Prevalence of Antibiotic-Resistant Bacteria Isolated from Fresh Vegetables Retailed in Eastern Spain

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Abstract : Antibiotic resistance is a growing public health concern worldwide, and it is now regarded as a critical issue within the "One Health" approach that affects human and animal health, agriculture, and environmental waste management. This concept focuses on the interconnected nature of human, animal and environmental health, and WHO highlights zoonotic diseases, food safety, and antimicrobial resistance as three particularly relevant areas for this framework. Fresh vegetables are garnering attention in the food chain due to the presence of pathogens and because they can act as a reservoir for Antibiotic Resistance Bacteria (ARB) and Antibiotic Resistance Genes (ARG). These fresh products are frequently consumed raw, thereby contributing to the spread and transmission of antibiotic resistance. Therefore, the aim of this research was to study the microbiological quality, the prevalence of ARB, and their role in the dissemination of ARG in fresh vegetables intended for human consumption. For this purpose, 102 samples of fresh vegetables (30 lettuce, 30 cabbage, 18 strawberries and 24 spinach) from different retail establishments in Valencia (Spain) have been analyzed to determine their microbiological quality and their role in spreading ARB and ARG. The samples were collected and examined according to standardized methods for total viable bacteria, coliforms, Shiga toxin-producing Escherichia coli (STEC), Listeria monocytogenes and Salmonella spp. Isolation was made in culture media supplemented with antibiotics (cefotaxime and meropenem). A total of 239 strains resistant to beta-lactam antibiotics (Third-Generation Cephalosporins and Carbapenems) were isolated. Thirty Gram-negative isolates were selected and biochemically identified or partial sequencing of 16S rDNA. Their sensitivity to 12 antibiotic discs was determined using the Kirby-Bauer disc diffusion technique to different therapeutic groups. To determine the presence of ARG, PCR assays for the direct sample and selected isolate DNA were performed for main expanded spectrum beta-lactamase (ESBL)-, carbapenemase-encoding genes and plasmid-mediated quinolone resistance genes. From the total samples, 68% (24/24 spinach, 28/30 lettuce and 17/30 cabbage) showed total viable bacteria levels over the accepted standard 10(2)-10(5) cfu/g range; and 48% (24/24 spinach, 19/30 lettuce and 6/30) showed coliforms levels over the accepted standard 10(2)-10(4) cfu/g range. In 9 samples (3/24 spinach, 3/30 lettuce, 3/30 cabbage; 9/102 (9%)) E. coli levels were higher than the standard 10(3) cfu/g limit. Listeria monocytogenes, Salmonella and STEC have not been detected. Six different bacteria species were isolated from samples. Stenotrophomonas maltophilia (64%) was the prevalent species, followed by Acinetobacter pitii (14%) and Burkholderia cepacia (7%). All the isolates were resistant to at least one tested antibiotic, including meropenem (85%) and ceftazidime (46%). Of the total isolates, 86% were multidrug-resistant and 68% were ESBL productors. Results of PCR showed the presence of resistance genes to beta-lactams blaTEM (4%) and blaCMY-2 (4%), to carbapenemes blaOXA-48 (25%), blaVIM (7%), blaIMP (21%) and blaKPC (32%), and to quinolones QnrA (7%), QnrB (11%) and QnrS (18%). Thus, fresh vegetables harboring ARB and ARG constitute a potential risk to consumers. Further studies must be done to detect ARG and how they propagate in non-medical environments.

Keywords : ESBL, β-lactams, resistances, fresh vegetables.
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