of antibiotic residues could be a health risk to the consumer due to potential involvement in the spread of AR. PCR represents a useful and effective approach to characterize and monitor AR carried by bacteria from the entire food chain.

Keywords : plant-based products, ARG, PCR, antibiotic residues

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